

REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: GF-3307 (S7K-3-3)

Product name(s): QUEEN

Chemical active substance(s):

Fenpicoxamid (XDE-777), 50 g/L

Prothioconazole, 100 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(extension of use)

Applicant: Corteva Agriscience

Submission date: March 2025

MS Finalisation date: July 2025 (initial Core Assessment)

March 2026 (final Core Assessment)

Version history

When	What
March 2025	Initial dRR Application for product authorisation of submission of GF-3307 (S7K-3-3) Sugar beet/Fodder beet Extension of Use in the Central Zone.
July 2025	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 struck through and shaded for transparency.
November 2025	The first update of aquatic risk assessment performed by the Applicant.
February 2026	The second risk assessment for aquatic organism performed by the Applicant.
March 2026	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. Not agreed or not relevant information are struck through and shaded for transparency.

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9.1.1.8	Effects on soil microbial activity (KCP 10.5)	Błąd! Nie zdefiniowano zakładek.
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9 Ecotoxicology (KCP 10)

This document reviews the environmental toxicology studies and risk calculations for the plant protection product GF-3307 (S7K-3-3), a formulation containing fenpicoxamid (XDE-777) (50 g as/L) and prothioconazole (100 g a.s./L) for the use on sugar beet/fodder beet.

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPsU

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use No.*	Member state(s)	Crop and/or situation	F, Fn, Fpn G, Gn, Gpn or I**	Pests or group of pests controlled	Application				Application rate			PHI ***	Remarks	Conclusion						
					Method/ kind	Timing/growth stage of crop & season	Max. number a) per use b) per crop/season	Min. interval between appn. (d)	L FP/ha a) max. rate per appn. b) max. total rate per crop/season	g as/ha a) max. rate per appn. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	PL, HU, RO, SK	Sugar Beet Fodder Beet	F	<i>Cercospora beticola</i> (CER-CBE) <i>Uromyces betae</i> (UROMBE) <i>Erysiphe betae</i> (ERYSBE) <i>Ramularia beticola</i> (RAMUBE)	Tractor mounted spray	BBCH 39-49	a) 1 b) 2	21 days	a) 1.5 L/ha b) 3 L/ha	a) 75 Fenpicoxamid + 150 Prothioconazole b) 150 Fenpicoxamid + 300 Prothioconazole	150-300	21	Range 1.2-1.5 L/ha	A	A	R	C	A	A	A
2	BE, CZ, AT, NL	Sugar Beet Fodder Beet	F	<i>Cercospora beticola</i> (CER-CBE) <i>Uromyces betae</i> (UROMBE) <i>Erysiphe betae</i> (ERYSBE) <i>Ramularia beticola</i> (RAMUBE)	Tractor mounted spray	BBCH 39-49	a) 1 b) 2	21 days	a) 1.5 L/ha b) 3 L/ha	a) 75 Fenpicoxamid + 150 Prothioconazole b) 150 Fenpicoxamid + 300 Prothioconazole	150-300	21		A	A	R	C	A	A	A

* 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).

Explanation for column 15 – 21 “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 table:

- (1) Numeration necessary to allow references
- (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/m³ 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
- (14) Remarks may include: Extent of use/economic importance/restrictions

9.1.1 Overall conclusions

9.1.1.1 Effects on birds (KCP 10.1.1), TERA and TERLT values are above the Annex VI trigger values, therefore, there is acceptable acute and chronic risk to birds from fenpicoxamid, prothioconazole, relevant metabolites, and GF-3307 (S7K-3-3). There is low risk to birds from drinking water or consuming contaminated prey items.

The acute and long-term risks of GF 3307 to birds was assessed by calculating toxicity exposure ratios between toxicity endpoints from studies with fenpicoxamid, prothioconazole and their calculated mixture toxicity with maximum residues estimated to occur on food items following applications according to the proposed use pattern. In the combined risk assessment also prothioconazole metabolite JAU 6476-desthio was taken into account due to its higher toxicity comparing to parent.

Based on combined mixture toxicity acceptable acute risk was shown and for all focal species relevant for the intended Central Zone use pattern and acceptable chronic risk was also determined.

There is low risk to birds from drinking water or consuming contaminated prey items.

Acceptable risk of secondary poisoning was demonstrated.

9.1.1.2 Effects on terrestrial vertebrates other than birds (KCP 10.1.2),

9.1.1.3 **TERA** and **TERLT** values are above the Annex VI trigger values, therefore, there is acceptable acute and chronic risk to birds and mammals from fenpicoxamid, prothioconazole and relevant metabolites. For GF-3307 (S7K-3-3), **TERA** values are above the Annex VI trigger values, therefore, there is acceptable acute risk for birds and mammals from GF-3307 (S7K-3-3). Chronic mixture toxicity was calculated based on fenpicoxamid and the prothioconazole metabolite JAU 6476-desthio, which is more toxic to birds and mammals than the parent; thereby, the lowest effect value from active substances and metabolites was chosen and compared to the summed application rate of the active substances. For birds, the **TERLT** value is above the Annex VI trigger value and therefore, there is acceptable chronic risk from GF-3307 (S7K-3-3). For mammals, the **TERLT** fell below the trigger for voles at BBCH > 40 and lagomorphs at BBCH 10 – 39 indicating potential chronic risk for these species. The risk for vole was refined using a refined deposition factor of 0.1 and the risk for lagomorphs was refined using the **NOAEL_{mix}** for fenpicoxamid and JAU 6476-desthio or alternatively the molecular conversion factor for the application rate of JAU 6576-desthio was considered. Based on these refinements, acceptable chronic risk for mammals from GF-3307 (S7K-3-3) is concluded.

There is low risk to mammals from drinking water or consuming contaminated prey items.

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

TER_A and **TER_{LT}** values are above the Annex VI trigger values, therefore, there is acceptable acute and chronic risk to birds and mammals from fenpicoxamid, prothioconazole and relevant metabolites. For GF-3307 (S7K-3-3), **TER_A** values are above the Annex VI trigger values, therefore, there is acceptable acute risk for birds and mammals from GF-3307 (S7K-3-3). Chronic mixture toxicity was calculated based on fenpicoxamid and the prothioconazole metabolite JAU 6476-desthio, which is more toxic to birds and mammals than the parent; thereby, the lowest effect value from active substances and metabolites was chosen and compared to the summed application rate of the active substances. For birds, the **TER_{LT}** value is above the Annex VI trigger value and therefore, there is acceptable chronic risk from GF-3307 (S7K-3-3). For mammals, the **TER_{LT}** fell below the trigger for voles at BBCH > 40 and lagomorphs at BBCH 10 – 39 indicating potential chronic risk for these species. The risk for vole was refined using a refined deposition factor of 0.1 and the risk for lagomorphs was refined using the **NOAEL_{mix}** for fenpicoxamid and JAU 6476-desthio or alternatively the molecular conversion factor for the application rate of JAU 6576-desthio. Based on these refinements, acceptable chronic risk for mammals from GF-3307 (S7K-3-3) is concluded.

There is low risk to mammals from drinking water or consuming contaminated prey items.

9.1.1.5 Effects on aquatic organisms (KCP 10.2)

Acceptable risk is demonstrated for fenpicoxamid, prothioconazole, relevant metabolites, and GF-3307 (S7K-3-3) in sugar beet/fodder beet at 1 to 2 applications of 1.5 L GF-3307/ha (1 to 2 x 75 g fenpicoxamid/ha + 150 g prothioconazole/ha) with a:

- 30 m no spray zone (NSZ) + 10 m Vegetative Filter Strip (VFS) or
- 10 m NSZ + 10 m VFS + 75% drift reducing nozzles (DRN).

9.1.1.6 Effects on bees (KCP 10.3.1)

Acute assessment

The SANCO Q_H values for fenpicoxamid, prothioconazole, relevant metabolites, and GF-3307 (S7K-3-3) in honeybee and bumble bee are below the Annex VI trigger of 50; therefore, the acute oral and contact risk to honeybees and bumble bees is acceptable. The EFSA acute contact Q_{HC} values and oral ETRs for fenpicoxamid, prothioconazole, and relevant metabolites for honeybees and bumble bees, where available, are below the trigger values indicating acceptable risk to bees. The EFSA Q_{HC} values for GF-3307 (S7K-3-3) for honeybees and bumble bees are below the trigger value indicating acceptable risk. The EFSA acute oral ETRs for GF-3307 (S7K-3-3) for honeybees and bumble bees only slightly exceeded the trigger values indicating low risk. Risk assessment for application of 2 L GF-3307/ha on flowering attractive bee crops *Phacelia tanacetifolia* is worst-case and protective for application of 1.5 L GF-3307/ha on sugar beet/fodder beet. Therefore, the risk to bees is considered acceptable. However, the final decision of using higher tier studies to refine the risk according to EFSA GD 2013 is left at MS level according to recommendation given during 7th CZHM in Warsaw, 2023.

Chronic assessment

The TER value for honeybee larvae is greater than the EPPO 2010 trigger of 1 indicating acceptable risk from GF-3307 (S7K-3-3). The chronic risk for adult honey bees and the risk assessment based on the EFSA 2013 bee guidance assessment were refined with an OECD 75 tunnel study and a colony feeding study in combination with pollen and nectar residue trials. These colony feeding and residue studies show that there is no effect at 2 L GF-3307/ha applied during flowering on oilseed rape on adult bee mortality, brood development, colony size and overwintering survival. Risk assessment for application of 2 L GF-3307/ha on flowering oilseed rape is worst case and protective for application of 1.5 L GF-3307/ha on sugar beet and fodder beet. Therefore, the risk is acceptable.

Risk assessment for application of 2 L GF-3307/ha on flowering attractive bee crops *Phacelia tanacetifolia* is worst-case and protective for application of 1.5 L GF-3307/ha on sugar beet/fodder beet. Therefore, the risk to bees is considered acceptable.

In conclusion: A low risk from application of 2 L GF-3307/ha on bee attractive crop (*Phacelia tanacetifolia*,) has been demonstrated. Therefore, a low risk to honey bees from GF-3307 (S7K-3-3) applied at the lower rate of 1.5 L/ha on sugar beet/ fodder beet, which are not attractive to bees, can be concluded.

However, the final decision of using higher tier studies to refine the risk according to EFSA GD 2013 is left at MS level according to recommendation given during 7th CZHM in Warsaw, 2023.

Acute assessment

9.1.1.7 The SANCO Q_H values for fenpicoxamid, prothioconazole, relevant metabolites, and GF-3307 (S7K-3-3) in honeybee and bumble bee are below the Annex VI trigger of 50; therefore, the acute oral and contact risk to honeybees and bumble bees is acceptable. The EFSA acute contact Q_{HC} values and oral ETRs for fenpicoxamid, prothioconazole, and relevant metabolites for honeybees and bumble bees, where available, are below the trigger values indicating acceptable risk to bees. The EFSA Q_{HC} values for GF-3307 (S7K-

3-3) for honeybees and bumble bees are below the trigger value indicating acceptable risk. The EFSA acute oral ETRs for GF-3307 (S7K-3-3) for honeybees and bumble bees only slightly exceeded the trigger values indicating low risk.

Chronic assessment

~~The TER value for honeybee larvae is greater than the EPPO 2010 trigger of 1 indicating acceptable risk from GF-3307 (S7K-3-3).~~ The chronic risk for adult honey bees and the risk assessment based on the EFSA 2013 bee guidance assessment were refined with an OECD 75 tunnel study and a colony feeding study in combination with pollen and nectar residue trials. These colony feeding and residue studies show that there is no effect at 2 L GF-3307/ha applied during flowering on oilseed rape on adult bee mortality, brood development, colony size and overwintering survival. Risk assessment for application of 2 L GF-3307/ha on flowering oilseed rape is worst-case and protective for application of 1.5 L GF-3307/ha on sugar beet and fodder beet. Therefore, the risk is acceptable.

In conclusion: A low risk from application of 2 L GF-3307/ha on bee attractive crop (*Phacelia tanacetifolia*,) has been demonstrated. Therefore, a low risk to honey bees from GF-3307 (S7K-3-3) applied at the lower rate of 1.5 L/ha on sugar beet/ fodder beet, which are not attractive to bees, can be concluded.

However, the final decision of using higher tier studies to refine the risk according to EFSA GD 2013 is left at MS level according to recommendation given during 7 th CZHM in Warsaw, 2023.

Commenting period process:

Some of MSs indicated that although the crop is not attractive to bees considering nectar and/or pollen, the crop may be attractive due to honeydew production.

Therefore this may indeed be an additional point on MS level.

9.1.1.8 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 prothioconazole, the tier 1 in-field HQ values are below the trigger of 2 for five of the six species tested, and the off-field HQ values are below the trigger of 2 for all six species tested. *A. rhopalosiphii* and *T. pyri* tier 2 in- and off-field HQ values are below the trigger of 1 indicating low risk to non-target arthropods in sugar beet/fodder beet at the proposed GAP.

Acceptable off-field risk is demonstrated for GF-3307 (S7K-3-3) when used according to proposed GAP. In-field risk to foliar-dwelling organisms (*Aphidius*, *Chrysoperla* and *Coccinella*) is acceptable 14 days post second application when used according to proposed GAP with no need for risk mitigation. Acceptable off-field risk is demonstrated for GF-3307 (S7K-3-3) when used according to proposed GAP. In-field risk to foliar-dwelling organisms (*Aphidius*, *Chrysoperla* and *Coccinella*) is acceptable 14 days post second application when used according to proposed GAP with no need for risk mitigation.

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

TERA values for prothioconazole and relevant metabolites are above the Annex VI trigger value of 10 indicating there is low acute risk to earthworms. TERLT values for fenpicoxamid, prothioconazole, relevant metabolites, and GF-3307 (S7K-3-3) are above the Annex VI trigger value of 5 indicating there is acceptable chronic risk to earthworms, meso-, and macrofauna at the proposed GAP.

9.1.1.10 Effects on soil microbial activity (KCP 10.5)

The maximum concentrations with less than 25% effects for fenpicoxamid, prothioconazole, 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.

9.1.1.11 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-3307 (S7K-3-3) in sugar beet/fodder beet according to good agricultural practice is acceptable.

9.1.1.12 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.

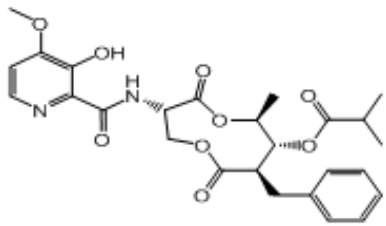
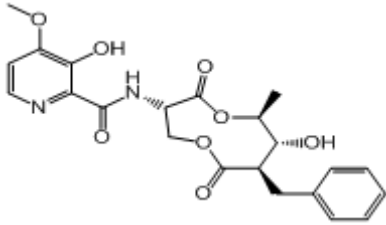
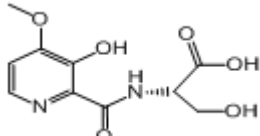
9.1.2 Grouping of intended uses for risk assessment

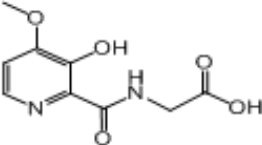
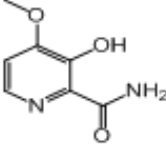
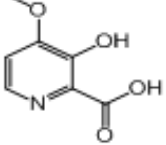
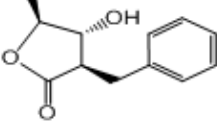
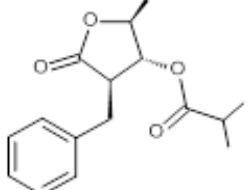
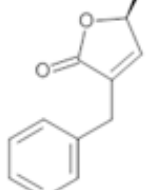
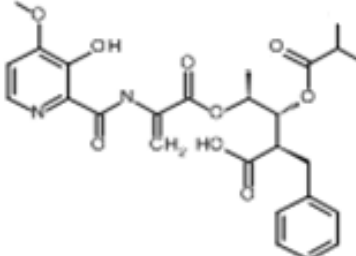
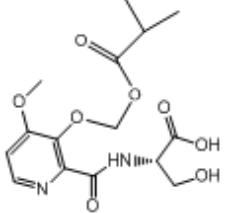
GF-3307 (S7K-3-3) is intended to be used as a post-emergence fungicide for the control of various diseases in sugar beet/fodder beet. No grouping for intended uses (i.e. risk envelope) is needed. The risks for birds, mammals, aquatic organisms, bees, non-target arthropods, soil organisms and micro-organisms, and non-target plants are assessed at the maximum rate of 2 x 1.5 L GF-3307/ha (2 x 75 g fenpicoxamid/ha + 2 x 150 g prothioconazole/ha).

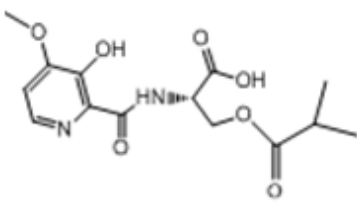
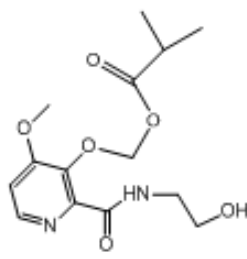
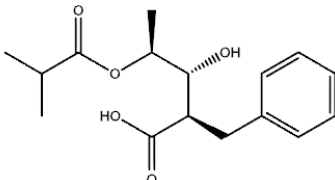
9.1.3 Consideration of metabolites

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-3307 (S7K-3-3) is indicated in the table.

Table 9.1-2 Major (>5% AR) metabolites of fenpicoxamid 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		Soil (aerobic), 39.2% Water, 8.9% Sediment, 10.6% [Wat/sed total, 19.5%]	PEC_{soil} PEC_{gw} PEC_{sw} PEC_{sed}
X696872	444.2		Soil (aerobic), 17.2%	PEC_{soil} PEC_{gw} PEC_{sw}^{\dagger}
X12264475	256.1		Soil (anaerobic), 49.4% Water, 25.8% Sediment, 46.9% [Wat/sed total, 65.3%]	PEC_{soil} PEC_{gw} PEC_{sw} PEC_{sed}

Metabolite	Molar mass (g/mol)	Chemical structure	Maximum observed occurrence (% AR) in compartments	Exposure assessment
X763024	226.1		Soil (aerobic), 5.7%	PECsoil PECgw PECsw [‡]
X12313581	168.0		Soil (field), 17.1% Water (aer. min.) 66.1% Sediment, 9.3% [Wat/sed total, 9.3%]	PECsoil PECgw PECsw PECsed
X696476	169.0		Soil (anaerobic), 46.9% Sediment, 67.1% [Wat/sed total, 67.1%]	PECsoil PECgw PECsw [‡] PECsed
X11963422	206.1		Soil (anaerobic), 80.3% Water, 40.2% Sediment, 7.7% [Wat/sed total, 45%]	PECsoil PECgw PECsw PECsed
X12314005	276.3		Soil (phot. irradi.), 5.4% Water (phot. irradi.), 61.6% [Wat/sed total, 35.1%]	PECsoil PECgw PECsw
X12019520	188.2		Soil (phot. irradi.), 9.8% Water (aer. min.) 74.0% Sediment 6.5% [Wat/sed total, 15.3%]	PECsoil PECgw PECsw PECsed
X12255349	514.5		Soil (phot. irradi.), 6.9%	PECsoil PECgw PECsw [‡]
X12335723	356		Water (phot. irradi.), 77.0%	PECsw

Metabolite	Molar mass (g/mol)	Chemical structure	Maximum observed occurrence (% AR) in compartments	Exposure assessment
X12386481	326		Water (aer. min.), 69.5%	See exclusion section below
X12446477	312		Water (phot. irradi.), 12.5%	PEC _{sw} (water column metabolite only)
X12433979	294		Water (hydrol. pH9), 35.7%	PEC _{sw} (water column metabolite only)

* Maximum in any individual replicate

‡ while not triggered by the criteria listed above regarding the % AR, metabolites are assessed for risk in surface water (run-off/drainage only, not drift) because of potential run-off from soil.

Major fenpicoxamid (XDE-777) metabolites excluded from assessment

Per the EFSA Peer Review Report (EFSA, 2017) commenting tables, per the Environmental fate and behavior comment No. 4, “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 include in FOCUS Surface water Step 1-2 calculation. However, worst case calculation done the worst case default parameters were kept in the LoEP,” (pg 720, excerpt below).

Member States' comments on the draft EFSA Conclusion on XDE-777 (RMS: UK)				(08.12.2017) 9/26
Pesticides Peer Review Written Procedure: November-December 2017				
Environmental fate and behaviour				
No.	Reference (e.g. conclusion text, list of endpoints, evaluation table etc)	Member State comment	EFSA response to comment	
		The unextractable sediment fraction was for both the phenyl and pyridine ring...		
4	Conclusion text, page 13	UK: In the list of metabolites included in the FOCUS Surface Water Step 1-2 calculations, metabolite X12386481 is missing.	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 the FOCUS Surface Water Step 1-2 calculation. However, worst case calculations done the worst case default parameters were kept in the LoEP. The conclusion was not changed.	

This is confirmed in the Ecotoxicology section of the commenting table EFSA responds in comment No. 23, “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, excerpt below).

Member States' comments on the draft EFSA Conclusion on XDE-777 (RMS: UK) (08.12.2017) 24/26 Pesticides Peer Review Written Procedure: November-December 2017			
Ecotoxicology			
No.	Reference (e.g. conclusion text, list of endpoints, evaluation table etc)	Member State comment	EFSA response to comment
		produced by the bacteria. Therefore, as the studies for x642188 were invalidated the tier 1 studies should be made a data gap. This was agreed at meeting 165, where EFSA insisted that acceptable tier 1 studies were required, despite the need for higher tiers studies to demonstrate an acceptable risk.	Changes in the conclusion are not needed.
23	LoEP: page 146; risk assessment for X12386481	UK: Please provide an explanation why the risk assessment for X12386481 has been removed.	The aquatic risk assessment for this metabolite was removed since this metabolite is no longer a metabolite requiring further assessment for the aquatic compartment.

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, excerpt below).

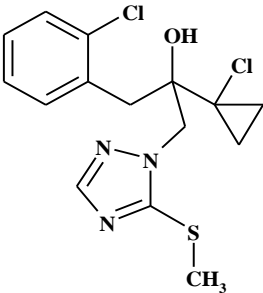
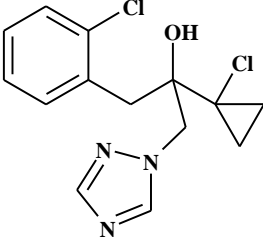
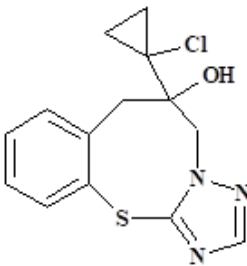
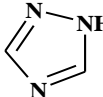
4(16)	Vol.3 (AS), B.8.1.1, Route and rate of degradation in soil, p.374	Applicant: In 2 nd paragraph, a description of kinetics is given for the photoproduct X12442397/X12399889, but this has been discounted as an artefact and should be removed as it does not need to be considered for exposure assessment.	RMS: The text has been altered to read, 'Metabolites X12255349, X12314005, X12019520 and X12442397 are present at >5 % at 2 consecutive timepoints. The applicant has submitted a justification for the excision of X12442397 from the exposure assessment, see 'Degradation of X12255349, X12314005, X12019520 and X12442397 in Four Soils Under Aerobic Conditions' below.' The RMS would therefore suggests that metabolite X12442397/X12399889 could be removed from the list of endpoints if it is considered to be an artefact. Addressed	REV.1: Open point: RMS to provide an amended LoEP removing metabolite X12442397/X12399889, which is considered an artefact formed in the photolysis dark control samples. See also open point in comment 4(74) and comment 4(46).
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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.

According to information performed in The EFSA Peer Review Report (EFSA, 2017) commenting tables the metabolite X12442397/X12399889 is considered an artefact formed in the photolysis dark control samples and for this reason the aquatic risk assessment for this metabolite is not required since this metabolite is no longer a metabolite requiring further assessment for the aquatic compartment.

Table 9.1-3 Metabolites of Prothioconazole relevant for exposure assessment (from Section 8.2)

Metabolite	Molar mass (g/mol)	Chemical structure	Maximum observed occurrence (% AR) in compartments	Exposure assessment required due to
JAU 6476-S-methyl (M01)	358.3		Soil (aerobic), 14.6% Sediment 9.6% [Wat/sed total, 12.7%]	PECsoil PECgw PECsw PECsed
JAU 6476-desthio (M04) Also referred to as prothioconazole-desthio	312.2		Soil (field), 57.1% Water, 32.3% Sediment, 26.9% [Wat/sed total, 54.4%]	PECsoil PECgw PECsw PECsed
JAU 6476-thiazocine (M12)	307.8		Water (phot. irradi.), 14.1%	-*
1,2,4-triazole (M13)	69.1		Water, 37.2% Sediment, 6.1% [Wat/sed total, 41.8%]	PECsw PECsed

* EFSA (2007) concluded that under environmental conditions M12 is unlikely to be formed at >10% AR in natural surface water based on information from photolysis and water/sediment studies; therefore, no PECsw/sed not required.

zRMS comments:

Metabolites relevant for soil and water compartment listed in Table 9.1-3 are the same as indicated in EFSA Scientific Report (2007) 106. It is noted that in the course of the EU review of prothioconazole metabolite JAU 6476-thiazocine was formed at >10% in photodegradation study in water, however according to EFSA Scientific Report (2007) 106, it was considered to be not relevant for evaluation in area of ecotoxicology.

With regard to the maximum occurrence, it is noted that part of the information (e.g. max occurrence of PTZ-desthio in soil) is in line with EFSA Scientific Report (2007) 106. However, part of the information has been taken from the new post Annex I studies. Nevertheless, as the maximum occurrence is relevant for exposure evaluation, for information agreed in this area please refer to the Core Assessment, Part B, Section 8, where all respective data are provided and used in calculation of PEC_{soil} and PEC_{sw/sed} values, considered further in the risk assessment. As the information on the maximum occurrence was not checked in detail, it was struck through in Table 9.1-3.

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with fenpicoxamid, prothioconazole, and relevant metabolites. Full details of these studies are provided in the respective EU DAR (fenpicoxamid: United Kingdom, 2017; prothioconazole: United Kingdom, 2007) and related documents as well as in Appendix 2 of this document (new studies).

Effects on birds of GF-3307 (S7K-3-3) 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 ₅₀ >2000 mg/kg bw LD₅₀ = 3228 mg/kg bw (extrapolated)	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.

zRMS comments:

Avian toxicity data for fenpicoxamid presented in Table 9.2-1 are in line with the EU agreed endpoints reported in EFSA Journal 2018;16(1):5146.

Table 9.2-2: Endpoints and effect values relevant for the risk assessment for birds – prothioconazole and JAU 6476-desthio

Species	Substance	Exposure System	Results	Reference
Bobwhite quail (<i>Colinus virginianus</i>)	Prothioconazole	Oral 1 d Acute	LD₅₀ >2000 mg/kg bw LD ₅₀ = 3776 mg/kg bw (extrapolated)	EFSA Scientific Report 2007; 106:1-98; EFSA B&M GD, 2009, 7(12), pg 16.
Mallard duck (<i>Anas platyrhynchos</i>)	Prothioconazole	Dietary 21 wks Reproductive toxicity	NOEL = 78 mg/kg bw/d	EFSA, 2007
Prothioconazole metabolites				
Bobwhite quail (<i>Colinus virginianus</i>)	JAU 6476-desthio	Oral 1 d Acute	LD ₅₀ >2000 mg/kg bw	EFSA, 2007
Bobwhite quail (<i>Colinus virginianus</i>)	JAU 6476-desthio	Short term dietary toxicity (5 day)	LD ₅₀ >297 mg p.m./kg bw/d	EFSA, 2007
Bobwhite quail (<i>Colinus virginianus</i>)	JAU 6476-desthio	Dietary 20 wks Reproductive toxicity	NOAEL = 14.8 mg/kg bw/d	EFSA, 2007

zRMS comments:

Avian toxicity data for prothioconazole and JAU 6476-desthio in Table 9.2-2 are in line with the EU agreed endpoints reported in EFSA Scientific Report (2007) 106, 1-98.

Table 9.2-3: Endpoints and effect values relevant for the risk assessment for birds - GF-3307 (S7K-3-3)

Species	Substance	Exposure System	Results	Reference
Bobwhite quail (<i>Colinus virginianus</i>)	GF-3307	Oral 1 d Acute	LD ₅₀ >2000 mg/kg bw LD₅₀ = 3228 mg/kg bw (extrapolated)*	█/2014/DAS# 140447

* extrapolated endpoint in line with EFSA Risk Assessment for Birds and Mammals (2009); extrapolation factor of 1.614 applied

zRMS comments:

Study on toxicity of GF-3307 to birds was evaluated and agreed by the zRMS-PL in the course of zonal autorisation of the product GF-3307 in cereals, Core Dossier, 2023.
For details of the evaluation please refer to Appendix 2. The endpoint presented in Table 9.2-3 is 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).

Acute avian mixture assessment

Per Appendix B of the Guidance Document on Risk Assessment for Birds and Mammals (EFSA Journal 2009; 7(12): 1438), because the formulation GF-3307 (S7K-3-3) contains two active ingredients, a mixtures calculation using the Finney equation is provided in Table 9.2-4. The concentration addition (CA) model was used to determine the predicted toxicity of GF-3307 (S7K-3-3), which is 4.8% fenpicoxamid and 9.4% prothioconazole. The predicted LD_{50-mix} was calculated as follows:

$$\text{Predicted } LD_{50-mix} = \left(\frac{\% \text{ active A}}{LD_{50-A}} + \frac{\% \text{ active B}}{LD_{50-B}} \right)^{-1}$$

where: the fraction of active A + active B in the formulation must equal 1.

Table 9.2-4 Predicted and measured acute avian endpoint comparison for fenpicoxamid and prothioconazole when combined in GF-3307 (S7K-3-3)

Species	Active substance	a.i. ratio	LD ₅₀ (mg/kg-bw)	LD _{50-mix} predicted	LD _{50-mix} measured
Bobwhite quail (<i>Colinus virginianus</i>)	Fenpicoxamid	0.34	>2000	>2000	>2000
	Prothioconazole	0.66	>2000		

Using the CA approach (Finney equation), the surrogate or predicted LD_{50mix} is the equivalent to the measured LD₅₀ of GF-3307 (S7K-3-3), i.e. greater than the limit dose. As the formulation components do not significantly alter the toxicity of the active ingredients, the measured LD₅₀ is used in the risk assessment with the appropriate extrapolation.

For the prothioconazole metabolite JAU 6476-desthio, a short term dietary toxicity study is available and the endpoint is lower than in the acute oral studies conducted with prothioconazole and JAU 6476-desthio. Therefore, an LD_{50mix} for the mixture of fenpicoxamid and JAU 6476-desthio, assuming that the metabolite represents the parent fraction as a worst case, was calculated. The combined acute endpoint calculated this way would also cover contribution of prothioconazole to the mixture toxicity. The relevant endpoint is calculated below.

Table 9.2-5 Predicted and measured acute avian endpoint comparison for fenpicoxamid and JAU 6476-desthio when combined in GF-3307 (S7K-3-3)

Species	Active substance	a.i. ratio	LD ₅₀ (mg/kg-bw)	LD _{50-mix} predicted	LD _{50-mix} measured
Bobwhite quail (<i>Colinus virginianus</i>)	Fenpicoxamid	0.34	>2000	>421.4	>2000
	JAU 6476-desthio	0.66 ¹⁾	>297		

¹⁾ Relative amount of the parent assuming immediate and complete conversion of prothioconazole to JAU 6476-desthio; this in combination with metabolite endpoint represents worst case and covers also contribution of prothioconazole to the mixture toxicity.

Table 9.2-6 Avian “tox per fraction” for the JAU 6476-desthio metabolite and fenpicoxamid when combined in GF-3307

	JAU 6476-desthio	Fenpicoxamid	“mix”
Content in the formulation (%)	9.4	4.8	
Fraction in mixture	0.66	0.34	
LD ₅₀ (mg/kg bw)	>297	>2000	LD _{50mix} =421.4
Tox per fraction	450	5882.35	
Contribution to predicted toxicity	93.7%	6.3%	

Based on calculations provided above fenpicoxamid contributes 6.3 % to mixture toxicity, while the JAU 6476-desthio metabolite has an impact on the predicted risk of 93 %, therefore, the combined acute toxicity is covered by acute risk of JAU 6476-desthio metabolite.

zRMS comments:

The LD₅₀ mix of 421.4 mg /kg bw has been accepted by zRMS -PL in the course of zonal autorisation of the product GF-3307 in cereals, Core Dossier, 2023.
This value is still valid. zRMS agrees that acute risk assessment is driven by JAU6476 metabolite. No additional calculations are required.

Chronic avian mixture assessment

Per Appendix B of the Birds and Mammals Guidance Document (EFSA Journal 2009; 7(12): 1438), for “the risk to reproduction from exposure to more than one active substance, it is currently not recommended to consider the use of predicted toxicity values as surrogates in the risk assessment.” None-the-less, a predicted chronic toxicity value has been calculated using the CA approach since an avian reproduction test was not run with the formulation, GF-3307.

The predicted NOAEL_{mix} was calculated to be 27.5 mg/kg bw/day as follows:

$$\text{Predicted NOAEL}_{\text{mix}} = \left(\frac{\% \text{ active A}}{\text{NOAEL}_A} + \frac{\% \text{ active B}}{\text{NOAEL}_B} \right)^{-1}$$

where: the fraction of active A + active B in the formulation must equal 1.

Table 9.2-7 Predicted chronic avian endpoint comparison for GF-3307

Species	Active substance	a.s. in GF-3307	a.s. ratio	NOEC (mg/kg-bw/day)	Predicted NOAEL _{mix} *
Bobwhite quail (<i>Colinus virginianus</i>)	Fenpicoxamid	4.8%	0.34	12.1	27.5
Mallard duck (<i>Anas platyrhynchos</i>)	Prothioconazole	9.4%	0.66	78	

*manual calculations may differ slightly using the a.s. ratio as presented with only 2 significant figures.

As shown in the endpoints table for prothioconazole (Table 9.2-2), relevant metabolite JAU 6476-desthio is more toxic than the parent; therefore a predicted chronic avian endpoint has also been generated using

the NOEC from the JAU 6476 desthio avian reproduction study and assumed 100% conversion to the metabolite as an overconservative approach. This alternative NOEL was calculated to be 13.8 mg/kg bw/day.

Table 9.2-8 Predicted chronic avian endpoint comparison for GF-3307 (S7K-3-3)

Species	Active substance	a.i. ratio	NOEC (mg/kg bw/day)	NOEL _{mix} predicted
Bobwhite quail (<i>Colinus virginianus</i>)	Fenpicoxamid	0.34	12.1	13.8
Bobwhite quail (<i>Colinus virginianus</i>)	JAU 6476-desthio	0.66	14.8	

In the registration report for GF-3307 (S7K-3-3) in cereals, the zRMS Poland conducted the combined chronic risk assessment for GF-3307 (S7K-3-3) by using the lowest NOEC (12.1 mg a.s./kg-bw/day for fenpicoxamid) and exposure to the sum of both active substances (0.225 kg a.s./ha). The zRMS Poland argued that this approach covers also the mixture toxicity for fenpicoxamid with either prothioconazole or its metabolite JAU 6476-desthio, since the endpoint for fenpicoxamid is lower than the NOEL_{mix} predicted. This approach is also recommended in the EFSA 2009 GD for compounds acting in the same way on defined molecular targets. This approach was used in the risk assessment as a worst case (Section 9.2.2.1).

zRMS comments:

zRMS -PL confirmed that combined long-term risk assessment performed with consideration of the cumulative application rate of fenpicoxamid and prothioconazole together with the lowest available NOEL of 12.1 mg/kg bw/d covers also exposure to JAU 6476-desthio in the mixture.
The approach using NOEL_{mix} is not considered acceptable in Core Dossier for GF-3307 in the course of zonal auto-risation of the product GF-3307 in cereals, Core Dossier, 2023.

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 9.2-9: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of fenpicoxamid in sugar beet/fodder beet

Intended use	Sugar beet/fodder beet				
Active substance	Fenpicoxamid				
Application rate (g/ha)	2 x 75, interval 21 days (MAF calculated according to Appendix H, EFSA 2009 B&M GD)				
Acute toxicity (mg/kg bw)	LD ₅₀ = 3228 (extrapolated); >2000 mg/kg (actual)				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a

Sugar beet/fodder beet	Indicator species for screening (small omnivore)	158.8	1.1	13.1	246 152.7
Reprod. toxicity (mg/kg bw/d)	NOAEL = 12.1				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Sugar beet/fodder beet	Indicator species for screening (small omnivore)	64.8	1.2 x 0.53	3.09	3.91
Sugar beet/fodder beet (summer, autumn) (BBCH 30-49)	Small granivorous bird “finch” Linnet (Carduelis cannabina)	11.4	1.2 x 0.53	0.544	22.3
Sugar beet/fodder beet (BBCH 20-49)	Small insectivorous bird “wagtail” Yellow wagtail (Motacilla flava)	2.8	1.2 x 0.53	0.134	90.6
Sugar beet/fodder beet (BBCH 20-49)	Small insectivorous bird “wagtail” Yellow wagtail (Motacilla flava)	9.7	1.2 x 0.53	0.463	26.2

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_A and tier 1 TER_{LT} 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_{multi} was determined using the following equation:

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

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 9.2-10: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of metabolite X12019520 in sugar beet/fodder beet

Intended use	Sugar beet/fodder beet				
Metabolite	X12019520				
Application rate (g/ha)	2 x 4.1 g/ha ¹ , interval 21 days (MAF calculated according to Appendix H, EFSA 2009 B&M GD)				
Acute toxicity (mg/kg bw)	LD ₅₀ = 3228 (parent)/10 = 322.8 LD ₅₀ > 2000 (parent)/10 = 200				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Sugar beet/fodder beet	Indicator species for screening (small omnivore)	158.8	1.1	0.721	448 277.4
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_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Sugar beet/fodder beet	Indicator species for screening	64.8	1.2 x 0.53	0.170	7.12

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. ¹ 75 g a.s./ha x 5.5% maximum formation = 4.1 g X12019520/ha

Screening level TER_A and TER_{LT} values are above the Annex VI trigger values, therefore, there is acceptable acute and chronic risk to birds from X12019520.

zRMS comments:

The risk assessment for the active substance Fenpicoxamid and metabolite X12019520 presented in Tables 9.2-9 and 9.2-10 with consideration both extrapolated and actual LD₅₀ values 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.

Overall, based on the performed calculations, acceptable acute and long-term dietary risk to birds from fenpicoxamid, its relevant plant metabolites was confirmed.

Table 9.2-11: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of prothioconazole in sugar beet/fodder beet

Use of prothioconazole in sugar beet/fodder beet						
Intended use		Sugar beet/fodder beet				
Active substance						
Application rate (g/ha)						
		Prothioconazole				
		2 x 150, interval 21 days (MAF calculated according to Appendix H, EFSA 2009 B&M GD)				
Acute toxicity (mg/kg bw)		LD ₅₀ = 2000				
TER criterion						
		10				
Crop scenario	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Growth stage						
Sugar beet/fodder beet	Indicator species for screening	158.8	1.1	26.2	76	
Reprod. toxicity (mg/kg bw/d)		NOAEL = 78				
TER criterion						
		5				
Crop scenario	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}	
Growth stage						
Sugar beet/fodder beet	Indicator species for screening	64.8	1.2 x 0.53	6.18	12.6	

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_A and TER_{LT} values are above the Annex VI trigger values, therefore, there is acceptable acute and chronic risk to birds from prothioconazole.

A molecular weight conversion factor was used to calculate the application rate of the metabolite JAU 6476-desthio as the endpoints shown are from testing done with the metabolite.

Table 9.2-12: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of JAU 6476-desthio in sugar beet/fodder beet

Intended use		sugar/fodder beet			
Active substance		JAU 6476-desthio			
Application rate (g/ha)		2 x 136 ¹ , interval 21 days (MAF calculated according to Appendix H, EFSA 2009 B&M GD)			
Acute toxicity (mg/kg bw)		LD ₅₀ >297			
TER criterion		10			
Crop scenario	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Growth stage					
Sugar/fodder beet	Indicator species for screening (small omnivore)	158.8	1.1	23.8	12.5

Reprod. toxicity (mg/kg bw/d)		NOAEL = 14.8			
TER criterion		5			
Crop scenario Growth stage	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Sugar/fodder beet	Indicator species for screening (small omnivore)	64.8	1.2 x 0.53	5.61	2.64
Sugar beet, late (summer, autumn) (BBCH 30-49)	Small granivorous bird “finch” Linnet (<i>Carduelis cannabina</i>)	11.4	1.2 x 0.53	0.986	15.0
Sugar beet (BBCH 20-49)	Small insectivorous bird “wagtail” Yellow wagtail (<i>Motacilla flava</i>)	2.8	1.2 x 0.53	0.242	61.1
Sugar beet (BBCH 20-49)	Small insectivorous bird “wagtail” Yellow wagtail (<i>Motacilla flava</i>)	9.7	1.2 x 0.53	0.839	17.6

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.

¹ 150 g a.s./ha x MW conversion factor of 0.907 x 100% formation = 136 g JAU 6476-desthio/ha

Screening level TER_A and tier 1 TER_{LT} values are above the Annex VI trigger values, therefore, there is acceptable acute and chronic risk to birds from JAU 6476-desthio.

zRMS comments:

Evaluation presented in Tables 9.2-11 and 9.2-12 above with consideration of the LD₅₀ of 2000 mg a.s./kg bw and >297 mg pm/kg bw/d values, respectively, has been accepted by zRMS.

The screening step and Tier 1 chronic risk assessment for metabolite JAU 6476-desthio are acceptable.

As described in the mixture calculations section 9.2.1, the predicted LD_{50mix} is the equivalent to the measured LD₅₀ for GF-3307 (S7K-3-3) (>2000 mg GF-3307/kg-bw), therefore the extrapolated value of the measured endpoint is used for the acute risk assessment. An avian chronic endpoint is not available for GF-3307, thus the lowest effect value (NOEC 12.1 mg a.s./kg-bw/day for fenpicoxamid) and exposure to the sum of both active substances (0.225 kg a.s./ha) are used for risk assessment.

Table 9.2-13: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of GF-3307 (S7K-3-3) in sugar beet/fodder beet

Intended use	sugar/fodder beet				
Active substance	GF-3307				
Application rate (g/ha)	2 x 1.57 kg product/ha (acute calculation) 2 x 0.075 kg fenpicoxamid + 0.150 kg prothioconazole/ha = 2 x 0.225 kg a.s./ha (chronic calc.) interval 21 days (MAF calculated according to Appendix H, EFSA 2009 B&M GD)				
Acute toxicity (mg/kg bw)	LD₅₀ = 3228 mg GF-3307/kg (extrapolated); >2000 mg GF-3307/kg (measured)				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Sugar/fodder beet	Indicator species for screening (small omnivore)	158.8	1.1	274	11.8
Reprod. toxicity (mg/kg bw/d)		NOAEL = 12.1			
TER criterion		5			
Crop scenario Growth stage	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}

Sugar/fodder beet	Indicator species for screening (small omnivore)	64.8	1.2 x 0.53	9.27	1.30
Sugar beet, late (summer, autumn) (BBCH 30-49)	Small granivorous bird “finch” Linnet (<i>Carduelis cannabina</i>)	11.4	1.2 x 0.53	1.631	7.4
Sugar beet (BBCH 20-49)	Small insectivorous bird “wagtail” Yellow wagtail (<i>Motacilla flava</i>)	2.8	1.2 x 0.53	0.401	30.2
Sugar beet (BBCH 20-49)	Small insectivorous bird “wagtail” Yellow wagtail (<i>Motacilla flava</i>)	9.7	1.2 x 0.53	1.338	8.7

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_A and tier 1 TER_{LT} values are above the Annex VI trigger values, therefore, there is acceptable acute and chronic risk to birds from GF-3307 (S7K-3-3).

zRMS comments:

Acute combined risk assessment:

The LD₅₀mix value of >2000 mg/kg bw calculated with consideration of the lowest acute endpoints for active substances prothioconazole and fenpicoxamid is essentially equivalent to the measured LD₅₀ value for GF-3307, therefore the risk assessment presented with LD₅₀ = 3228 mg GF-3307/kg bw (extrapolated value for product) has been accepted by zRMS.

In case of combined risk of fenpicoxamid and metabolite JAU 6476-desthio the acute risk assessment is covered by JAU 6476-desthio.

Long-term combined risk assessment:

As already indicated in the zRMS comments above, consideration of the lowest toxicity endpoint of 12.1 mg/kg bw/d together with cumulative application rate of both active substances represents worst case and accounts also for conversion of prothioconazole to metabolite JAU 6476-desthio.

Overall conclusion on the combined risk assessment:

Based on performed calculations acceptable acute and long-term risk may be concluded for birds exposed to the mixture of fenpicoxamid, prothioconazole and JAU 6476-desthio following application of GF-3307.

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-3307 (S7K-3-3) is not intended to be applied on leafy vegetables forming heads or other leaf vegetables 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 L/kg (geomean, EFSA, 2018), fenpicoxamid belongs to the group of more sorptive substances.

Application rate (g/ha)	2 x 75 g fenpicoxamid/ha; interval 21 days		
Effective application rate (g/ha) =	76.2 (based on a soil DT_{50} of 3.5 days, geomean (n=4); EFSA, 2018)		
Acute toxicity (mg/kg bw) =	>3228	quotient =	0.02
Reprod. toxicity (mg/kg bw/d) =	12.1	quotient =	6.30

With a $K(f)_{oc}$ of 1765 (EFSA, 2007), prothioconazole belongs to the group of more sorptive substances.

Application rate (g/ha)	2 x 150 g prothioconazole/ha; interval 21 days		
Effective application rate (g/ha) =	150.0 (based on a soil DT_{50} of 1.27 days; range: 0.07 to 1.27 days, median: 0.5 days; EFSA, 2007)		
Acute toxicity (mg/kg bw) =	>2000	quotient =	0.08
Reprod. toxicity (mg/kg bw/d) =	78	quotient =	1.92

With a K_{oc} of 523-625 mL/g (EFSA, 2007), JAU 6476-desthio belongs to the group of more sorptive substances.

Application rate (g/ha)	2 x 136 g JAU 6476-desthio/ha; interval 21 days		
Effective application rate (g/ha) =	207.6 (based on a soil DT_{50} of 34.0 days; range 7.0 to 34.0 days; median: 24.1 days; EFSA, 2007)		
Acute toxicity (mg/kg bw) =	>297	quotient =	0.76
Reprod. toxicity (mg/kg bw/d) =	14.8	quotient =	15.18

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

zRMS comments:

The screening step of the drinking water risk assessment performed for fenpicoxamid, prothioconazole and metabolite prothioconazole JAU 6476-desthio 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- fenpicoxamid. 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 [%] ¹⁾	Application rate [g/ha]	Ratio for acute risk 2)	Ratio for acute risk 2)	Ratio for long-term risk 3)	Ratio for long-term risk 3)	Trigger ⁴⁾
Fenpicoxamid	614.6	parent	parent	75	0.023	see above	see above	6.30	see above
X642188	514.2	0.837	39.2	24.6	0.076	see above	see above	20.33	3000
X696872	444.2	0.723	17.2	9.3	0.029	see above	see above	7.70	3000
X12264475	256.1	0.417	49.4	15.4	0.048	see above	see above	12.76	50
X763024	226.1	0.368	5.7	1.6	0.005	see above	see above	1.30	50
X12313581	168.0	0.273	17.1	3.50	0.011	see above	see above	2.90	3000
X696476	169.0	0.275	46.9	9.7	0.030	see above	see above	7.99	3000
X11963422	206.1	0.335	80.3	20.2	0.063	see above	see above	16.69	50

X12314005	276.3	0.450	5.4	1.8	0.006	1.20	1.45	1.50	50
X12019520	188.2	0.306	9.8	2.2	0.007	1.10	1.55	1.86	50
X12255349	514.5	0.837	6.9	4.3	0.013	1.20	1.55	3.58	594

¹⁾ See EFSA Journal 2018;16(1):5146 or Table 8.2-1 in the Core Assessment, Part B, Section 8
²⁾ Based on LD₅₀ of 322.8 mg/kg bw (parent endpoint divided by 10)
³⁾ Based on NOEL of 1.21 mg/kg bw (parent endpoint divided by 10)
⁴⁾ Determined based on geometric mean Kfoc 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_{ow} of fenpicoxamid, prothioconazole, JAU 6476-desthio, and prothioconazole-S-methyl amounts to 4.4, 3.82 (pH 7), 3.04, and 4.19, respectively, 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_{soil} x 0.53 or the 21-d TWA PEC_{soil} to be used for secondary poisoning; in this assessment the 21-d TWA PEC_{soil} was used. The application rates used for calculation of PEC_{soil} values are aligned to the GAP of 2 x 75 g fenpicoxamid and 150 g prothioconazole/ha.

Table 9.2-14: Assessment of the risk for earthworm-eating birds due to exposure to fenpicoxamid via bioaccumulation in earthworms (secondary poisoning) for the intended use in sugar beet/fodder beet

Parameter	Fenpicoxamid	comments
PEC _{soil} 21d TWA (mg/kg soil)	0.0266	Section 8.7 @ 2 x 75 g a.s./ha
log P _{ow} / P _{ow}	4.4/25119	
Koc	5776	EFSA, 2018
foc	0.02	Default
BCF _{worm}	2.62	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC _{worm}	0.070	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.073	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	12.1	
TER _{lt}	166	

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

X642188 exhibits measured values of LogK_{ow} >3. While this metabolite was observed in the hen metabolism study and exhibited LogK_{ow} 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 9.2-15: Assessment of the risk for earthworm-eating birds due to exposure to X642188 via bioaccumulation in earthworms (secondary poisoning) for the intended use in sugar beet/fodder beet

Parameter	X642188	comments
PEC _{soil} 21d TWA (mg/kg soil)	0.016	Section 8.7 @ 2 x 75 g a.s./ha

Parameter	X642188	comments
$\log P_{ow} / P_{ow}$	3.58/3802	QSAR estimate
Koc	4518	EFSA, 2018
foc	0.02	Default
BCF _{worm}	0.514	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + \mathbf{0.012} \times P_{ow}) / foc \times Koc$
PEC _{worm}	0.0082	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.0086	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	1.21	Parent/10
TER _{lt}	140	

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

The soil photolysis metabolite X12255349 was predicted to exhibit a $\log K_{ow}$ 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 9.2-16: Assessment of the risk for earthworm-eating birds due to exposure to X12255349 via bioaccumulation in earthworms (secondary poisoning) for the intended use in sugar beet/fodder beet

Parameter	X12255349	comments
PEC _{soil} 21d TWA (mg/kg soil)	0.0006	Section 8.7 @ 2 x 75 g a.s./ha
$\log P_{ow} / P_{ow}$	3.89/7762	QSAR estimate
Koc	594	EFSA, 2018
foc	0.02	Default
BCF _{worm}	7.91	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC _{worm}	0.0047	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.0050	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	1.21	Parent/10
TER _{lt}	243	

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

Table 9.2-17: Assessment of the risk for earthworm-eating birds due to exposure to prothioconazole via bioaccumulation in earthworms (secondary poisoning) for the intended use in sugar beet/fodder beet

Parameter	Prothioconazole	comments
PEC _{soil} 21d TWA (mg/kg soil)	0.013	Section 8.7 @ 2 x 150 g a.s./ha
$\log P_{ow} / P_{ow}$	3.82/6607	EFSA, 2007
Koc	1765	Aged leaching study
foc	0.02	Default
BCF _{worm}	2.27	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC _{worm}	0.030	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.031	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	78	

Parameter	Prothioconazole	comments
TER _{lt}	2518	

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

Table 9.2-18: Assessment of the risk for earthworm-eating birds due to exposure to JAU 6476-des-thio via bioaccumulation in earthworms (secondary poisoning) for the intended use in sugar beet/fodder beet

Parameter	JAU 6476-des-thio	comments
PEC _{soil 21d TWA} (mg/kg soil)	0.0511	Section 8.7 @ 2 x 150 g a.s./ha
log P _{ow} / P _{ow}	3.04/1096	EFSA, 2007
Koc	575.4	Mean adsorption
foc	0.02	Default
BCF _{worm}	1.22	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC _{worm}	0.062	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.065	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	14.8	
TER _{lt}	227	

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

Prothioconazole metabolite prothioconazole-S-methyl was predicted to exhibit a LogK_{ow} of 4.19 (EFSA, 2007), 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 9.2-19: Assessment of the risk for earthworm-eating birds due to exposure to prothioconazole-S-methyl via bioaccumulation in earthworms (secondary poisoning) for the intended use in sugar beet/fodder beet

Parameter	Prothioconazole-S-methyl	comments
PEC _{soil initial} (mg/kg soil)	0.0134	Section 8.7 @ 2 x 150 g a.s./ha
LogK _{ow} / K _{ow}	4.19/15488	Predicted; EFSA, 2007
Koc	2556.3	Mean adsorption; EFSA, 2007
foc	0.02	Default
BCF _{worm}	3.65	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC _{worm}	0.049	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.051	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	7.8	Parent/10
TER _{lt}	152	

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

LogK_{ow} a synonym of LogP_{ow}

TER_{LT} values for birds eating earthworms are above the Annex VI trigger value of 5, therefore, there is acceptable chronic risk to birds from fenpicoxamid, prothioconazole, and relevant metabolites.

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 $PEC_{sw} \times 0.53$ or the 21-d TWA PEC_{sw} to be used for secondary poisoning; in this assessment the FOCUS Step 1 PEC_{sw-max} was used. The application rates used for calculation of PEC_{sw-max} values are aligned to the current GAP of 2 x 75 g fenpicoxamid and 2 x 150 g prothioconazole/ha.

The zebrafish BCF study (Schlechtriem, 2014) found no detectable accumulation of fenpicoxamid, therefore the BCF_{ss} was < 5 L/kg lipid uncorrected and < 2.6 L/kg 5% lipid corrected. Moreover, the DT_{90} of fenpicoxamid is less than 24 hours. Owing to its fast degradation in the aquatic compartment, fenpicoxamid is unlikely to bioaccumulate and therefore consideration of secondary poisoning from the active substance is not required. 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 9.2-20: Assessment of the risk for fish-eating birds due to exposure to fenpicoxamid via bioaccumulation in fish (secondary poisoning) for the intended use in sugar beet/fodder beet

Parameter	Fenpicoxamid	comments
PEC_{sw-max} (mg/L)	0.007126	Focus Step 1 PEC_{sw} max, Section 8.9 @ 2 x 75 g a.s./ha
BCF_{fish}	18.36 L/kg	QSAR estimate
BMF	--	biomagnification factor (relevant for $BCF \geq 2000$)
PEC_{fish}	0.131	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.0208	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	12.1	
TER_{lt}	582	

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 9.2-21: Assessment of the risk for fish-eating birds due to exposure to X12255349 via bioaccumulation in fish (secondary poisoning) for the intended use in sugar beet/fodder beet

Parameter	X12255349	comments
PEC_{sw-max} (mg/L)	0.001613	Focus Step 1 PEC_{sw} max, Section 8.9 @ 2 x 75 g a.s./ha
BCF_{fish}	9.63 L/kg	QSAR estimate
BMF	--	biomagnification factor (relevant for $BCF \geq 2000$)
PEC_{fish}	0.01553	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.00247	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	1.21	Parent/10
TER_{lt}	490	

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

Table 9.2-22: Assessment of the risk for fish-eating birds due to exposure to prothioconazole via bioaccumulation in fish (secondary poisoning) for the intended use in sugar beet/fodder beet

Parameter	Prothioconazole	comments
PEC_{sw-max} (mg/L)	0.03258	Focus Step 1 PEC_{sw} max, Section 8.9 @ 2 x 150 g/ha
BCF_{fish}	19.7	EFSA (2007)

Parameter	Prothioconazole	comments
BMF	--	biomagnification factor (relevant for $BCF \geq 2000$)
PEC_{fish}	0.642	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.1021	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	78	EFSA (2007)
TER_{It}	764	

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

Table 9.2-23: Assessment of the risk for fish-eating birds due to exposure to JAU 6476-desthio via bioaccumulation in fish (secondary poisoning) for the intended use in sugar beet/fodder beet

Parameter	JAU 6476-desthio	comments
PEC_{sw-max} (mg/L)	0.05857	Focus Step 1 PEC_{sw} max, Section 8.9 @ 2 x 150 g/ha
BCF_{fish}	65	EFSA (2007)
BMF	--	biomagnification factor (relevant for $BCF \geq 2000$)
PEC_{fish}	3.807	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.60535	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	14.8	EFSA (2007)
TER_{It}	24	

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

Due to the low predicted environmental concentrations of prothioconazole-S-methyl in surface water no fish-BCF-study was conducted even though this metabolite has a LogK_{ow} of 4.19. The BCF for this metabolite can be estimated from its LogK_{ow} and BCF and the LogK_{ow} of the parent compound with a sufficient degree of precision. The chemical structure of prothioconazole-S-methyl is very similar to the one of parent compound prothioconazole. The only modification of the parent molecule during the formation of prothioconazole-S-methyl is the methylation of the sulphur atom. This methylation results in an increase of the K_{ow} of prothioconazole-S-methyl that is 15488 ($\text{LogK}_{ow} = 4.19$) in comparison to 6607 ($\text{LogK}_{ow} = 3.82$) of prothioconazole (EFSA, 2007). Therefore it can be anticipated that the BCF for S-methyl is not more than 2.34 times higher ($15488/6607 = 2.34$) than the BCF_{parent} (whole fish, normalised to 6% fat) for prothioconazole that has been determined to be 18.8. An estimated BCF for prothioconazole-S-methyl of 44 ($18.8 \times 2.34 = 43.992$) is also in line with the results of the fish BCF study that has been conducted for the parent compound. In the prothioconazole BCF study, prothioconazole-S-methyl was detected as a minor metabolite in edibles and viscera of fish. No significant increase of the level and the portion of the total radioactive residue (TRR) was observed for the metabolite prothioconazole-S-methyl between day 7 and day 14. Therefore it can be concluded that formation and degradation/depuration of this metabolite, which had been formed in the fish, were in balance. If the metabolite would have a much higher bioaccumulation potential than the parent compound, a further increase of its level and portion of the TRR would have to be expected from day 7 to day 14 in the BCF study. Therefore a BCF of 44 seems to be a realistic estimate for the BCF of prothioconazole-S-methyl in fish.

In the registration report of GF-3307 for cereals, the zRMS Poland suggested using the the significantly higher BCF of 764.8 agreed during the expert meeting in the renewal process of prothioconazole. Although the process itself is not finalised yet, this value is used as a worst case for the risk assessment.

Table 9.2-24: Assessment of the risk for fish-eating birds due to exposure to prothioconazole-S-methyl via bioaccumulation in fish (secondary poisoning) for the intended use in sugar beet/fodder beet

Parameter	JAU 6476-S-methyl	Comments
PEC _{sw} (mg/L)	0.006809	Focus Step 1 PEC _{sw max} , Section 8.9 @ 2 x 150 g/ha
BCF _{fish}	764.8	BCF agreed during renewal process, already peer-reviewed and agreed, although the renewal process itself is not finalised yet
BMF	-	Biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	5.208	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	0.8280	DDD = PEC _{fish} × 0.159
NOEL (mg/kg bw/d)	7.8	Parent/10
TER _{lt}	9	

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

TER_{LT} values for birds eating fish and earthworms are above the Annex VI trigger value of 5, therefore, there is acceptable chronic risk to birds from fenpicoxamid, prothioconazole, and relevant metabolites.

zRMS comments:

The Applicants' approach in evaluation of the risk of secondary poisoning is in line with EFSA (2009). Compounds selected for this assessment are agreed by the zRMS. Evaluation was not triggered for remaining metabolites of both active substances due to their log Pow <3.

Based on calculations performed by the Applicant from Table 9.2-14 to Table 9.2-24 an acceptable risk of secondary exposure from all relevant compounds could 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_A and TER_{LT} values are above the Annex VI trigger values, therefore, there is acceptable acute and chronic risk to birds from fenpicoxamid, prothioconazole, relevant metabolites, and GF-3307 (S7K-3-3). There is low risk to birds from drinking water or consuming contaminated prey items.

The acute and long-term risks of GF 3307 to birds was assessed by calculating toxicity exposure ratios between toxicity endpoints from studies with fenpicoxamid, prothioconazole and their calculated mixture toxicity with maximum residues estimated to occur on food items following applications according to the proposed use pattern. In the combined risk assessment also prothioconazole metabolite JAU 6476-desthio was taken into account due to its higher toxicity comparing to parent.

Based on combined mixture toxicity acceptable acute risk was shown and for all focal species relevant for the intended Central Zone use pattern and acceptable chronic risk was also determined.

There is low risk to birds from drinking water or consuming contaminated prey items.

Acceptable risk of secondary poisoning was demonstrated.

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, prothioconazole, and relevant metabolites. Full details of these studies are provided in the respective EU DAR (fenpicoxamid: United Kingdom, 2017; prothioconazole: United Kingdom, 2007) and related documents as well as in Section 6 (Mammalian Toxicology) of this report (new studies).

Effects on mammals of GF-3307 (S7K-3-3) were not evaluated as part of the EU assessment of fenpicoxamid. However, the provision of further data on the GF-3307 (S7K-3-3) formulation is not considered essential, because mixture calculations are provided below in order to generate a surrogate LD₅₀ for the assessment of acute risk to mammals. Additionally, data on acute toxicity of GF-3307 (S7K-3-3) to rat are provided.

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 ₅₀ >2000 mg/kg bw	EFSA, 2018
NZ White rabbit	Fenpicoxamid	Dietary Developmental toxicity (long-term)	NOAEL = 495 mg/kg bw/d	EFSA, 2018

zRMS comments:

Mammalian toxicity data for fenpicoxamid presented in Table 9.3-1 are in line with the EU agreed end-points reported in EFSA Journal 2018;16(1):5146.

Table 9.3-2: Endpoints and effect values relevant for the risk assessment for mammals – prothioconazole and JAU 6476-desthio

Species	Substance	Exposure System	Results	Reference
Rat	Prothioconazole	Oral 1 d Acute	LD ₅₀ >6200 mg/kg bw	EFSA, 2007
Rat	Prothioconazole	Dietary Reproductive toxicity Two-generation study, gavage	NOAEL = 95.6 mg/kg bw/d (reduced pup weight gain, reduced litter size)	EFSA, 2007
Prothioconazole metabolites				
Mouse	JAU 6476-desthio	Oral 1 d Acute	LD_{50-male} = 2235 mg/kg bw LD _{50-female} = 3459 mg/kg bw	EFSA, 2007
Rat	JAU 6476-desthio	Dietary Reproductive toxicity Two-generation study	NOAEL = 10 mg/kg bw/d (reproductive effects)	EFSA, 2007

zRMS comments:

Mammalian toxicity data for prothioconazole and JAU 6476-desthio in Table 9.3-2 are in line with the EU agreed endpoints reported in EFSA Scientific Report (2007) 106, 1-98.

Table 9.3-3: Endpoints and effect values relevant for the risk assessment for mammals – GF-3307 (S7K-3-3)

Species	Substance	Exposure System	Results	Reference
Rat	GF-3307	Oral 2 d Acute	LD ₅₀ = 2000-5000 mg/kg bw	Prajapati/2021/Corteva #211324

zRMS comments:

The study has been evaluated and accepted in Section 6 (please ss in details).

Acute mammalian mixture assessment (no formulation endpoints available)

Per Appendix B of the Birds and Mammals GD (EFSA/2009/1438), a mixture calculation using the Finney equation is provided below to generate a surrogate/predicted GF-3307 mammalian endpoint. The concentration addition (CA) model was used to determine the predicted toxicity of the GF-3307 (S7K-3-3), which is 4.8% fenpicoxamid and 9.4% prothioconazole. The predicted LD_{50-mix} was calculated as follows:

$$\text{Predicted } LD_{50-mix} = \left(\frac{\% \text{ active } A}{LD_{50-A}} + \frac{\% \text{ active } B}{LD_{50-B}} \right)^{-1}$$

where: the fraction of active A + active B in the formulation must equal 1.

Table 9.3-4: Predicted and measured acute mammalian endpoints for GF-3307 (S7K-3-3)

Species	Active substance	a.i. ratio	LD ₅₀ (mg/kg-bw)	LD _{50-mix} predicted	LD _{50-mix} measured
Rat	Fenpicoxamid	0.34	>2000	>3626	2000-5000
	Prothioconazole	0.66	>6200		

Since the measured endpoint is a range and the predicted endpoint falls into this range, the mixture is not more toxic than predicted based on the toxicity of the active substances.

As shown in the endpoints table for prothioconazole (Table 9.3-2), relevant metabolite JAU 6476-desthio is more toxic than the parent; therefore a predicted acute mammalian endpoint has also been generated using the LD₅₀ from the JAU 6476-desthio reproduction study and assuming 100% conversion to the metabolite.

Table 9.3-5: Predicted acute mammalian endpoints for GF-3307 _metabolite considered

Species	Active substance	a.i. ratio	LD ₅₀ (mg/kg-bw)	Predicted LD _{50-mix} *
Rabbit	Fenpicoxamid	0.34	>2000	2150
Rat	JAU 6476-desthio	0.66	2235	

The predicted LD₅₀ value based on fenpicoxamid and the metabolite JAU 6476-desthio also falls into the range of the measured value; this LD_{50-mix} of 2150 mg/kg-bw was considered in the acute mammalian risk assessment.

zRMS comments:

The LD_{50mix} presented in Table 9.3-4 and Table 9.3-5 has been validated by the zRMS and it is confirmed to be correct.

Chronic mammalian mixture assessment

Per Appendix B of the Birds and Mammals Guidance Document (EFSA Journal 2009; 7(12): 1438), for “the risk to reproduction from exposure to more than one active substance, it is currently not recommended to consider the use of predicted toxicity values as surrogates in the risk assessment.” None the less, a predicted chronic toxicity value has been calculated as a courtesy.

An mammalian reproduction test was not run with the formulation, GF-3307, thus a predicted value was generated using the CA approach (NOAEL = 131.5 mg/kg bw/day) and used in the risk assessment (Section 9.3.2.1). The predicted NOAEL_{mix} was calculated as follows:

$$\text{Predicted NOAEL}_{\text{mix}} = \left(\frac{\% \text{ active A}}{\text{NOAEL}_A} + \frac{\% \text{ active B}}{\text{NOAEL}_B} \right)^{-1}$$

where: the fraction of active A + active B in the formulation must equal 1.

Table 9.3-6: Predicted chronic mammalian endpoints for GF-3307

Species	Active substance	a.s. ratio	NOEC (mg/kg-bw/day)	Predicted NOAEL _{mix}
Rabbit	Fenpicoxamid	0.34	495	131.5
Rat	Prothioconazole	0.66	95.6	

The next step is to evaluate the ‘tox per fraction’ to determine if one active contributes ≥90% to the mixture toxicity, therefore the other components will only have a marginal impact on the predicted risk.

Table 9.3-7: Contribution of the active substances of GF-3307 to the predicted toxicity

Compound	Fenpicoxamid	Prothioconazole
NOEC	495	95.6
Content in formulation	4.8%	9.4%
Fraction in mixture	33.8%	66.2%
Tox per fraction	1464	144
GF-3307	NOAEL(mix)=	131.5
Contribution to predicted toxicity	8.98%	91.0%

As the risk contribution from prothioconazole is 91.0%, the chronic avian risk assessment for prothioconazole is sufficient to address the risk from GF-3307 (S7K-3-3) and no further consideration is needed.

As shown in the endpoints table for prothioconazole (Table 9.3-2), relevant metabolite JAU 6476-desthio is more toxic than the parent; therefore a predicted chronic mammalian endpoint has also been generated using the NOAEL from the JAU 6476-desthio reproduction study and assumed 100% conversion to the metabolite. Additionally the “tox per fraction” has been assessed.

Table 9.3.8: Predicted chronic mammalian endpoints for GF-3307 (S7K-3-3) metabolite considered

Species	Active substance	a.s.-ratio	NOEC (mg/kg-bw/day)	Predicted NOAEL _{-mix}
Rabbit	Fenpicoxamid	0.34	495	15.0
Rat	JAU-6476-desthio	0.66	10	

Table 9.3.9: Contribution of fenpicoxamid and JAU-6476-desthio to the predicted toxicity

Compound	Fenpicoxamid	JAU-6476-desthio
NOEC	495	10
Content in formulation	4.8%	9.4%
Fraction in mixture	33.8%	66.2%
Tox per fraction	1464	15.1
GF-3307	NOAEL(mix)=	15.0
Contribution to predicted toxicity	1.00%	99.0%

As the risk contribution from JAU-6476-desthio is 99.0%, the chronic mammal assessment for JAU-6476-desthio is sufficient to address the risk from GF-3307 and no further consideration is needed.

In the registration report for GF-3307 (S7K-3-3) in cereals, the zRMS Poland conducted the combined chronic risk assessment for GF-3307 (S7K-3-3) by using the lowest effect value (10 mg pm/kg-bw/day for JAU-6476-desthio) and exposure to the sum of both active substances (0.225 kg a.s./ha). This approach is also recommended in the EFSA 2009 GD for compounds acting in the same way on defined molecular targets. This approach was used in the risk assessment as a worst case (Section 9.3.2.1).

zRMS comments:

As already indicated in the zRMS comments above, consideration of the lowest toxicity endpoint of 10 12.1 mg/kg bw/d together with cumulative application rate of both active substances represents worst case and accounts also for conversion of prothioconazole to metabolite JAU-6476-desthio.

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 Birds 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 screening and first-tier risk assessments are summarised in the following tables.

Table 9.3-10: Screening and first-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of fenpicoxamid in sugar beet/fodder beet

Intended use		Sugar beet/fodder beet				
Active substance		Fenpicoxamid				
Application rate (g/ha)		2 x 75, interval 21 days (MAF calculated according to Appendix H, EFSA 2009 B&M GD)				
Acute toxicity (mg/kg bw)		>2000				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀	TER_a	
Growth stage				(mg/kg bw/d)		
Sugar beet/fodder beet	Indicator species for screening	118.4	1.1	9.8	>205	
Reprod. toxicity (mg/kg bw/d)		495				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m	TER_{lt}	
Growth stage				(mg/kg bw/d)		
Sugar beet/fodder beet	Indicator species for screening	48.3	1.2 x 0.53	2.30	215	
Sugar beet, BBCH ≥ 20	Small insectivorous mammal “shrew”	1.9	1.2 x 0.53	0.09	5500	
Sugar beet, BBCH ≥ 40	Small herbivorous mammal “vole”	18.1	1.2 x 0.53	0.8	618.8	
Sugar beet, BBCH 10 - 39	Large herbivorous mammal “lagomorph”	14.3	1.2 x 0.53	0.7	707.2	
Sugar beet, BBCH ≥ 40	Large herbivorous mammal “lagomorph”	3.6	1.2 x 0.53	0.2	2475	
Sugar beet, BBCH ≥ 10 - 39	Small omnivorous mammal “mouse”	7.8	1.2 x 0.53	0.4	1237.5	
Sugar beet, BBCH ≥ 40	Small omnivorous mammal “mouse”	1.9	1.2 x 0.53	0.09	5500	

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_A and TER_{LT} 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_{multi} and TERs:

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

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 9.3-11: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of metabolite X696476 in sugar beet/fodder beet

Intended use		Sugar beet/fodder beet				
Metabolite		X696476				
Application rate (g/ha)		2 x 75; max % formation = 1.4%; = 2 x 1.1 g/ha, interval 21 days (MAF calculated according to Appendix H, EFSA 2009 B&M GD)				
Acute toxicity (mg/kg bw)		> 2000 (parent)/10 = 200				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Growth stage						
Sugar beet/fodder beet	Indicator species for screening	118.4	1.1	0.137	>1463	
Reprod. toxicity (mg/kg bw/d)		495 (parent)/10 = 49.5				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}	
Growth stage						
Sugar beet/fodder beet	Indicator species for screening	48.3	1.2 x 0.53	0.032	1535	

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_A and TER_{LT} values are above the Annex VI trigger values, therefore, there is acceptable acute and chronic risk to mammals from X696476.

zRMS comments:

The risk assessment for the active substance and metabolite X696476 presented in Tables 9.3-10 and 9.3-11 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 fenpicoxamid 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).

Overall, based on the performed calculations, acceptable acute and long-term dietary risk to mammals from fenpicoxamid and its relevant plant metabolites may be concluded following the intended Central Zone uses of GF-3307.

Table 9.3-12: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of prothioconazole in sugar beet/fodder beet

Intended use		Sugar beet/fodder beet				
Active substance		Prothioconazole				
Application rate (g/ha)		2 x 150, interval 21 days (MAF calculated according to Appendix H, EFSA 2009 B&M GD)				
Acute toxicity (mg/kg bw)		> 6200				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀	TER_a	
Growth stage				(mg/kg bw/d)		
Sugar beet/fodder beet	Indicator species for screening	118.4	1.1	19.5	>317	
Reprod. toxicity (mg/kg bw/d)		95.6				
TER criterion		5				

Crop scenario Growth stage	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{it}
Sugar beet/fodder beet	Indicator species for screening	48.3	1.2 x 0.53	4.61	20.7
Sugar beet, BBCH ≥ 20	Small insectivorous mammal “shrew”	1.9	1.2 x 0.53	0.18	531.1
Sugar beet, BBCH ≥ 40	Small herbivorous mammal “vole”	18.1	1.2 x 0.53	1.73	55.3
Sugar beet, BBCH 10 - 39	Large herbivorous mammal “lagomorph”	14.3	1.2 x 0.53	1.36	70.3
Sugar beet, BBCH ≥ 40	Large herbivorous mammal “lagomorph”	3.6	1.2 x 0.53	0.34	281.2
Sugar beet, BBCH ≥ 10 - 39	Small omnivorous mammal “mouse”	7.8	1.2 x 0.53	0.75	127.5
Sugar beet, BBCH ≥ 40	Small omnivorous mammal “mouse”	1.9	1.2 x 0.53	0.18	531.1

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_A and TER_{LT} values are above the Annex VI trigger values, therefore, there is acceptable acute and chronic risk to mammals from prothioconazole.

A molecular weight conversion factor was used to calculate the application rate of the metabolite JAU 6476-desthio as the endpoints shown are from testing done with the metabolite.

zRMS comments:

The screening step risk assessment for prothioconazole is agreed by the zRMS.
Acceptable acute and long-term risk may be concluded for mammals exposed to prothioconazole in GF-3307.

Table 9.3-13: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of JAU 6476-desthio in sugar beet/fodder beet

Intended use	Sugar beet/fodder beet				
Metabolite	JAU 6476-desthio				
Application rate (g/ha)	2 x 136 g/ha ¹ , interval 21 days (MAF calculated according to Appendix H, EFSA 2009 B&M GD)				
Acute toxicity (mg/kg bw)	2235				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
Sugar beet	Indicator species for screening	118.4	1.1	17.7	126
Reprod. toxicity (mg pm/kg bw/d)	10				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{it}
Sugar beet	Indicator species for screening	48.3	1.2 x 0.53	4.18	2.39
Sugar beet, BBCH ≥ 20	Small insectivorous mammal “shrew”	1.9	1.2 x 0.53	0.164	60.8
Sugar beet, BBCH ≥ 40	Small herbivorous mammal “vole”	18.1	1.2 x 0.53	1.566	6.39
Sugar beet, BBCH 10 - 39	Large herbivorous mammal “lagomorph”	14.3	1.2 x 0.53	1.237	8.08

Sugar beet, BBCH \geq 40	Large herbivorous mammal “lagomorph”	3.6	1.2 x 0.53	0.31	32.1
Sugar beet, BBCH \geq 10 - 39	Small omnivorous mammal “mouse”	7.8	1.2 x 0.53	0.67	14.8
Sugar beet, BBCH \geq 40	Small omnivorous mammal “mouse”	1.9	1.2 x 0.53	0.16	60.8

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.

¹ 150 g a.s./ha x MW conversion factor of 0.907 x 100% formation = 136 g JAU 6476-desthio/ha

The screening level TER_A and tier 1 TER_{LT} are above the Annex VI trigger values of 10 and 5, respectively, therefore, there is acceptable acute and chronic risk to mammals from JAU 6476-desthio.

zRMS comments:

The screening step and Tier 1 risk assessment for metabolite JAU 6476-desthio has been accepted by the zRMS.

Based on performed calculations acceptable acute and chronic risk may be concluded for mammals exposed to the metabolite.

As described in the mixture calculations section 9.3.1, the lowest effect value (10 mg pm/kg-bw/day for JAU 6476-desthio) and exposure to the sum of both active substances (0.225 kg a.s./ha) was used in the risk assessment for GF-3307 (S7K-3-3) as a worst case.

Table 9.3-14: First-tier assessment of the acute and chronic risk for mammals due to the use of GF-3307 (S7K-3-3) in sugar beet/fodder beet

Intended use	Sugar beet/fodder beet				
Product	GF-3307				
Application rate (kg/ha)	Calculated rate: 2 x 0.075 kg fenpicoximid + 0.150 kg prothioconazole/ha = 2 x 0.225 kg a.s./ha, interval 21 days (MAF calculated according to Appendix H, EFSA 2009 B&M GD)				
Acute toxicity (mg/kg bw)	2150 (predicted; fenpicoxamid + JAU 6476-desthio)				
TER criterion	10				
Crop scenario	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_A
Sugar beet/fodder beet	Indicator species for screening	118.4	1.1	29.3	73.4
Reprod. toxicity (mg pm/kg bw/d)	10 (NOAEL for JAU 6476-desthio as worst case)				
TER criterion	5				
Crop scenario	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{LT}
Sugar beet/fodder beet	Indicator species for screening	48.3	1.2 x 0.53	6.01	1.45
Sugar beet, BBCH \geq 20	Small insectivorous mammal “shrew”	1.9	1.2 x 0.53	0.272	36.8
Sugar beet, BBCH \geq 40	Small herbivorous mammal “vole”	18.1	1.2 x 0.53	2.590	3.86
Sugar beet, BBCH 10 - 39	Large herbivorous mammal “lagomorph”	14.3	1.2 x 0.53	2.046	4.89
Sugar beet, BBCH \geq 40	Large herbivorous mammal “lagomorph”	3.6	1.2 x 0.53	0.52	19.4
Sugar beet, BBCH \geq 10 - 39	Small omnivorous mammal “mouse”	7.8	1.2 x 0.53	1.12	9.0
Sugar beet, BBCH \geq 40	Small omnivorous mammal “mouse”	1.9	1.2 x 0.53	0.27	36.8

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.

The screening level TER_A value is above the Annex VI trigger value of 10, therefore, there is acceptable acute risk to mammals from GF-3307 (S7K-3-3) (using the JAU 6476-desthio endpoint).

The Tier 1 TER_{LT} values are above the trigger of 5 for shrew, mouse and lagomorphs at BBCH >40 indicating acceptable risk. For vole at BBCH > 40 and lagomorphs at BBCH 10 – 39, the TER falls below the trigger of 5 indicating potential risk for these species.

The risk for the vole can be refined using the interception of 90% for sugar beet (deposition factor of 0.1) as reported in the latest ‘Generic Guidance for Tier-1 FOCUS Ground Water Assessment’ (vers. 2.2; May 2014) instead of deposition factor 0.25 at stages at BBCH > 40 (growth stages relevant for the common vole). This approach has been used by the zRMS Poland in the RR for GF-3307 in cereals.

Table 9.3-15¹⁵: Refined assessment of the chronic risk for mammals (vole) due to the use of GF-3307 (S7K-3-3) in sugar beet/fodder beet

Intended use	Sugar beet/ fodder beet BBCH 39 - 49								
Application rate (g/ha)	0.075 kg fenpicoximid + 0.150 kg prothioconazole/ JAU 6476-desthio /ha mixture = 0.225 kg a.s./ha								
MAF	1.2								
Long-term toxicity (mg/kg bw/d)	10 (JAU 6476-desthio)								
TER criterion	5								
Generic focal species	Food item	FIR/bw	RUD	MAF	TWA	fdep	PT	DDD_m (mg/kg bw/d)	TER_{LT}
Small herbivore (vole), BBCH ≥ 40	100 % grass	1.33	54.2	1	0.53	0.1	1	1.03	9.69

The Tier 1 TER_{LT} value for vole at BBCH > 40 is above the trigger of 5, when the interception of 90% is considered, indicating acceptable risk.

For lagomorphs at BBCH 10 – 39 refinement based on interception is not possible, since rabbits eat 100% crop leaves. However, the TER falls below the trigger of 5 very slightly (4.89 vs. trigger of 5) for short time (BBCH 39 only) and mixture assessment based on the toxicity of the metabolite JAU 6476-desthio (NO-AEL 10) for both active substances is very conservative, since fenpicoxamid has a NOAEL of > 2000.

Using the above calculated mixture value for fenpicoxamid and JAU 6476-desthio of NOAEL_{mix} 15 mg/kg bw/d, the TER_{LT} of 7.33 is above the trigger of 5 indicating low chronic risk for lagomorphs at BBCH 10 – 39. Independently, if the conservative NOAEL of 10 for JAU 6476-desthio is used, also the molecular conversion factor (150 g a.s./ha x MW conversion factor of 0.907 x 100% formation = 136 g JAU 6476-desthio/ha) should be used; resulting in an application rate of 0.211 kg a.s./ha, which leads to a TER_{LT} of 5.21; which also falls above the trigger indicating acceptable risk for lagomorphs at BBCH 10 – 39.

Therefore, it was concluded that the chronic risk for mammals due to the use of GF-3307 in sugar beet/fodder beet is acceptable.

zRMS comments:

Combined acute risk assessment:

As metabolite JAU 6476-desthio should be also considered in the combined risk assessment due to its higher toxicity comparing to the parent, the zRMS calculated the LD₅₀mix with consideration of relevant toxicity endpoint for the metabolite and the acute risk assessment in Table 9.3-14 has been accepted.

Combined long-term risk assessment

As already indicated in the zRMS comments above, in order to cover the risk from the mixture of fenpicoximid, prothioconazole and metabolite JAU 6476-desthio, the lowest endpoint of all compounds should be taken into account, i.e. NOAEL of 10 mg pm/kg bw/d derived for metabolite JAU 6476-desthio together with cumulative application rate of both active substances accounting also for conversion of prothioconazole to metabolite JAU 6476-desthio. The relevant calculations performed in the Table 9.3-14 has been accepted by zRMS.

The Tier 1 TER_{LT} values are above the trigger of 5 for shrew, mouse and lagomorphs at BBCH >40 indicating acceptable risk. For vole at BBCH > 40 and lagomorphs at BBCH 10 – 39, the TER falls below the trigger of 5 indicating potential risk for these species.

The risk for the vole can be refined using the interception of 90% for sugar beet (deposition factor of 0.1) as reported in the latest ‘Generic Guidance for Tier-1 FOCUS Ground Water Assessment’ (vers. 2.2; May 2014) instead of deposition factor 0.25 at stages at BBCH > 40 (growth stages relevant for the common vole).

Based on performed calculations presented in the Table 9.3-15 an acceptable combined long-term risk to mammals exposed to the mixture of both active compounds and metabolite JAU 6476-desthio may be concluded.

In case of species lagomorph at BBCH 10-39, when the molecular conversion factor for the application rate of JAU 6576-desthio was considered resulting in an application rate of 0.211 kg a.s./ha the TER_{LT} of 5.21 is estimated, indicating an acceptable risk for lagomorph.

Table 9.3-16-1: First-tier assessment of the acute and chronic risk for mammals – lagomorph due to the use of GF-3307 (S7K-3-3) in sugar beet/fodder beet

Intended use	Sugar beet/fodder beet				
Product	GF-3307				
Application rate (kg/ha)	Calculated rate: 2 x 0.075 kg fenpicoximid + 0.136 kg prothioconazole desthio/ha = 2 x 0.211 kg a.s./ha, interval 21 days (MAF calculated according to Appendix H, EFSA 2009 B&M GD)				
Reprod. toxicity (mg pm/kg bw/d)	10 (NOAEL for JAU 6476-desthio as worst case)				
TER criterion	5				
Crop scenario	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Growth stage					
Sugar beet, BBCH 10 - 39	Large herbivorous mammal “lagomorph”	14.3	1.2 x 0.53	2.046	5.21

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.

For concerned Member States preferring simplified approach (TER_{mix}), respective calculation based on the lowest TER values is presented below.

				Σ1/TER	Σ1/TER ⁻¹	Trigger
Fenpicoximid		Prothioconazole				
¹⁾ 707.2	0.0014	¹⁾ 70.3	0.014	0.0154	65.0	5
²⁾ 618.8	0.0016	²⁾ 55.3	0.018	0.0196	51.0	5

¹⁾ Lowest Tier 1 for lagomorph

²⁾ Lowest Tier 1 for vole

				Σ1/TER	Σ1/TER ⁻¹	Trigger
Fenpicoximid		JAU 6476-desthio				
¹⁾ 707.2	0.0014	¹⁾ 8.08	0.123	0.125	8.0	5
²⁾ 618.8	0.0016	²⁾ 6.39	0.156	0.158	6.33	5

¹⁾ Lowest Tier 1 for lagomorph

²⁾ Lowest Tier 1 for vole

Based on performed calculation, acceptable combined long-term risk to mammals exposed to the mixture of both active compounds and as well as active substance with metabolite JAU 6476-desthio may be concluded.

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), fenpicoxamid belongs to the group of more sorptive substances.

Application rate (g/ha)	2 x 75 g fenpicoxamid/ha; interval 21 days		
Effective application rate (g/ha) =	76.2 (based on a soil DT_{50} of 3.5 days, geomean (n=4); EFSA, 2018)		
Acute toxicity (mg/kg bw) =	>2000	quotient =	0.04
Reprod. toxicity (mg/kg bw/d) =	495	quotient =	0.15

With a K_{oc} of 1765 (EFSA, 2007), prothioconazole belongs to the group of more sorptive substances.

Application rate	2 x 150 g prothioconazole/ha; interval 21 days		
Effective application rate (g/ha) =	150.0 (based on a soil DT_{50} of 1.27 days; range: 0.07 to 1.27 days, median: 0.5 days; EFSA, 2007)		
Acute toxicity (mg/kg bw) =	>6200	quotient =	0.02
Reprod. toxicity (mg/kg bw/d) =	95.6	quotient =	1.57

With a K_{oc} of 523-625 mL/g (EFSA, 2007), JAU 6476-desthio belongs to the group of more sorptive substances.

Application rate (g/ha)	2 x 136 g JAU 6476-desthio/ha; interval 21 days		
Effective application rate (g/ha) =	207.6 (based on a soil DT_{50} of 34.0 days; range 7.0 to 34.0 days; median: 24.1 days; EFSA, 2007)		
Acute toxicity (mg/kg bw) =	2235	quotient =	0.10
Reprod. toxicity (mg/kg bw/d) =	10	quotient =	22.46

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

zRMS comments:

The screening step of the drinking water risk assessment performed for fenpicoxamid, prothioconazole and JAU 6476-desthio metabolite 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- fenpicoxamid. 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).

The calculations are provided below.

Substance	Molar	Molar	Peak	Application	Ratio for	Ratio for	Trigger ⁴⁾
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	mass [g/mol]	ratio	occurrence [%] ¹⁾	rate [g/ha]	acute risk ²⁾	long-term risk ³⁾	
Fenpicoxamid	614.6	parent	parent	75	see above	see above	see above
X642188	514.2	0.837	39.2	24.6	0.123	0.50	3000
X696872	444.2	0.723	17.2	9.3	0.046	0.18	3000
X12264475	256.1	0.417	49.4	88.8	0.44	1.80	50
X763024	226.1	0.368	5.7	1.6	0.008	0.032	50
X12313581	168.0	0.273	17.1	3.50	0.017	0.070	3000
X696476	169.0	0.275	46.9	9.7	0.048	0.19	3000
X11963422	206.1	0.335	80.3	20.2	0.10	0.40	50
X12314005	276.3	0.450	5.4	1.8	0.009	0.036	50
X12019520	188.2	0.306	9.8	2.2	0.011	0.044	50
X12255349	514.5	0.837	6.9	4.3	0.021	0.087	3000

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

²⁾ Based on LD₅₀ of 200 mg/kg bw (parent endpoint divided by 10)

³⁾ Based on NOEL of 49.5 mg/kg bw (parent endpoint divided by 10)

⁴⁾ Determined based on geometric mean K_{foc} 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.

9.3.2.4 Effects of secondary poisoning

The LogK_{ow} of fenpicoxamid, prothioconazole, JAU 6476-desthio, and prothioconazole-S-methyl amounts to 4.4, 3.82 (pH 7), 3.04, and 4.19, respectively, 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_{soil} x 0.53 or the 21-d TWA PEC_{soil} to be used for secondary poisoning; in this assessment the FOCUS Step 1 21-d TWA PEC_{soil} was used. The application rates used for calculation of PEC_{soil} is aligned to the current GAP of 2 x 75 g fenpicoxamid and 2 x 150 g prothioconazole/ha.

Table 9.3-17: Assessment of the risk for earthworm-eating mammals due to exposure to fenpicoxamid via bioaccumulation in earthworms (secondary poisoning) for the intended use in sugar beet/fodder beet

Parameter	Fenpicoxamid	comments
PEC _{soil} 21d TWA (mg/kg soil)	0.0266	Section 8.7 @ 2 x 75 g a.s./ha
log P _{ow} / P _{ow}	4.4/25119	
K _{oc}	5776	EFSA, 2018
f _{oc}	0.02	Default
BCF _{worm}	2.62	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + \mathbf{0.012} \times P_{ow}) / f_{oc} \times K_{oc}$
PEC _{worm}	0.070	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.089	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	495	
TER _{It}	5556	

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

X642188 exhibits measured values of $\text{LogK}_{\text{ow}} > 3$. While this metabolite was observed in the hen metabolism study and exhibited LogK_{ow} 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 9.3-18: Assessment of the risk for earthworm-eating mammals due to exposure to X642188 via bioaccumulation in earthworms (secondary poisoning) for the intended use in sugar beet/fodder beet

Parameter	X642188	comments
PEC _{soil} 21d TWA (mg/kg soil)	0.016	Section 8.7 @ 2 x 75 g a.s./ha
log P _{ow} / P _{ow}	3.58/3802	QSAR estimate
K _{oc}	4518	EFSA, 2018
f _{oc}	0.02	Default
BCF _{worm}	0.514	$\text{BCF}_{\text{worm/soil}} = (\text{PEC}_{\text{worm,ww}} / \text{PEC}_{\text{soil,dw}})$ $= (0.84 + \mathbf{0.012} \times \text{P}_{\text{ow}}) / \text{f}_{\text{oc}} \times \text{K}_{\text{oc}}$
PEC _{worm}	0.0082	$\text{PEC}_{\text{worm}} = \text{PEC}_{\text{soil}} \times \text{BCF}_{\text{worm/soil}}$
Daily dietary dose (mg/kg bw/d)	0.0105	$\text{DDD} = \text{PEC}_{\text{worm}} \times 1.28$
NOEL (mg/kg bw/d)	49.5	parent/10
TER _{It}	4701	

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

The soil photolysis metabolite X12255349 was predicted to exhibit a Log K_{ow} 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 9.3-19: Assessment of the risk for earthworm-eating mammals due to exposure to X12255349 via bioaccumulation in earthworms (secondary poisoning) for the intended use in sugar beet/fodder beet

Parameter	X12255349	comments
PEC _{soil} 21d TWA (mg/kg soil)	0.0006	Section 8.7 @ 2 x 75 g a.s./ha
log P _{ow} / P _{ow}	3.89/7762	QSAR estimate
K _{oc}	594	EFSA, 2018
f _{oc}	0.02	Default
BCF _{worm}	7.91	$\text{BCF}_{\text{worm/soil}} = (\text{PEC}_{\text{worm,ww}} / \text{PEC}_{\text{soil,dw}})$ $= (0.84 + 0.012 \times \text{P}_{\text{ow}}) / \text{f}_{\text{oc}} \times \text{K}_{\text{oc}}$
PEC _{worm}	0.0047	$\text{PEC}_{\text{worm}} = \text{PEC}_{\text{soil}} \times \text{BCF}_{\text{worm/soil}}$
Daily dietary dose (mg/kg bw/d)	0.0061	$\text{DDD} = \text{PEC}_{\text{worm}} \times 1.28$
NOEL (mg/kg bw/d)	49.5	parent/10
TER _{It}	8147	

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

Table 9.3-20: Assessment of the risk for earthworm-eating mammals due to exposure to prothioconazole via bioaccumulation in earthworms (secondary poisoning) for the intended use in sugar beet/fodder beet

Parameter	Prothioconazole	comments
PEC _{soil} 21d TWA (mg/kg soil)	0.013	Section 8.7 @ 2 x 150 g a.s./ha
log P _{ow} / P _{ow}	3.82/6607	EFSA, 2007
K _{oc}	1765	Aged leaching study

Parameter	Prothioconazole	comments
foc	0.02	Default
BCF _{worm}	2.27	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC _{worm}	0.030	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.038	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	95.6	
TER _{lt}	2531	

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

Table 9.3-21: Assessment of the risk for earthworm-eating mammals due to exposure to JAU 6476-desthio via bioaccumulation in earthworms (secondary poisoning) for the intended use in sugar beet/fodder beet

Parameter	JAU 6476-desthio	comments
PEC _{soil} 21d TWA (mg/kg soil)	0.0511	Section 8.7 @ 2 x 150 g a.s./ha
log P _{ow} / P _{ow}	3.04/1096	EFSA, 2007
Koc	575.4	Mean adsorption
foc	0.02	Default
BCF _{worm}	1.22	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC _{worm}	0.0622	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.0796	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	10	
TER _{lt}	126	

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

Prothioconazole metabolite prothioconazole-S-methyl was predicted to exhibit a LogK_{ow} of 4.19, thus a bioaccumulation and food chain transfer risk assessment for fish-eating and earthworm-eating mammals was conducted. As no toxicity data is available for the metabolite, the toxicity of the metabolite is assumed to be 10X the parent.

Table 9.3-22: Assessment of the risk for earthworm-eating mammals due to exposure to prothioconazole-S-methyl via bioaccumulation in earthworms (secondary poisoning) for the intended use in sugar beet/fodder beet

Parameter	Prothioconazole-S-methyl	comments
PEC _{soil} initial (mg/kg soil)	0.0134	Section 8.7 @ 2 x 150 g a.s./ha
LogK _{ow} / K _{ow}	4.19/15488	Predicted; EFSA, 2007
Koc	2556.3	Mean adsorption; EFSA, 2007
foc	0.02	Default
BCF _{worm}	3.65	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC _{worm}	0.0489	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.0626	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	9.56	Parent/10
TER _{lt}	153	

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

LogK_{ow} a synonym of LogP_{ow}

TER_{LT} values for mammals eating earthworms are above the Annex VI trigger values, therefore, there is acceptable chronic risk to mammals from fenpicoxamid, prothioconazole, and relevant metabolites.

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 $PEC_{sw} \times 0.53$ or the 21-d TWA PEC_{sw} to be used for secondary poisoning; however, in this assessment the FOCUS Step 1 PEC_{sw-max} was used, except for Prothioconazole-S-methyl, where the PEC_{sw-21d} TWA value was used. The application rates used for calculation of PEC_{sw-max} values are aligned to the current GAP of 1 x 75 g fenpicoxamid and 150 g prothioconazole/ha.

Table 9.3-23: Assessment of the risk for fish-eating mammals due to exposure to fenpicoxamid via bioaccumulation in fish (secondary poisoning) for the intended use in sugar beet/fodder beet

Parameter	Fenpicoxamid	comments
PEC_{sw-max} (mg/L)	0.007126	Focus Step 1 PEC_{sw} max, Section 8.9 @ 2 x 75 g a.s./ha
BCF_{fish}	18.36 L/kg	QSAR estimate
BMF	--	biomagnification factor (relevant for $BCF \geq 2000$)
PEC_{fish}	0.1308	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.0186	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	495	
TER_{lt}	26645	

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

Table 9.3-24: Assessment of the risk for fish-eating mammals due to exposure to X12255349 via bioaccumulation in fish (secondary poisoning) for the intended use in sugar beet/fodder beet

Parameter	X12255349	comments
PEC_{sw-max} (mg/L)	0.001613	Focus Step 1 PEC_{sw} max, Section 8.9 @ 2 x 75 g a.s./ha
BCF_{fish}	9.63 L/kg	QSAR estimate
BMF	--	biomagnification factor (relevant for $BCF \geq 2000$)
PEC_{fish}	0.01553	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.00221	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	49.5	parent/10
TER_{lt}	22442	

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

Table 9.3-25: Assessment of the risk for fish-eating mammals due to exposure to prothioconazole via bioaccumulation in fish (secondary poisoning) for the intended use in sugar beet/fodder beet

Parameter	Prothioconazole	comments
PEC_{sw-max} (mg/L)	0.03258	Focus Step 1, Section 8.9 @ 2 x 150 g a.s./ha
BCF_{fish}	19.7	EFSA (2007)
BMF	--	biomagnification factor (relevant for $BCF \geq 2000$)
PEC_{fish}	0.642	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.0911	$DDD = PEC_{fish} \times 0.142$

NOEL (mg/kg bw/d)	95.6	
TER _{It}	1049	

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

Table 9.3-26: Assessment of the risk for fish-eating mammals due to exposure to JAU 6476-desthio via bioaccumulation in fish (secondary poisoning) for the intended use in sugar beet/fodder beet

Parameter	JAU 6476-desthio	comments
PEC _{sw-max} (mg/L)	0.05857	Focus Step 1, Section 8.9 @ 2 x 150 g a.s./ha
BCF _{fish}	65	EFSA (2007)
BMF	--	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	3.807	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	0.54063	DDD = PEC _{fish} × 0.142
NOEL (mg/kg bw/d)	10	
TER _{It}	18	

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

Due to the low predicted environmental concentrations of prothioconazole-S-methyl in surface water no fish-BCF-study was conducted even though this metabolite has a LogK_{ow} of 4.19. The BCF for this metabolite can be estimated from its LogK_{ow} and BCF and the LogK_{ow} of the parent compound with a sufficient degree of precision. The chemical structure of prothioconazole-S-methyl is very similar to the one of parent compound prothioconazole. The only modification of the parent molecule during the formation of prothioconazole-S-methyl is the methylation of the sulphur atom. This methylation results in an increase of the K_{ow} of prothioconazole-S-methyl that is 15488 (LogK_{ow} = 4.19) in comparison to 6607 (LogK_{ow} = 3.82) of prothioconazole. Therefore, it can be anticipated that the BCF for S-methyl is not more than 2.34 times higher (15488/6607 = 2.34) than the BCF_{parent} (whole fish, normalised to 6% fat) for prothioconazole that has been determined to be 18.8. An estimated BCF for prothioconazole-S-methyl of 44 (18.8 x 2.34 = 43.992) is also in line with the results of the fish BCF study that has been conducted for the parent compound. In the prothioconazole BCF study, prothioconazole-S-methyl was detected as a minor metabolite in edibles and viscera of fish. No significant increase of the level and the portion of the total radioactive residue (TRR) was observed for the metabolite prothioconazole-S-methyl between day 7 and day 14. Therefore, it can be concluded that formation and degradation/depuration of this metabolite, which had been formed in the fish, were in balance. If the metabolite would have a much higher bioaccumulation potential than the parent compound, a further increase of its level and portion of the TRR would have to be expected from day 7 to day 14 in the BCF study. Therefore, a BCF of 44 seems to be a realistic estimate for the BCF of prothioconazole-S-methyl in fish.

In the registration report of GF-3307 (S7K-3-3) for cereals, the zRMS Poland suggested using the significantly higher BCF of 764.8 agreed during the expert meeting in the renewal process of prothioconazole. Although the process itself is not finalised yet, this value is used as a worst case for the risk assessment.

Table 9.3-27: Assessment of the risk for fish-eating mammals due to exposure to prothioconazole-S-methyl via bioaccumulation in fish (secondary poisoning) for the intended use in sugar beet/fodder beet

Parameter	JAU 6476-S-methyl	Comments
PEC _{sw} (mg/L)	0.006809	Focus Step 1 PEC _{SW max} , Section 8.9 @ 2 x 150 g/ha
BCF _{fish}	764.8	BCF agreed during renewal process, already peer-reviewed and agreed, although the renewal process itself is not finalised yet
BMF	-	Biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	5.208	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	0.7395	DDD = PEC _{fish} × 0.142
NOEL (mg/kg bw/d)	9.56	Parent/10
TER _{lt}	13	

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

TER_{LT} values for mammals eating fish and earthworms are above the Annex VI trigger values, therefore, there is acceptable chronic risk to mammals from fenpicoxamid, prothioconazole, and relevant metabolites.

zRMS comments:

The Applicants' approach in evaluation of the risk of secondary poisoning is in line with EFSA (2009). Compounds selected for this assessment are agreed by the zRMS. Evaluation was not triggered for remaining metabolites of both active substances due to their log Pow <3.
Based on the calculations performed from Table 9.3-16 to Table 9.3-26 acceptable risk of secondary exposure from all relevant compounds could 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_A and TER_{LT} values are above the Annex VI trigger values, therefore, there is acceptable acute and chronic risk to birds and mammals from fenpicoxamid, prothioconazole and relevant metabolites. For GF-3307 (S7K-3-3), TER_A values are above the Annex VI trigger values, therefore, there is acceptable acute risk for birds and mammals from GF-3307 (S7K-3-3). Chronic mixture toxicity was calculated based on fenpicoxamid and the prothioconazole metabolite JAU 6476-desthio, which is more toxic to birds and mammals than the parent; thereby, the lowest effect value from active substances and metabolites was chosen and compared to the summed application rate of the active substances. For birds, the TER_{LT} value is above the Annex VI trigger value and therefore, there is acceptable chronic risk from GF-3307 (S7K-3-3). For mammals, the TER_{LT} fell below the trigger for voles at BBCH > 40 and lagomorphs at BBCH 10 – 39 indicating potential chronic risk for these species. The risk for vole was refined using a refined deposition factor of 0.1 and the risk for lagomorphs was refined using the NOAEL_{mix} for fenpicoxamid and JAU 6476-desthio or alternatively the molecular conversion factor for the application rate of JAU 6576-desthio was considered. Based on these refinements, acceptable chronic risk for mammals from GF-3307 (S7K-3-3) is concluded.

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, prothioconazole, and relevant metabolites. Full details of these studies are provided in the respective EU DAR (fenpicoxamid: United Kingdom, 2017; prothioconazole: United Kingdom, 2007) and related documents, as well as in Appendix 2 of this document (new studies).

Effects on aquatic organisms of GF-3307 (S7K-3-3) 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	LC₅₀ = 1.79 µg a.s./L_{mm}	EFSA, 2018
<i>Oncorhynchus mykiss</i> (trout)	Fenpicoxamid	96 h, f	LC ₅₀ = 2.2 µg a.s./L _{mm}	EFSA, 2018
<i>Cyprinus carpio</i> (carp)	Fenpicoxamid	96 h, f	LC ₅₀ = 5.41 µg a.s./L _{gm}	EFSA, 2018
<i>Lepomis macrochirus</i> (bluegill)	Fenpicoxamid	96 h, f	LC ₅₀ = 13.8 µg a.s./L _{mm}	EFSA, 2018
<i>Danio rerio</i> (zebrafish)	Fenpicoxamid	96 h, f	LC ₅₀ = 104 µg a.s./L _{mm}	EFSA, 2018; excluded from geomean per EFSA, 2017
Acute geomean, 4 species			LC₅₀ = 4.1 µg a.s./L	See justification in

Species	Substance	Exposure System	Results	Reference
(fenpicoxamid endpoints only)				Section 9.5.1.1
<i>O. mykiss</i>	GF-2925	96 h, ss	LC ₅₀ = 9.15 µg prep/L _{gm} (1.1 µg a.s./L)	EFSA, 2018
Trout geomean for fenpicoxamid and GF-2925			LC ₅₀ = 1.56 µg a.s./L	--
Acute geomean, 4 species (includes GF-2925)			LC ₅₀ = 3.82 µg a.s./L	EFSA, 2018
<i>P. promelas</i>	Fenpicoxamid	32 d (ELS), f	NOEC = 0.37 µg a.s./L _{mm} EC₁₀ (total length) = 0.91 µg a.s./L_{mm} EC ₂₀ = NA	EFSA, 2018
<i>Daphnia magna</i>	Fenpicoxamid	48 h, ss Rec. <LOQ-110%	EC₅₀ = 0.93 µg a.s./L_{gm}*	EFSA, 2018
<i>D. magna</i>	Fenpicoxamid	21 d, ss	NOEC = 0.53 µg a.s./L _{twa} Study invalidated; not considered a data gap	EFSA, 2018
<i>Pseudokirchneriella subcapitata</i>	Fenpicoxamid	72 h, s	ErC₅₀ >522 µg a.s./L_{gm} EyC₅₀ >522 µg a.s./L NOEC >522 µg a.s./L	EFSA, 2018
Fenpicoxamid metabolites				
<i>O. mykiss</i>	X642188	96 h, f	LC ₅₀ = 7.3 µg/L _{mm}	EFSA, 2018
<i>D. magna</i>		48 h, ss (24 hr renewals) Rec. <MQL-168%	EC₅₀ = 1.3 µg/L_{gm} Study invalidated; data gap	EFSA, 2018
<i>D. magna</i>		48 h, f	EC ₅₀ = 0.79 µg/L _{mm}	Goudie/2018/ DAS#180562
<i>Chironomus riparius</i>		28 d, s, sediment-spiked	NOEC = 1.9 mg/kg _{im} EC ₁₀ = 1.8 mg/kg NOEC = 0.63 mg/kg _{twmm} EC₁₀ = 0.58 mg/kg	Beasley/2018/DAS# 180563
<i>P. subcapitata</i>		Parent/10	ErC ₅₀ = 52.2 µg/L	EFSA, 2018
<i>O. mykiss</i>	X11963422	96 h, ss Rec. 92-103%	LC₅₀ >10000 µg/L_{nom} LC₅₀ >9800 µg/L_{mm}	EFSA, 2018
<i>D. magna</i>		48 h, ss Rec. 70-100%	EC ₅₀ >9100 µg/L _{mm, gm}	EFSA, 2018
<i>P. subcapitata</i>		72 h, s	ErC₅₀ >9000 µg/L_{mm} EyC ₅₀ >9000 µg/L NOEC >9000 µg/L	EFSA, 2018
<i>O. mykiss</i>	X12264475	96 h, ss Rec. 78-106%	LC ₅₀ >9980 µg/L _{gm}	EFSA, 2018
<i>D. magna</i>		48 h, ss Rec. 96-108%	EC ₅₀ >10000 µg/L _{nom}	EFSA, 2018
<i>P. subcapitata</i>		72 h, s	ErC₅₀ = 4440 µg/L_{gm} EyC ₅₀ = 2750 µg/L NOEC = 243 µg/L	EFSA, 2018
<i>O. mykiss</i>	X12313581	96 h, ss Rec. 92-111%	LC₅₀ >10000 µg/L_{nom} LC₅₀ >9800 µg/L_{mm}	EFSA, 2018
<i>D. magna</i>		48 h, ss Rec. 92-114%	EC₅₀ >10000 µg/L_{nom} EC₅₀ >9800 µg/L_{mm}	EFSA, 2018
Algae		QSAR	ErC ₅₀ = 15000 µg/L	EFSA, 2018
<i>O. mykiss</i>	X696872	96 h, ss	LC ₅₀ >2000 µg/L _{gm}	EFSA, 2018

Species	Substance	Exposure System	Results	Reference
		Rec. <MQL-102%		
<i>D. magna</i>		48 h, ss Rec. <MQL-90%	EC ₅₀ = 545 µg/L _{gm} *	EFSA, 2018
Algae		Parent**/410	ErC ₅₀ = 522, 522 µg/L	EFSA, 2018
<i>O. mykiss</i>	X696476	96 h, ss Rec. 89-98%	LC ₅₀ >10000 µg/L _{nom} LC ₅₀ >9210 µg/L _{mm}	EFSA, 2018
<i>D. magna</i>		48 h, ss Rec. 79-109%	EC ₅₀ >9500 µg/L _{gm}	EFSA, 2018
Algae		QSAR	ErC ₅₀ = 350000 µg/L	EFSA, 2018
<i>O. mykiss</i>	X12314005	96 h, ss Rec. <MQL - 81%	LC ₅₀ >1900 µg/L _{gm}	EFSA, 2018
<i>D. magna</i>		48 h, ss Rec. <MQL-83%	EC ₅₀ >8500 µg/L _{gm}	EFSA, 2018
Algae		Parent**/410	ErC ₅₀ = 522, 522 µg/L	EFSA, 2018
<i>D. magna</i>	X12386481	48 h, ss Rec. 64-85%	EC ₅₀ >7800 µg/L _{mm, gm}	EFSA, 2018
Fish	X763024	QSAR	LC ₅₀ = 568000 µg/L	EFSA, 2018
<i>D. magna</i>		48 h, ss Rec. 82-91%	EC ₅₀ >10000 µg/L _{nom}	EFSA, 2018
Algae		QSAR	ErC ₅₀ = 275000 µg/L	EFSA, 2018
Fish	X12019520	parent, GF-2925	LC ₅₀ = 1.1 µg/L	EFSA, 2018
<i>O. mykiss</i>		96 h, ss Rec. 94-103%	LC ₅₀ >10000 µg/L _{nom} LC ₅₀ >9800 µg/L _{mm}	Huges/2018/DAS# 180560
<i>D. magna</i>		48 h, ss Rec. 95-111%	EC ₅₀ >10000 µg/L _{mm, nom}	EFSA, 2018
Algae		Parent**/410	ErC ₅₀ = 522, 522 µg/L	EFSA, 2018
Fish	X12335723	QSAR	LC ₅₀ = 1.27 x 10 ⁷ µg/L	EFSA, 2018
<i>D. magna</i>		48 h, ss Rec. 67-102%	EC ₅₀ >8600 µg/L _{gm} EC ₅₀ >8700 µg/L _{mm} †	EFSA, 2018
<i>C. riparius</i>		28 d, s, sediment-spiked	NOEC = 6.8 mg/kg _{im} LOEC > 6.8 mg/kg EC ₁₀ = NA mg/kg NOEC = 2.2 mg/kg _{twmm}	Leak/2018/DAS# 180564
Algae		QSAR	ErC ₅₀ = 1100000 µg/L	EFSA, 2018
<i>D. magna</i>	X12393285	48 h, ss Rec. 49-96%	EC ₅₀ >7600 µg/L _{gm}	EFSA, 2018
<i>O. mykiss</i>	X12255349	96 h, ss Rec. 87-102%	LC ₅₀ = 7100 µg/L _{nom}	EFSA, 2018
<i>D. magna</i>		48 h, ss Rec. <MQL-100%	EC ₅₀ = 11 µg/L _{gm}	EFSA, 2018
<i>P. subcapitata</i>		72 h, s	ErC ₅₀ >10000 µg/L _{nom} * ErC ₅₀ > 8600 µg/L _{mm} EyC ₅₀ > 10000 nom NOEC = 10000 nom	EFSA, 2018
Fish	X12446477	parent, GF-2925	LC ₅₀ = 1.1 µg/L	EFSA, 2018
<i>O. mykiss</i>		96 h, ss	LC ₅₀ >10000 µg/L _{nom}	2018

Species	Substance	Exposure System	Results	Reference
		Rec. 92-106%	LC₅₀ > 9600 µg/L mm	
<i>D. magna</i>		48 h, ss Rec. 86-110%	EC ₅₀ = 1100 µg/L mm	EFSA, 2018
Algae		Parent**/10	ErC ₅₀ = 5.22, 5.22 µg/L	EFSA, 2018
<i>D. magna</i>	X12399889 (X12442397)	48 h, ss Rec. 79-104%	EC ₅₀ >9000 µg/L gm	EFSA, 2017; artifact, remove from LoEP
<i>D. magna</i>	X122442403	48 h, ss Rec.68-80%	EC ₅₀ = 2400 µg/L gm	EFSA, 2018
Fish	X12433979	QSAR	LC ₅₀ = 81990 µg/L	Blickley/2018/DAS# 180910
Invertebrate		QSAR	EC ₅₀ = 48857 µg/L	
Algae		QSAR	ErC ₅₀ = 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 im EC ₁₀ -juveniles = 0.770 µg/Lim (reduction on day 7) EC ₁₀ -neonates = 1.11 µg/Lim (reduction on day 7)	Hicks/2017/DAS# 160125
<i>D. magna</i>	X642188	21 d, indoor population, static, single pulse	NOEC _{day 2 and 21} = 35 µg/L NOEC _{day 7 and 14} = 17 µg/L NOEC _{overall} = 17 µg/L im	Lamichhane/2015/Corteva 131295
Invertebrate mesocosm (outdoor)	GF-2925	133 d, static	NOEAEC = 3.3 µg a.s./L NOEC = 0.1 µg a.s./L im	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

† The EFSA Conclusion LoEP (2018) and DAR (United Kingdom, 2017) incorrectly lists an EC₅₀ of > 8700 gm for X12335723. The correct 48 hr EC₅₀ 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.

*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)

** EFSA LoEP, 2018: Considering the available information on the two most sensitive taxonomic groups, the toxicity of this metabolite was considered comparable to the parent toxicity

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.

The fish geomean LC₅₀ was agreed by the zRMS in the course of zonal autorisation of the product GF-3308 in Core Dossier , 2023.

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 in course of zonal registration of GF-3308 and was 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-3307 in order to demonstrate that the active substance is more toxic than GF-3307. 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.

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 – prothioconazole and relevant metabolites

Species	Substance	Exposure System	Results	Reference
<i>O. mykiss</i>	Prothioconazole	96 h, s	LC ₅₀ = 1.83 mg a.s./L _{mm}	EFSA, 2007
<i>L. macrochirus</i>		96 h, s	LC ₅₀ = 4.59 mg a.s./L _{mm}	
<i>C. carpio</i>		96 h, s	LC ₅₀ = 6.91 mg a.s./L _{nom}	
Geomean of <i>O. mykis</i> , <i>L. macrochirus</i> , and <i>C. carpio</i>			LC ₅₀ = 3.87 mg a.s./L	--
<i>O. mykiss</i>	Prothioconazole	97 d ELS	NOEC = 0.308 mg a.s./L _{mm} (reduction in swim-up, increase in time to swim-up)	EFSA, 2007
<i>D. magna</i>		48 h, s	EC ₅₀ = 1.3 mg a.s./L _{nom}	EFSA, 2007
<i>D. magna</i>		21 d, ss	NOEC = 0.56 mg a.s./L _{nom} (reduction of offspring)	EFSA, 2007
<i>C. riparius</i>		28 d, s, spiked water	NOEC = 9.14 mg/L _{nom} (emergence,	EFSA, 2007

Species	Substance	Exposure System	Results	Reference
			development rate)	
<i>P. subcapitata</i>		72 h, s	ErC₅₀ = 2.18 mg a.s./L_{im} EbC ₅₀ = 1.1 mg a.s./L _{im}	EFSA, 2007
<i>Lemna gibba</i>		7 d, s	ErC₅₀ = 0.1776 mg a.s./L, mm	Kern/2004b/Report# 200488
Prothioconazole metabolites				
<i>O. mykiss</i>	JAU 6476-desthio	96 h, s	LC₅₀ = 6.63 mg/L, nom	EFSA, 2007
<i>Leuciscus idus melanotus</i>		96 hr, s	LC ₅₀ = 13.2 mg/L	EFSA, 2007
<i>O. mykiss</i>		96 d ELS	NOEC = 0.00334 mg/L, mm (deformities)	EFSA, 2007
<i>D. magna</i>		48 h, s	EC ₅₀ >10 mg/L, nom	EFSA, 2007
<i>D. magna</i>		21 d, ss	NOEC = 0.10 mg a.s./L, nom (offspring reduction)	EFSA, 2007
<i>C. riparius</i>		28 d, s, spiked water	NOEC = 2.0 mg/L, nom EC₁₀ = 3.77 mg/L	EFSA, 2007 (NOEC); Draft RAR, UK, 2018 (EC₁₀)
<i>C. riparius</i>		28 d, s, spiked sediment	NOEC = 50 mg/kg, nom	Picard/2008/Report# EBJAY008
<i>Scenedesmus subspicatus</i>		72 h, s	EbC ₅₀ = 0.073 mg/L nom ErC₅₀ = 0.55 mg/L nom	EFSA, 2007
<i>Lemna gibba</i>		7 d, s	ErC ₅₀ = 0.0809 mg a.s./L, mm	Kern/2003/Report# 200469
<i>O. mykiss</i>	JAU 6476-S-methyl	96 h, ss	LC ₅₀ = 1.8 mg/L, mm	EFSA, 2007
<i>D. magna</i>		48 h, s	EC ₅₀ = 2.8 mg/L, nom	EFSA, 2007
<i>C. riparius</i>		28 d, s, spiked water	NOEC = 0.1 mg/L, nom EC₁₀ = 0.071 mg/L	Bruns/2006b/Report# EBJAX303
<i>P. subcapitata</i>		72 h, s	EbC ₅₀ = 3.77 mg/L, im ErC₅₀ = 47.4 mg/L im	EFSA, 2007
<i>O. mykiss</i>	1,2,4-Trizaole	96 h, s	LC ₅₀ = 498 mg/L	EFSA, 2007
<i>O. mykiss</i>		97 d ELS	NOEC = 3.2 mg/L	EFSA, 2007
<i>D. magna</i>		48 h, s	EC ₅₀ = 900 mg/L mm	EFSA, 2007
<i>P. subcapitata</i>		72 h, s	EbC ₅₀ = 8.2 mg/L ErC₅₀ = 22.5 mg/L	EFSA, 2007

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

Table 9.5-3: Endpoints and effect values relevant for the risk assessment for aquatic organisms – GF-3307 (S7K-3-3)

Species	Substance	Exposure System	Results	Reference
<i>O. mykiss</i>	GF-3307	96 h, ss Rec. <MQL-86% XDE-777	LC ₅₀ = 33.8 µg/L gm	█ 2014 █ 2017
<i>O. mykiss</i>	GF-3307	96 h, f	LC₅₀ = 72 µg prep/L gm	█ 2018/

Species	Substance	Exposure System	Results	Reference
<i>D. magna</i>	GF-3307	48 h, ss (daily renewals) Rec. <MQL-75% XDE-777	EC ₅₀ = 8.29 µg/L _{gm}	Hadsell/2014/DAS# 140489 ; amended Hoover/2018
<i>D. magna</i>	GF-3307	48 h, ss (8 hr renewals) Rec. <LOQ - 98.9% XDE-777	LC ₅₀ = 15 µg prep/L _{twgm}	Goudie/2020/DAS# 191366
<i>P. subcapitata</i>	GF-3307	72 h, s Rec. <MQL-109% XDE-777 Rec. 39-115% Prothioconazole	ErC ₅₀ = 25 mg/L _{im} EyC ₅₀ = 3.6 mg/L _{im} ErC ₅₀ = 8.0 mg/L _{twgm} EyC ₅₀ = 0.71 mg/L _{twgm}	Hicks/2014/DAS# 140491; addendum 2020
Higher-tier studies (micro- or mesocosm studies)				
<i>D. magna</i>	GF-3308	21d, indoor population, static, single pulse	NOEC = 550 µg prep/L _{im} (27 µg XDE-777/L) LOEC > 550 µg prep/L _{im}	Hicks/2016/DAS# 160126
<i>D. magna</i>	GF-3307	21d, indoor population, static, duel pulse (days 0 and 14)	NOEC = 180.0 µg prep/L _{im} (8.46 µg XDE-777/L) LOEC = 532.0 µg prep/L _{im} (25.0 µg XDE-777/L)	Bruggemann/2020/DAS# 181382

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

zRMS comments:

Endpoints presented in Table 9.5-2 are in line with the EU agreed endpoints reported in EFSA Scientific Report (2007) 106, 1-98.

No studies on effects of prothioconazole and metabolite JAU 6476-desthio to *Lemna gibba* were available during the first EU review and endpoints for this species reported in Table 9.5-2 above originate from new studies, performed post-approval.

The studies are owned by Bayer and Applicant for GF-3307 has access only to their results, but not study reports. For this reason, the studies could not be summarised in this document. It is noted that testing of aquatic macrophytes was not required for prothioconazole being a fungicide.

Furthermore, these are new active substance data and therefore should not be considered at the zonal level, especially they are not necessary to finalize the risk assessment.

Taking this into account, endpoints for *Lemna gibba* were struck through in Table 9.5-2 above.

The same approach was taken in zonal evaluation of the product GF-3307 in cereals (Core dossier, 2023).

Studies on toxicity of GF-3307 to aquatic organisms were evaluated and agreed by the zRMS in Core Dossier for GF-3307 in the course of zonal authorisation of the product GF-3307 in cereals, Core Dossier, 2023.

For details of the evaluation please refer to Appendix 2.

Acute aquatic organism mixture toxicity

Per pages 147 - 155 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,” a mixtures assessment is necessary when formulations contain more than one active substance. As shown in the table above, studies are available for GF-3307 (S7K-3-3) for *O. mykiss*, *D. magna*, and *P. subcapitata*. Using the Concentration Addition (CA) model, the predicted acute toxicity of GF-3307 (S7K-3-3), which is 4.8% fenpicoxamid and 9.4% prothioconazole, was calculated for each taxa using the following formula (Eq. 13):

$$\text{Predicted } EC_{50-mix} = \left(\frac{\% \text{ active A}}{EC_{50-A}} + \frac{\% \text{ active B}}{EC_{50-B}} \right)^{-1}$$

where: the fraction of active A + active B in the formulation must equal 1.

Using the predicted EC_{50-mix} and the measured EC₅₀ values, the model deviation ratios (MDRs) were calculated as follows (Eq. 15):

$$MDR = \frac{EC_{predicted}}{EC_{measured}}$$

Using the CA approach, MDRs greater than 5 suggest potential synergism, whereas MDRs between 0.2 and 5 indicate that there is agreement between the measured and predicted values.

Table 9.5-4: Predicted and measured aquatic endpoint comparison for GF-3307 (S7K-3-3)

Species	Test substance	a.i. ratio	Active EC ₅₀ (µg a.s./L)	EC or LC _{50-mix} Predicted (µg prep/L)	EC or LC _{50-mix} Measured (µg prep/L)	EC _{50-mix} Measured based on Sum of actives*	MDR
<i>O. mykiss</i>	Fenpicoxamid	0.34	2.2	6.5	72	10.2	0.635
	Prothioconazole	0.66	1830				
<i>D. magna</i>	Fenpicoxamid	0.34	0.93	2.7	15	2.13	1.29
	Prothioconazole	0.66	1300				
<i>P. subcapitata</i> (Growth rate)	Fenpicoxamid	0.34	>522	>1051	8000	1136	0.925
	Prothioconazole	0.66	2180				

Note: the lowest measured EC₅₀ is displayed versus the EC₅₀ based on the EFSA conclusion endpoint for prothioconazole.

*GF-3307 is 4.8% fenpicoxamid and 9.4% prothioconazole = 14.2% active substance

The MDR values for all three taxa are between 0.2 and 5, i.e. the measured and predicted mix-ture toxicity are considered in agreement. However, the proportions of active substances do not maintain their roughly 1:2 fenpicoxamid:prothioconazole ratio for every FOCUS scenario evaluated, specifically for some of the FOCUS Step 4 R scenarios, thus further evaluation is needed.

The toxic unit (TU) approach makes use of the existing data by comparing the ratio between the concentration of a mixture component and the endpoint. The TU is derived as follows (Eq. 14):

$$Toxic\ Unit\ (TU) = \frac{C_A}{EC_{50-A}} + \frac{C_B}{EC_{50-B}}$$

where: C_A + C_B must equal 1.

If the toxicity of the mixture is largely explained by the toxicity of a single active substance (i.e. ≥ 90%), it can be concluded that this component drives the overall mixtures toxicity and the risk assessment can be based on the toxicity data for the single “driver.”

Table 9.5-5: Mixture assessment for aquatic organisms using the TU approach

Test organism	Active substance	a.i. ratio (C)	EC ₅₀ or LC ₅₀ (µg/L)	C/ EC ₅₀ or LC ₅₀	TU	%
<i>O. mykiss</i>	Fenpicoxamid	0.34	2.2	0.155	0.155	99.8
	Prothioconazole	0.66	1830	0.0004		0.2
<i>D. magna</i>	Fenpicoxamid	0.34	0.93	0.366	0.366	99.9

Test organism	Active substance	a.i. ratio (C)	EC ₅₀ or LC ₅₀ (µg/L)	C/ EC ₅₀ or LC ₅₀	TU	%
	Prothioconazole	0.66	1300	0.0005		0.1
<i>P. subcapitata</i> (Growth rate)	Fenpicoxamid	0.34	> 522	0.0007	0.001	68.3
	Prothioconazole	0.66	2180	0.0003		31.7

Using the TU approach, it is apparent that fenpicoxamid drives the risk assessment for fish and invertebrates as it accounts for greater than $\geq 99.8\%$ of the toxicity, **therefore the aquatic risk assessment for fish and invertebrates exposed to GF-3307 (S7K-3-3) should be based on that of fenpicoxamid.**

Note that metabolite JAU 6476-desthio is less acutely toxic than parent for these taxa, so the impact of this metabolite does not need to be considered in the TU approach.

However, the TU approach does not hold for algae, so a mixtures risk assessment is provided in Section 9.5.2 using the measured GF-3307 (S7K-3-3) algal endpoint.

Chronic aquatic organism mixture toxicity

According to the EFSA Aquatic GD (2013), the above mixtures assessment scheme for EC_x values may equally be applied to NOEC data if these are pragmatically considered as low effect concentrations.

The Tier 1 chronic *D. magna* endpoint for fenpicoxamid was invalidated per EFSA (2018), but no other Tier 1 endpoint exists and the GF-2925 invertebrate mesocosm is not an appropriate surrogate for the prothioconazole Tier 1 chronic daphnid study endpoint, thus the invalidated fenpicoxamid endpoint is used for the mixture toxicity assessment. **However, upon request, a mixture assessment using the GF-2925 invertebrate mesocosm as surrogate for the fenpicoxamid Tier 1 chronic daphnid study endpoint has been added.**

As no chronic Tier 1 data for the formulation is available, the TU approach is used to evaluate mixture toxicity as noted above.

Table 9.5-6: Mixture assessment for aquatic organisms using the TU approach_chronic

Test organism	Active substance	a.s. ratio (C)	EC ₅₀ or LC ₅₀ (µg/L)	C/ EC ₅₀ or LC ₅₀	TU	%
Fish	Fenpicoxamid	0.34	0.91	0.374	0.376	99.4
	Prothioconazole	0.66	308	0.002		0.6
<i>D. magna</i>	Fenpicoxamid	0.34	0.1 (mesocosm NOEC)	3.4 0.642	3.401 0.643	99.97 99.8
	Prothioconazole	0.66	560 (D.magna NOEC)	0.001		0.03 0.2

Using the TU approach, it is apparent that fenpicoxamid drives the chronic risk assessment for fish and invertebrates as it accounts for greater than $\geq 99.4\%$ of the toxicity, **therefore the aquatic risk assessment for fish and invertebrates exposed to GF-3307 (S7K-3-3) should be based on that of fenpicoxamid.**

However, metabolite JAU 6476-desthio is more toxic than parent in Tier 1 chronic toxicity studies, therefore it is considered in the TU assessment below using the proportions of the parent (i.e. assuming 100% conversion).

Table 9.5-7: Mixture assessment for aquatic organisms using the TU approach_chronic (desthio)

Test organism	Active substance	a.i. ratio (C)	EC ₅₀ or LC ₅₀ (µg/L)	C/ EC ₅₀ or LC ₅₀	TU	%
Fish	Fenpicoxamid	0.34	0.91	0.374	0.571	65.4
	JAU 6476-desthio	0.66	3.34	0.198		34.6

Test organism	Active substance	a.i. ratio (C)	EC ₅₀ or LC ₅₀ (µg/L)	C/ EC ₅₀ or LC ₅₀	TU	%
<i>D. magna</i>	Fenpicoxamid	0.34	0.1 (mesocosm NOEC) 0.53	3.4 0.642	3.407 0.648	99.8 99.0
	JAU 6476-desthio	0.66	100	0.007		0.2 1.0

Using the TU approach, even if JAU 6476-desthio is considered, it is apparent that fenpicoxamid drives the chronic risk assessment for invertebrates as it accounts for 99.0% of the toxicity, **therefore the aquatic risk assessment for invertebrates exposed to GF-3307 (S7K-3-3) should be based on that of fenpicoxamid.**

However, the TU approach does not hold for chronic fish, so a mixtures risk assessment is provided in Section 9.5.2.

In general, the predicted GF-3307 fish NOEC would be derived from the proportion of each active substance in the formulation ‘as is’ to arrive at a NOEC of 1.76 µg GF-3307/L. However, given the high toxicity of fenpicoxamid and JAU 6476-prothio to fish, it was necessary to refine the predicted NOEC based on the actual fenpicoxamid and JAU 6476-desthio proportions at each FOCUS Step and scenario, therefore within the chronic fish risk assessment provided in Section 9.5.2 is a predicted GF-3307 fish NOEC for FOCUS Steps 1-4 based on the proportion of fenpicoxamid and JAU 6476-desthio resulting from the FOCUS PEC_{sw} values. Note: EFSA recently released the AGD_Aquamix_v115.xlsm Excel Aquatic MixTox tool, which can be found at <https://eng.mst.dk/chemicals/pesticides/applications-for-authorisation-after-14-june-2011/evaluation-framework/>. While the above mixture toxicity assessment combined with the risk assessments in Section 9.5.2 indicate safe use is demonstrated with appropriate mitigations at the proposed GAP, the AGD input and outcome tables are included in Appendix 3 as a courtesy, and confirm acceptable risk of GF-3307 at the proposed GAP with appropriate mitigations.

zRMS comments:

The zRMS agrees with the Applicant approach with the mixture toxicity risk assessment. The AGD input and outcome tables are included in Appendix 3.

Commenting period process:

Additionally, to the risk assessment following the toxic driver approach based on the content of the active substances in the formulated product, a risk assessment based on the ratio of the substances during the actual expected exposure in surface water has been conducted using the AGD_Aquamix_v122.xlsm Excel Aquatic MixTox tool, calculations for the following mixture assessments have been submitted with the (Final version Core Dossier, March 2026):

- 75g fenpicoxamid +150g prothioconazole _acute risk assessment
- 75g fenpicoxamid +150g prothioconazole _chronic risk assessment
- 75g fenpicoxamid +150g prothioconazole - desthio_chronic risk assessment

The result tables have been copied into Appendix 3 of the Core dossier.

In step 5 of the calculations, fenpicoxamid has been confirmed as toxic driver for

- Acute risk to fish and aquatic invertebrates from fenpicoxamid + prothioconazole
- Chronic risk to fish and aquatic invertebrates from fenpicoxamid + prothioconazole
- Chronic risk to aquatic invertebrates from fenpicoxamid + prothioconazole-desthio

There is no clear driver for

- Chronic risk to fish from fenpicoxamid + prothioconazole-desthio
- Risk to algae

That means the outcome for the toxic driver assessment was identical when the ratio of the substances in the formulated product versus the ratio during the actual expected exposure in surface water was used.

Nevertheless, a complete mixture assessment for all scenarios has been calculated in step 8b and presented in Appendix 3 of the core dossier. It shows acceptable risk for all scenarios with the following risk mitigation.

- 30 m NSZ + 10 m NSZ and
20 m NSZ + 10 m VFS + 75% DRN or
- 10 m NSZ + 10 m VFS + 75% DRN.

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_{sw/sed} values are presented in Section 8.9 based upon generic assumptions for the metabolite. X124433979 does not contain the toxicophore and QSAR predicted fish 96 hr LC₅₀, daphnid 48 hr EC₅₀, and green algae 96 hr EC₅₀ 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 sugar beet/fodder beet, calculated PEC/RAC ratios for X12433979 did not indicate an unacceptable risk for any group of aquatic organisms in FOCUS Step 1 and 2 scenarios (Table 9.5-30). Therefore, no further assessment is necessary.

zRMS comments:

The justification of new endpoints were evaluated by zRMS in course of zonal authorisation of the product GF-3308 and GF-3307 in cereals (Core Dossier, 2023) and they are relevant for the current evaluation of GF-3307 for extended uses.

The zRMS's conclusion (Core Dossier 2023) is presented below.

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.

zRMS comments:

The information were evaluated by zRMS in course of zonal authorisation of the product GF-3308, GF-3307 in cereals (Core Dossier 2023) and is relevant for the current evaluation of GF-3307 for extended uses.

The zRMS's conclusion (Core Dossier , 2023) is presented below.

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 sub-stance 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.

Table 9.5-8: 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, <i>Pseudokirchneriella subcapitata</i>	120383	After 72 hours of exposure, mean cell density in the control was 84.2×10^4 cells/mL, or 168 times the initial nominal cell density. The mean cell density in the vehicle control was 84.5×10^4 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, <i>Pseudokirchneriella subcapitata</i>	120380	After 72 hours of exposure, mean cell density in the control was 61.4×10^4 cells/mL, or 123 times the initial nominal cell density. The mean cell density in the vehicle control was 61.2×10^4 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, <i>Pseudokirchneriella subcapitata</i>	130385	After 72 hours of exposure, mean cell density in the control was 123×10^4 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, <i>Pseudokirchneriella subcapitata</i>	130384	After 72 hours of exposure, mean cell density in the control was 132×10^4 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, <i>Pseudokirchneriella subcapitata</i>	141001	After 72 hours of exposure, mean cell density in the control was 132×10^4 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, <i>Pseudokirchneriella subcapitata</i>	120376	After 72 hours of exposure, mean cell density in the control was 77.1×10^4 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
GF-3308	GF-3308 Growth Inhibition Test with the Unicellular Green Alga, <i>Pseudokirchneriella subcapitata</i>	160103	After 72 hours of exposure, mean cell density in the control was 69.0×10^4 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

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-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 55.2×10^4 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

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.

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_{im}; AF=3; RAC = 0.033 µg/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).

In the GF-2925 invertebrate mesocosm study, organisms were exposed to both fenpicoxamid and its 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 9.5-9 and 9.5-10 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. DT₅₀ 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 9.5-9: Mean measured concentrations of fenpicoxamid (XDE-777) and X642188 in water samples (µg/L)

Time ¹ (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
1st application of test item							
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
2nd application of test item							
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 analyzed 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 analyzed 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 9.5-10: Mean measured concentrations of fenpicoxamid (XDE-777) and X642188 in sediment (µg/kg)

Time ¹ (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/kg¹ 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 were they particularly sensitive in Tier 1 sediment-spiked chronic *Chironomus* and *Lumbriculus* studies (Beasley, 2018; Dinehart, 2019).

While an outdoor invertebrate mesocosm study is not available for GF-3307 (S7K-3-3) (4.8% fenpicoxamid and 9.4% prothioconazole), an indoor daphnid population study is available for GF-3307 (S7K-3-3) (Bruggemann, 2020; DAS# 181382). This study had two applications of GF-3307 (S7K-3-3) with a 14 day interval. Daphnid abundance for juvenile and adult daphnids as well as for the sum of all age groups was affected two days after the 2nd test item application (day 16), resulting in a NOEC of 180 µg/L and a LOEC of 532 µg/L GF-3307 (S7K-3-3), corresponding to 8.46 and 25.0 µg/L XDE-777, based on arithmetic mean measured initial concentrations. Afterwards, no significant differences to controls were found until the end of the test.

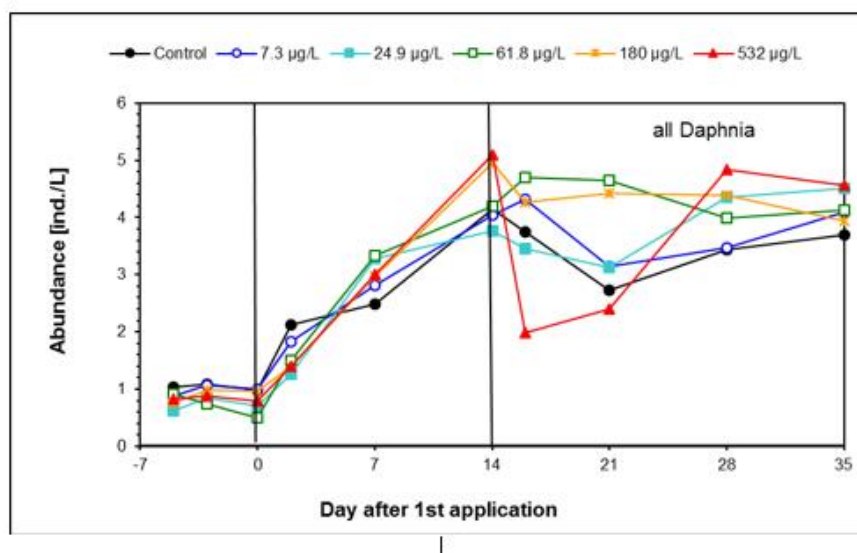


Figure 9.5-1. Diagram showing the $\ln(2y+1)$ transformed data of abundance (means) of daphnids of all age groups during the 35-day exposure period. (Concentrations based on arithmetic mean measured initial concentrations of GF-3307).

The MDD (minimum detectable difference in percent of control) for the sum of all age groups was $< 50\%$ on most sampling days, which allows the determination of small effects according to the current aquatic guidance document (EFSA PPR 2013). For the single development stages, MDDs were higher, but it should be noted that if mean abundances in treated vessels were higher than in the controls, direct effects can be excluded without statistical testing.

Indoor daphnid population studies are also available for fenpicoxamid and GF-3308 (Hicks, 2017 and 2016, respectively). GF-3308 EC contains the same percentage of fenpicoxamid (4.8%) and the same co-formulants (full comparison is available in dRR Part C). The endpoints are directly compared in the table below and summaries are provided in Appendix 2. Note the discrepancy in the length of the fenpicoxamid and GF-3308 daphnid population studies; this is due to the range of concentrations tested and the objective to determine recovery, when necessary. 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 ($550 \mu\text{g prep/L}$), thus the study was terminated. There were effects from fenpicoxamid on daphnid abundance (various age classes) at concentrations $\geq 4.75 \mu\text{g/L}$ on days 2, 7, and 14, but no effects at any concentration tested on days 21, 28, and 35, thus population structure was deemed recovered and the study terminated.

The fenpicoxamid, GF-2925, GF-3308, and GF-3307 endpoints (acute and chronic) are compared in the table below and the summaries are located in Appendix 2. What is evident in the acute studies is that GF-3307 is of comparable toxicity to fenpicoxamid and two orders of magnitude less toxic than GF-2925. In the available chronic studies, GF-3307 is of comparable toxicity to fenpicoxamid and an order of magnitude less toxic than GF-2925.

Table 9.5-11: Comparison of aquatic invertebrate endpoints - fenpicoxamid and formulations

Species	Substance	Exposure System	Results	Reference
Acute invertebrates				
Acute daphnid studies with daily renewals				
<i>Daphnia magna</i>	Fenpicoxamid	48 h, ss (daily renewals) Rec. <LOQ - 110%	$EC_{50} = 0.93 \mu\text{g a.s./L}_{\text{gm}^*}$	EFSA, 2018

<i>D. magna</i>	GF-2925	48 h, ss (daily renewals) Rec. <MQL - 184%	EC ₅₀ = 0.068 µg GF-2925/L _{gm} equivalent to 0.00823 µg fencicoxamid/L (12.1% a.i.)	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% fencicoxamid	EC ₅₀ = 0.029 µg GF-2925/L _{gm} , equivalent to 0.00363 µg fencicoxamid/L (12.5% a.i.)	Hadsell/2014/DAS# 140489; amended Hoover/2018
Acute daphnid studies with 8 hour renewals				
<i>D. magna</i>	GF-2925	48 h, ss (8 hr renewals)	***terminated, SANCO compliant analytical results could not be obtained***	Goudie/20##/DAS# 191365
<i>D. magna</i>	GF-2925	48 h, ss (8 hr renewals)	EC ₅₀ < 1.65 µg prep/L _{twgm} (0.203 µg fencicoxamid/L based on 12.3% a.i.)	Goudie/2021/DAS# 202284
<i>D. magna</i>	GF-3307	48 h, ss (8 hr renewals) Rec. <LOQ - 98.9% fencicoxamid	LC ₅₀ = 15 µg prep/L _{twgm} (0.71 µg fencicoxamid/L based on 4.7% a.i.)	Goudie/2020/DAS# 191366
<i>D. magna</i>	GF-3308	48 h, ss (8 hr renewals) Rec. <MQL – 108%	EC ₅₀ = 48 µg GF-3308/L _{twgm} (2.3 µg fencicoxamid/L based on 4.8% a.i.) (supportive information)	Goudie/2016/DAS# 160102
Chronic invertebrates				
<i>D. magna</i>	Fencicoxamid	35 d, indoor population, static	NOEC = 1.88 µg/L _{im} EC ₁₀ -juveniles = 0.770 µg/L _{im} (reduction on day 7) EC ₁₀ -neonates = 1.11 µg/L _{im} (reduction on day 7) LOEC = 4.75 µg/L LOEC = 4.75 µg/L	Hicks/2017/ DAS# 160125
Invertebrate mesocosm (outdoor)	GF-2925	133 d, static, 2 applications w/14 d interval	Class 3A = 3.3 µg a.s./L Class 1 = 0.1 µg a.s./L_{im}	EFSA, 2018
<i>D. magna</i>	GF-3308	21d, indoor population, static, single pulse	NOEC = 550 µg prep/L _{im} (27 µg fencicoxamid/L) LOEC > 550 µg prep/L _{im}	Hicks/2016/DAS# 160126
<i>D. magna</i>	GF-3307	21d, indoor population, static, duel pulse (days 0 and 14)	NOEC = 180.0 µg prep/L _{im} (8.46 µg fencicoxamid /L) LOEC = 532.0 µg prep/L _{im} (25.0 µg fencicoxamid/L)	Bruggemann/2020/DAS# 181382

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)

Numerous attempts were made to generate an acute daphnid EC_{50} with GF-2925 with 8 hour renewals (Goudie, DAS# 191365) to directly compare with the results from the GF-3308 and GF-3307 studies with 8 hour renewals, on an active substance (fenpicoxamid) basis; however, analytical confirmation of the GF-2925 test concentrations in accordance with SANCO guidelines proved unsuccessful at nominal concentration levels bracketing the 48h EC_{50} .

Given this complication, with the agreement with CRD another acute daphnid GF-2925 study with 8 hour renewals was commissioned (Goudie, DAS# 202284) with the implicit purpose of testing at comparable nominal concentrations of GF-2925, on an active substance basis, to evaluate the percent immobility as compared to GF-3308 and GF-3307. The new daphnid acute toxicity study with GF-2925 (DAS# 202284) shows that under equivalent testing conditions (8 hour renewals; time-weighted geomean measured concentrations, twgm) GF-2925 is more toxic to *Daphnia* than GF-3307. The lowest GF-2925 and GF-3307 concentrations that caused 100% immobility were 0.203 and 2.50 μg fenpicoxamid/L twgm, 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 twgm. Therefore, **the Hommen et al. (2014) mesocosm endpoint can be used in the risk assessment for GF-3307.**

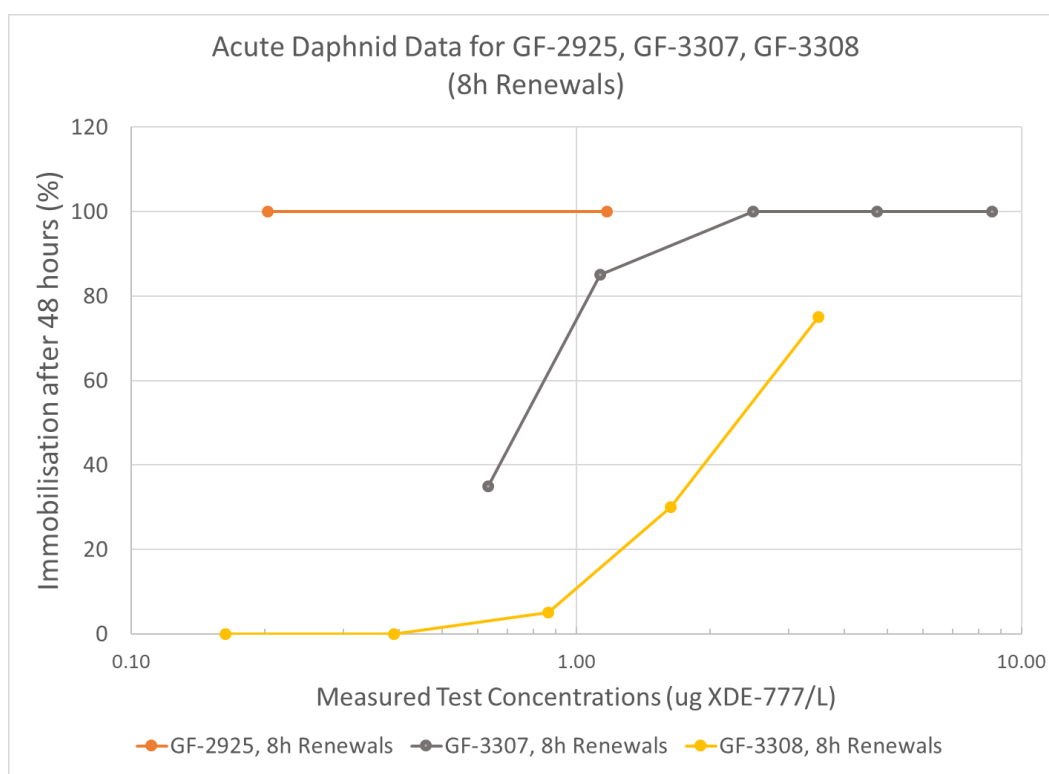


Figure 9.5-2. Acute daphnid immobilization (%) for GF-2925, GF-3307, and GF-3308 under 8 hour static renewal conditions. Concentrations are in μg fenpicoxamid/L, time-weighted geomean, and displayed on a log scale.

Note: Figure 9.5-2 lists the immobilization after 48 hours (%) on the y-axis, however, only GF-3307 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.

zRMS comments:

The information have been evaluated previously in Core Dossier for product GF-3307 for cereals.

zRMS 's conclusions GF-3307) are conducted:

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 GF-3307 concentrations that caused 100% immobility was 2.50 µg fenpicoxamid/L and 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 9.5-11 formulation GF-2925 is also considerably more toxic than the active compound.

In addition, two *Daphnia* population studies were performed in a microcosm test under laboratory conditions with a.s. – fenpicoxamid, (Hick, 2017) and with formulation GF33-07 (Bruggemann, 2020).

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. Obtained results from microcosm tests with *Daphnia* clearly showed that the lowest NOEC for GF-3307 (8.46 µg fenpicoxamid/L) is 4.5 times higher comparing to lowest NOEC for fenpicoxamid (1.88 µg a.s./L). Therefore, the daphnid population studies in conjunction with the acute invertebrate studies for the parent and formulation indicate that the formulated product GF-3307 is not more acutely or chronically toxic than the active substance-fenpicoxamid.

All available evidence also shows that formulation GF-2925 is 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-3307, 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.

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 was used for formulation GF-3307 which is intended to be applied in double application in sugar beet at BBCH 30-69, the application rate is lower (75 g a.s./ha).

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 Step 4 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 (EC₅₀ = 1.3 µg/L); 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/EN-924/2015). A new acute daphnid study has been conducted under flow-through conditions (48 hr EC₅₀ = 0.79 µg/L_{mm}) and is used in the risk assessment (see Section 9.5.2).

Additionally, an indoor daphnid population study is available for X642188 (Lamichhane, 2015). Abundance across each life-stage (neonates, juveniles, and adults) was measured on days 0, 2, 7, 14, and 21 and exposure concentrations were measured at 0, 1, 2, 4, 8, 24, 48, and 168 hours (i.e. until all concentrations were <MQL, min. quantitation limit). A NOEC of 17 µg/L has been calculated. As 55-58% of the test material was lost from the water column at 1 hour post-exposure and 77-82% within 2 hours, one assumes that X642188 is not available chronically (as with the parent) and that the NOEC could be considered in the overall weight-of-evidence regarding the acute toxicity of the metabolite.

To address the risk to sediment dwellers, a sediment-spiked chironomid chronic study was conducted according to OECD 218. 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. However, theoretical concentrations in the sediment (PEC_{sed}) from the FOCUS modelling are low.

Table 9.5-12: Steps 1, 2, and 3 PEC_{sw/sed} for X642188 on sugar beet/fodder beet (Southern Zone) - GF-3307

Compound	FOCUS scenario	Max. PEC _{sw} (µg/L)	Max. PEC _{sed} (µg/kg)
		1x or 2x (worst case) 75 g fenpicoxamid/ha	
X642188	Step 1	3.72	185
	Step 2 - NZ	0.13	5.67
	Step 2 - SZ	0.25	11.1
		1x or 2x (worst case) 75 g fenpicoxamid/ha on sugar beet	
	Step 3 (R1 stream)	0.0294	0.8269

The very low PEC_{sed} values for X642188 (despite strong sorption) are due to its fast degradation in water/sediment systems (DT₅₀ 2.7 days for whole system). Furthermore, the PEC_{sed} 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 study with X642188 has been conducted. In accordance with page 9 of the OECD 218 test guideline, effect concentrations are expressed, “based on measured sediment concentrations at the beginning of the test.” The X642188 *Chironomus riparius* NOEC and EC₁₀ values are 1.9 and 1.8 mg/kg initial measured, respectively (Beasley, 2018). Per the risk assessment for X642188 (Table 9.5-16), acceptable risk is demonstrated at the proposed GAP at FOCUS Step 1 when using the EC_{10 im}.

X12019520 fish: A new acute trout study was conducted according to OECD 203 (Hughes, 2018) and has a LC₅₀ > 10000 µg/L. Given the low PEC_{sw-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 (EC₅₀ > 10000 µg/L) indicating low hazard from the metabolite.

X12446477 fish: A new acute trout study was conducted according to OECD 203 (Hughes, 2018) and has a LC₅₀ > 10000 µg/L. Given the low PEC_{sw-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 (EC₅₀ = 1100 µg/L) indicating moderate hazard from the metabolite.

zRMS comments:

The above information were evaluated by zRMS in course of zonal authorisation of the product GF-3307 for cereals (Core Dossier ,2023) and is relevant for the current evaluation of GF-3307 for extended uses.

The zRMS's conclusion (Core dossier , 2023) is presented below.

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 during zonal evaluation of GF-3308 in Central Zone (please see at Circa platform). Summaries of the studies may be found in Appendix 2 together with zRMS evaluation (copied from GF-3308 Core Dossier available on Circa platform). 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 9.5-3) with boxes and can be divided into two pathways—the first stemming from X12335723 and the other from X642188.

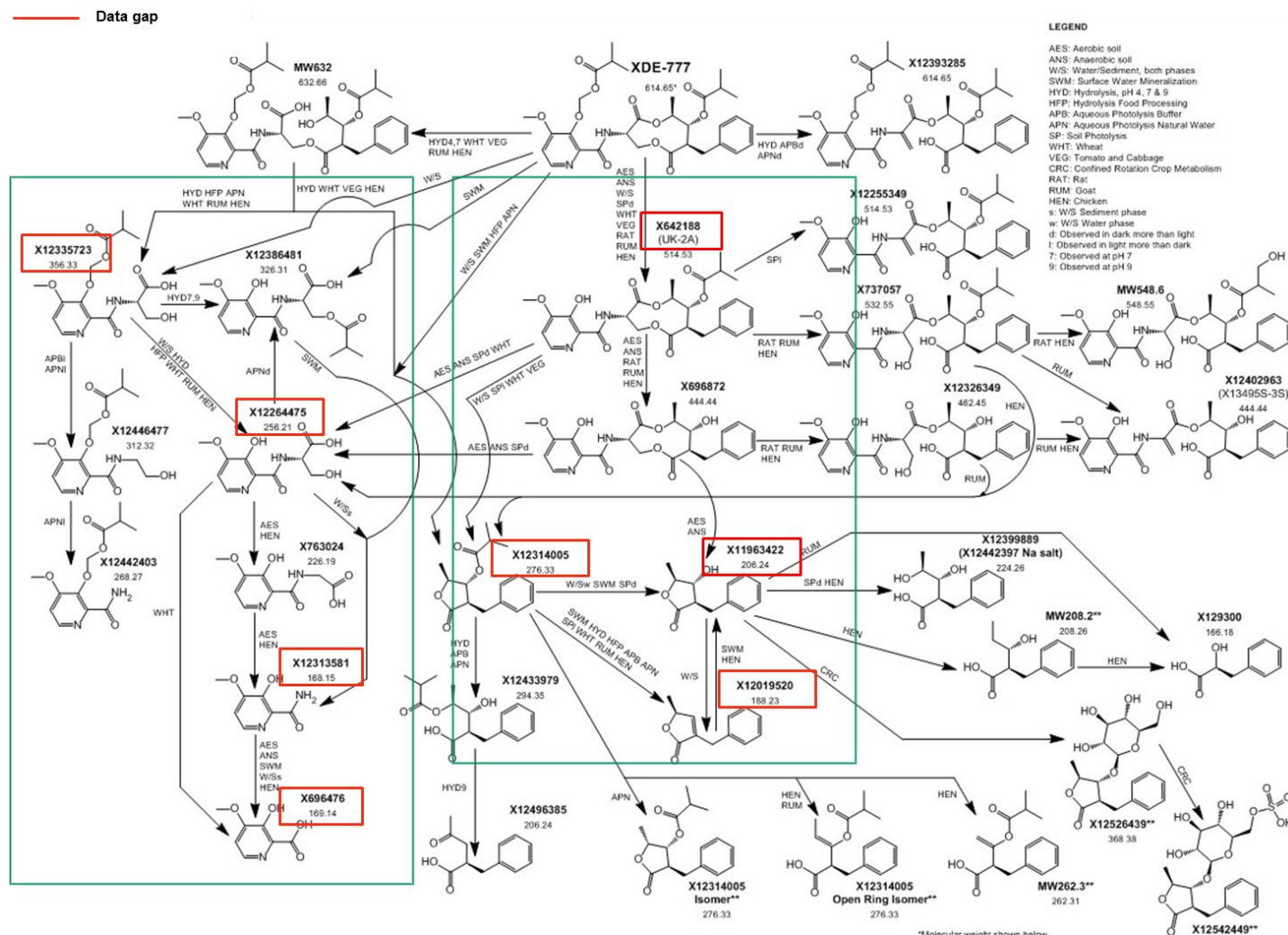


Figure 9.5-3. Global view of the metabolism of fencicoxamid.

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_i site of the cytochrome bc₁ 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 9.5-4).



Figure 9.5-4. 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 9.5-5). While the EC₅₀s for fenpicoxamid and X642188 are around 1 µg/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 9.5-6), where available, further confirming the loss of the toxicophore.

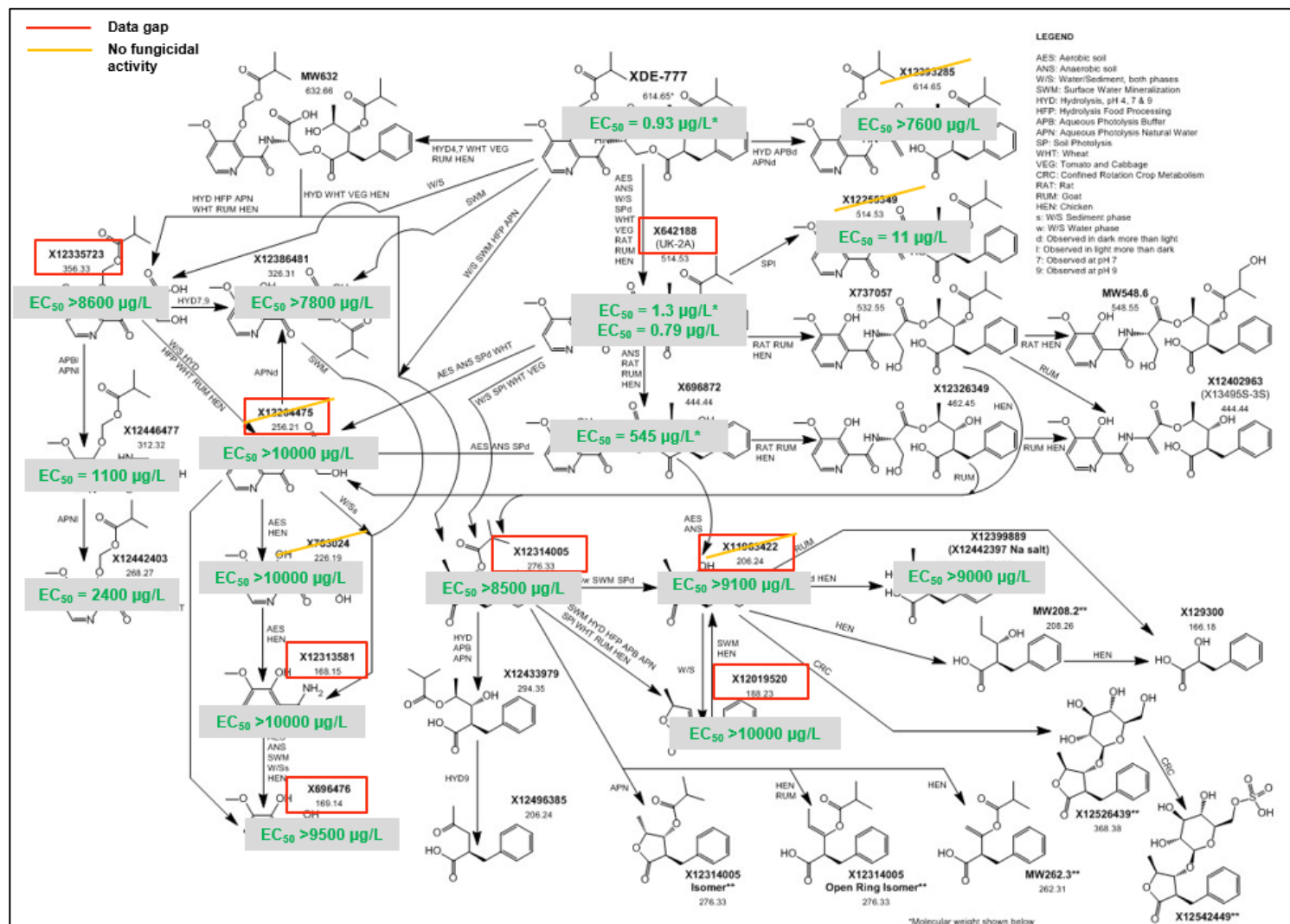
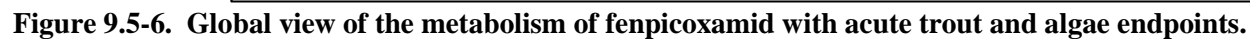


Figure 9.5-5. Global view of the metabolism of fencixoxamid with acute daphnid endpoints.



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 9.5-16), acceptable risk is demonstrated at the proposed GAP of 2 x 75 g a.s./ha at FOCUS Step 1 when using the EC₁₀ value of 0.58 mg/kg_{im}. 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 6.8 mg/kg_{im}, or 2.2 mg/kg_{mm}, the highest concentration tested. Per the risk assessment for X12335723 (Table 9.5-27) 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 above information were evaluated by zRMS in course of zonal authorisation of the product GF-3308, GF-3307 in cereals (Core Dossier, 2023) is relevant for the extended uses of GF-3307.

The zRMS's conclusion (Core Dossier 2023) is presented below.

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. It is also noted that both metabolites included in testing of toxicity to sediment dwellers are precursors of other metabolites to which the sediment dwellers may be also exposed to:

- X642188 for metabolites X12314005, X11963422 and X12019520,
- X12335723 for metabolites X12264475, X12313581 and X696476.

Available EU agreed toxicity data for metabolites X12314005, X11963422, X12019520, X12264475, X12313581 and X696476 do not indicate any increased toxicity comparing to their precursors (see Figures 9.5-4 and 9.5-5 above). Actually, a decrease in toxicity is observed for metabolites X12314005, X11963422 and X12019520 formed from X642188 due to loss of the toxophore, which is present only in metabolite X642188 (sharing the toxicity of the parent). Taking this into account, in opinion of the zRMS it is justified to consider the precursor endpoints in evaluation of the risk to sediment dwellers exposed to metabolites formed from the two tested metabolites, i.e.:

- EC10 of 0.58 mg/kg dw sediment for X12314005, X11963422 and X12019520 (formed from X642188),
- NOEC of 2.2 mg/kg dw sediment for X12264475, X12313581 and X696476 (formed from X12335723).

It is noted that in the zonal evaluation performed for formulation GF-3308 (containing fenpicoxamid only) extremely conservative approach was taken and the risk assessment was performed with assumption of 10 times higher toxicity of metabolites listed above comparing to their precursors. However, after reconsideration the zRMS came to the conclusion that although the approach taken represented worst case, it was not substantiated by the available data, which clearly indicate that toxicity of metabolites X12314005, X11963422, X12019520, X12264475, X12313581 and X696476 is either at the same level as their precursor (pathway from X12335723 downward) or are of lower toxicity comparing to their precursor (pathway from X642188 downwards). Taking this into account, consideration of 10 times toxicity of precursors was overconservative and it was decided to take modify the approach in case of evaluation performed for GF-3307.

Acute fish endpoint recalculation

Per the EFSA Conclusion (2018), the tier 1 acute rainbow trout (*O. mykiss*) LC₅₀ 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 9.5-13). However, GF-3307 EC is less toxic (based on fenpicoxamid concentrations) than GF-2925 SC. Therefore, we have used the lowest acute fenpicoxamid endpoint—*P. promelas* LC₅₀ = 1.79 µg/L—for the Tier 1 risk assessment, and have included the monoformulation 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. Note that GF-3307 was not included in trout geomean as it is not a monoformulation.

Table 9.5-13: Comparison of acute fish endpoints – fenpicoxamid, GF-2925, and GF-3307

Species	Substance	Exposure System	Results	Reference
Rainbow trout studies with active or monoformulations				
<i>O. mykiss</i>	Fenpicoxamid	96 h, f	LC ₅₀ = 2.2 µg a.s./L _{mm}	EFSA, 2018
<i>O. mykiss</i>	GF-2925	96 h, ss	LC ₅₀ = 9.15 µg prep/L _{gm} (1.1 µg a.s./L)	EFSA, 2018
<i>O. mykiss</i>	GF-3308*	96 h, f	LC ₅₀ = 78 µg prep/L _{mm} (3.8 µg a.s./L)	████/2016/████
Trout <u>geomean</u> for fenpicoxamid, GF-2925, GF-3308			LC ₅₀ = 2.1 µg a.s./L	--
<i>C. carpio</i>	Fenpicoxamid	96 h, f	LC ₅₀ = 5.41 µg a.s./L _{gm}	EFSA, 2018
<i>P. promelas</i>	Fenpicoxamid	96 h, f	LC ₅₀ = 1.79 µg a.s./L _{mm}	EFSA, 2018
<i>L. macrochirus</i>	Fenpicoxamid	96 h, f	LC ₅₀ = 13.8 µg a.s./L _{mm}	EFSA, 2018
New acute <u>geomean</u> , 4 species			LC₅₀ = 4.1 µg a.s./L	--

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

zRMS comments:

The above information were evaluated by zRMS in course of zonal authorisation of the product GF-3308, GF-3307 for cereals (Core Dossier, 2023) and is relevant for the current evaluation of GF-3307 for extended uses.

Calculation of the fish geomean LC₅₀ is agreed by the zRMS during course of zonal authorization of the product GF-3308. Since toxicity endpoint for GF-2925 was included in calculation of the EU agreed geometric mean and fenpicoxamid and both formulations (GF-2925 and GF-3308 containing fenpicoxamid only) are of comparable acute toxicity to rainbow trout, calculation of geomean LC₅₀ for rainbow trout from all 3 endpoints and its inclusion into

the calculated overall geomean LC50 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 LC₅₀ 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

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. Again, a ETR below 1 indicates acceptable risk.

The application rates used for calculation of PEC_{sw}/sed values are aligned to the current GAP of 2 x 75 g fenpicoxamid and 150 g prothioconazole/ha.

Risk assessment for fenpicoxamid at 1 to 2 x 75 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 9.5-14: 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-3307 (S7K-3-3) in sugar beet/fodder beet

Group		Fish acute		Fish prolonged	Inverteb. acute	Inverteb. pro- longed	Higher-tier info	Algae
Test species		<i>P. promelas</i> , fen- picoxamid	4. species ge- omean	<i>P. promelas</i>	<i>D. magna</i>	<i>D. magna</i>	Invert. Mesocosm, GF-2925	<i>P. subcapitata</i>
Endpoint		LC ₅₀	LC ₅₀	EC ₁₀	EC ₅₀	NA	Class 1	E _r C ₅₀
(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 ^{gl-max} (µg/L)	ETR = PEC/RAC, ETR < 1 is acceptable risk						
Step 1 @ 1X or 2X (whichever is worst case) sugar beet @ 75 g a.s./ha								
	7.1257	398	174	78	766	NA	214	0.14
Step 2 @ 1X or 2X (whichever is worst case) sugar beet @ 75 g a.s./ha								
N-Europe	0.6898	39	17	8	74	NA	21	0.01
S-Europe	0.6898	39	17	8	74	NA	21	0.01
Step 3 - @ 1X or 2X (whichever is worst case) sugar beet @ 75 g a.s./ha								
D3 ditch	0.3863	22	9	4	42	NA	12	0.01
D4 pond	0.0160	0.89	0.39	0.18	2	NA	0.48	<0.01
D4 stream	0.3211	18	8	4	35	NA	10	0.01
R1 pond	0.0156	0.87	0.38	0.17	2	NA	0.47	<0.01
R1 stream	0.2654	15	6	3	29	NA	8	0.01
R3 stream	0.3775	21	9	4	41	NA	11	0.01

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.

For the intended uses in sugar beet/fodder beet, calculated PEC/RAC ratios for fenpicoxamid did not indicate an acceptable risk for fish and invertebrates in several FOCUS Step 1-3 scenarios. The GF-2925 invertebrate mesocosm has the lowest RAC at 0.033 µg/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 as the most sensitive species.

Table 9.5-15: Aquatic organisms: PEC calculation and acceptability of risk (PEC < RAC) for fenpicoxamid based on FOCUS Step 4 calculations and toxicity data for invertebrates (higher tier) with mitigation options for the use of GF-3307 (S7K-3-3) in sugar beet/fodder beet

Intended use	Sugar beet/fodder beet			
Active substance	Fenpicoxamid			
Application rate (g/ha)	1-2 x 75			
No-spray buffer	30 m	10 m	20 m	10 m
Vegetated filter strip	10 m	10 m	10 m	10 m-
Nozzle reduction	-	-	75%	75%
Step 4 - @ 1X or 2X (worst case) sugar beet @ 75 g a.s./ha				
D3 ditch	0.0234	0.0667	0.0086	0.0166
D4 pond	0.005	0.0101	0.0016	0.0025
D4 stream	0.025	0.0713	0.0092	0.0177
R1 pond	0.005	0.0098	0.0017	0.0025
R1 stream	0.0206	0.0589	0.0076	0.0146
R3 stream	0.0294	0.0838	0.0108	0.0208
RAC (µg/L), GF-2925 invert. mesocosm	ETR = PEC/RAC, ETR < 1 is acceptable risk			
0.033				
No-spray buffer	30 m	10 m	20 m	10 m
Vegetated filter strip	10 m	10 m	10 m	10 m-
Nozzle reduction	-	-	75%	75%
Step 4 - @ 1X or 2X (worst case) sugar beet @ 75 g a.s./ha				
D3 ditch	0.71	2	0.26	0.50
D4 pond	0.15	0.31	0.05	0.08
D4 stream	0.76	2	0.28	0.54
R1 pond	0.15	0.30	0.05	0.08
R1 stream	0.62	2	0.23	0.44
R3 stream	0.89	3	0.33	0.63

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 all taxa, acceptable risk for fenpicoxamid is demonstrated for sugar beet/fodder beet at 1 to 2 x 75 g a.s./ha using a:

- 30 m NSZ + 10 m VFS;
- 20 m NSZ + 10 m VFS + 75% DRN;
- 10 m NSZ + 10 m VFS + 75% 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.

Based on the performed calculations acceptable risk from fenpicoxamid following application of GF-3307 according to the Central Zone GAP (beet/fodder beet at 1 to 2 x 75 g a.s./ha) and relevant scenarios D3, D4, R1 and R3 calculated by program FOCUS STEP 4 may be concluded provided that:

- 30 m unsprayed buffer zone to surface water bodies is respected, or
- 20 m NSZ + 10 m VFS + 75% DRN 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 2 x 75 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 (Hughes, 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 hydrolyzes very rapidly (DT₉₀ of 0.73 days) therefore satisfying the condition that it exhibits a hydrolysis DT₉₀ 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 ETR.

In the following table, the ratios between PEC_{SW} and/or PEC_{SED} and RAC for aquatic organisms are given per intended use for each FOCUS scenario and each organism group.

The GAP modelled in sugar beet/fodder beet at FOCUS Step 1 and 2 was aligned to the current GAP of 1 to 2 x 75 g a.s./ha.

Table 9.5-16: 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-3307 (S7K-3-3) in sugar beet/fodder beet

Group		Fish acute	Inverteb. acute	Algae		Sed. dwell. pro-longed
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>P. subcapitata</i>		<i>C. riparius</i>
Endpoint		LC ₅₀	EC ₅₀	E _r C ₅₀		EC ₁₀
(µg/L)		7.3	0.79	52.2		580
AF		100	100	10		10
RAC (µg/L)		0.073	0.0079	5.22		58
FOCUS Scenario	PEC ^{gl-max} (µg/L)	ETR = PEC/RAC, ETR < 1 is acceptable risk			PEC ^{gl-max} (µg/kg)	
Step 1 @ 1 x 75 g a.s./ha						
	3.7234	51	471	0.71	158.05	3
Step 2 @ 1X or 2X (worst case) sugar beet/fodder beet @ 75 g a.s./ha						
N-Europe	0.1281	2	16	0.02	5.6721	0.10
S-Europe	0.2490	3	32	0.05	11.1307	0.19
Step 3 @ 1X or 2X (worst case) sugar beet/fodder beet @ 75 g a.s./ha						
D3 ditch	0.0013	0.02	0.16	0.00	0.1504	<0.01
D4 pond	0.0018	0.02	0.23	0.00	0.0404	<0.01
D4 stream	0.0020	0.03	0.25	0.00	0.0076	<0.01
R1 pond	0.0134	0.18	2	0.00	0.0786	<0.01
R1 stream	0.0294	0.40	4	0.01	0.8269	0.01
R3 stream	0.0447	0.61	6	0.01	0.4226	0.01

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.

For the intended uses in sugar beet/fodder beet, calculated PEC/RAC ratios for X642188 did not indicate an acceptable risk for invertebrates at FOCUS Step 3 using the tier 1 daphnid endpoint. Further PEC/RAC ratios (ETRs) were calculated for the failing scenarios based on FOCUS Step 4 PEC_{sw} considering reduced

exposure of surface water bodies.

Table 9.5-17: Aquatic organisms: PEC calculation and acceptability of risk (PEC < RAC) for X642188 based on FOCUS Step 4 calculations and toxicity data for invertebrates (Tier 1) with mitigation options for the use of GF-3307 (S7K-3-3) in sugar beet/fodder beet

Intended use	Sugar beet/fodder beet			
Active substance	X642188			
Application rate (g a.s./ha)	1-2 x 75			
No-spray buffer	30 m	10 m	20 m	10 m
Vegetated filter strip	10 m	10 m	10 m-	10 m-
Nozzle reduction	-	-	75%	75%
Step 4 - @ 1X or 2X (worst case) sugar beet @ 75 g a.s./ha; PEC_{sw}				
D3 ditch	0.00064	0.0002	0.0000	0.0000
D4 pond	0.0005	0.0011	0.0003	0.0003
D4 stream	0.0020	0.0020	0.0020	0.0020
R1 pond	0.0055	0.0057	0.0054	0.0054
R1 stream	0.0134	0.0134	0.0134	0.0134
R3 stream	0.0204	0.0204	0.0204	0.0204
RAC (µg/L), acute invertebrate; Tier 1	ETR = PEC/RAC, ETR < 1 is acceptable risk			
0.0079				
No-spray buffer	30 m	10 m	20 m	10 m
Vegetated filter strip	10 m	10 m	10 m-	10 m-
Nozzle reduction	-	-	75%	75%
Step 4 - @ 1X or 2X (worst case) sugar beet @ 75 g a.s./ha				
D3 ditch	0.01	0.03	0.00	0.00
D4 pond	0.06	0.14	0.04	0.04
D4 stream	0.25	0.25	0.25	0.25
R1 pond	0.70	0.72	0.68	0.68
R1 stream	1.70	1.70	1.70	1.70
R3 stream	2.58	2.58	2.58	2.58

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 sugar beet/fodder beet, calculated PEC/RAC ratios for X642188 did indicate an acceptable risk for acute invertebrates in the FOCUS Step 4 D scenarios and in the R1 pond scenario but not for the FOCUS Step 4 R1 and R3 stream scenarios using the OECD Tier 1 EC₅₀ at the proposed GAP of 1-2 x 75 g fenpicoxamid/ha. Therefore, a ‘summed’ approach risk assessment for fenpicoxamid and X642188, using the GF-2925 invertebrate mesocosm endpoint, is presented below.

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 calculation of PEC/RAC ratios for X642188 for sugar beet acceptable risk could be concluded for algae at FOCUS Step 1, sediment dwellers at FOCUS Step 2 and acute fish for all FOCUS Step 3 scenarios

For the intended uses in sugar beet, calculated PEC/RAC ratios for X642188 not acceptable risk was identified for acute invertebrates in three FOCUS Step 3 winter cereals R-scenarios (R1, R3).

Further calculations based on Step 4 PEC_{sw} 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.

Further assessment is presented below.

Therefore, a ‘summed’ approach risk assessment for fenpicoxamid and X642188, using the GF-2925 invertebrate mesocosm endpoint, is presented below.

Risk assessment for fenpicoxamid + metabolite X642188 (‘summed’ approach) at 1-2 x 75 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 4 R1 pond scenario for sugar beet/fodder beet, the FOCUS Step 4 R1 and R3 stream scenarios for sugar beet/fodder beet 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 9.5-4);
- X642188 and fenpicoxamid are of comparable toxicity in aquatic invertebrates (acute daphnid EC₅₀ 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

The PEC_{sw} values for fenpicoxamid and the metabolite X642188 were summed. As a refinement, for FOCUS R3 also the hourly PEC_{sw} values were summed. For the “summed” approach it was necessary to convert the X642188 PEC_{sw} to a parent equivalent (x 614.2/514.2) which could be added to the parent PEC_{sw}. The “summed” PEC_{sw} values were then compared to the invertebrate mesocosm RAC as shown below. ETRs less than 1 indicate acceptable risk.

~~For illustration, the EPAT profiles of the hourly PEC_{sw} values (B8, pp 66-67) are presented below and compared to the exposure profile of the GF-2925 invertebrate mesocosm:~~

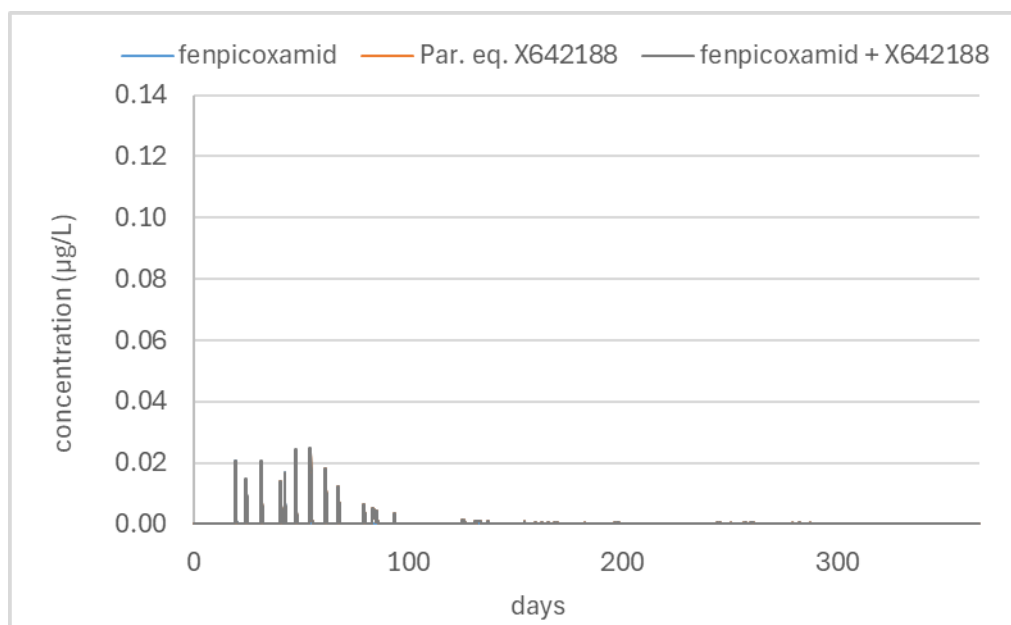


Figure 9.5-7: Modelled exposure (EPAT) for R3 stream at 1 x and 2 x 75 g fenpicoxamid/ha_10 m NSZ, 75% DRN and 10 m VFS – hourly summed PEC_{sw} values (fenpicoxamid + X642188)

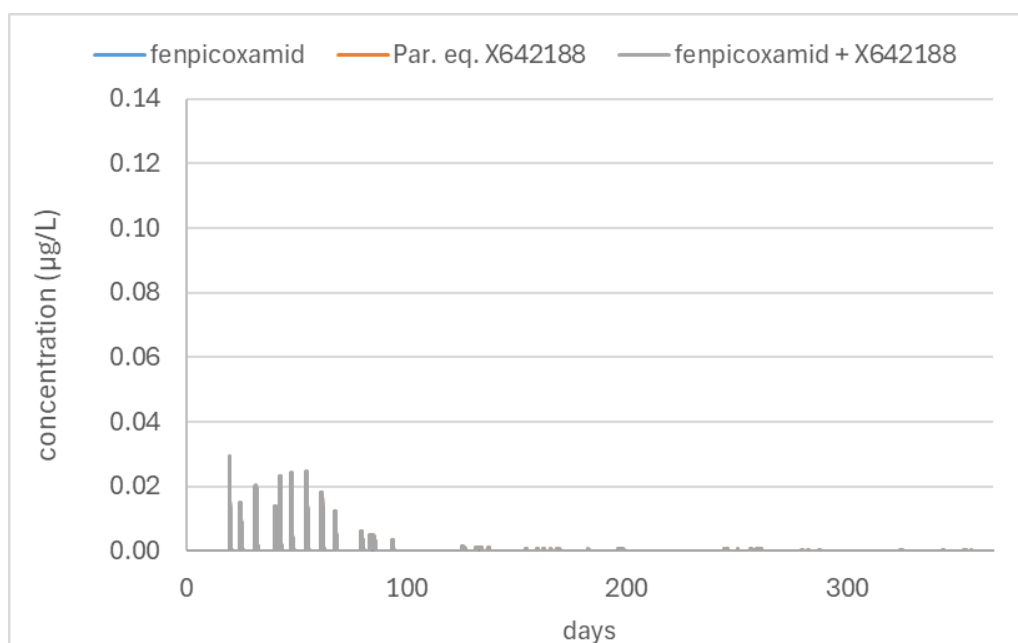


Figure 9.5-8: Modelled exposure (EPAT) for R3 stream at 1 x and 2 x 75 g fenpicoxamid/ha_30 m NSZ and 10 m VFS – hourly summed PEC_{sw} values (fenpicoxamid + X642188)

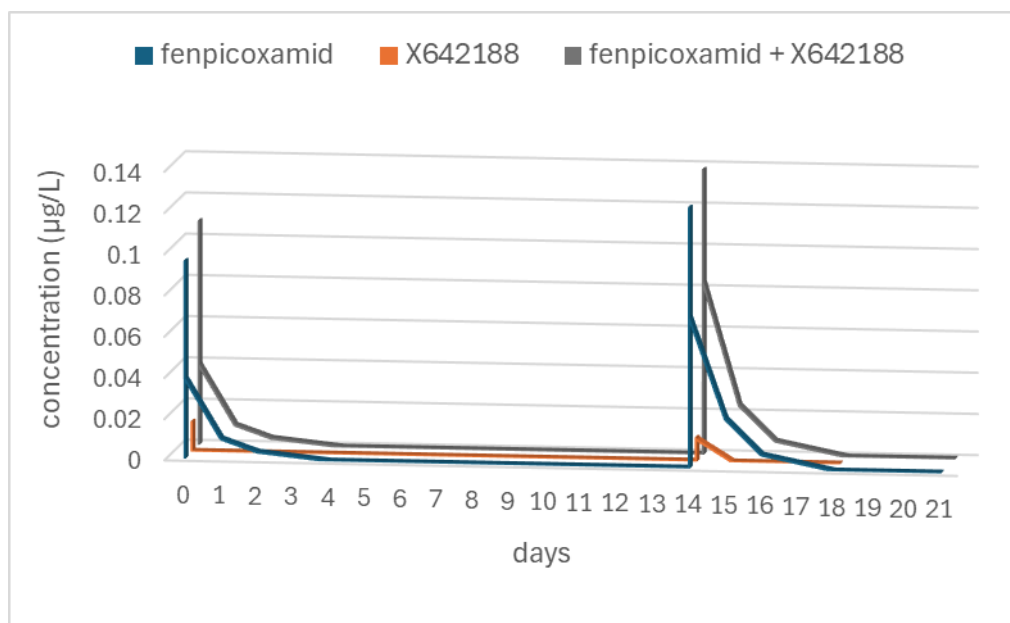


Figure 9.5-9. Concentrations of fenpicoxamid and X642188 in water at the NOEC (0.1 µg fenpicoxamid/L) of the GF-2925 invertebrate mesocosm study (Study ID 130984)

Table 9.5.17a: Concentrations of fenpicoxamid and X642188 in water at the NOEC (0.1 µg fenpicoxamid/L) of the GF-2925 invertebrate mesocosm study (Study ID 130984)

Days (Applications at 0 and 14 days)	Concentration (µg/L)		
	fenpicoxamid	X642188	fenpicoxamid + X642188
0.007 (10 min)	0.096	0.013	0.109
0.042 (1 h)	0.078	0.015	0.092
0.25 (6h)	0.039	n.d.	0.039
1	0.010	n.d.	0.010
2	0.004	n.d.	0.004
4	n.d.	n.d.	n.d.
7	n.d.	n.d.	n.d.
14	n.d.	n.d.	n.d.
14.007	0.099	0.008	0.107
14.042	0.127	0.012	0.138
14.25	0.073	0.011	0.084
15	0.024	n.d.	0.024
16	0.007	n.d.	0.007
18	n.d.	n.d.	n.d.
21	n.d.	-	n.d.

In the mesocosm study, there were two applications with a 14 day interval and a maximum exposure of 0.138 µg fenpicoxamid + X642188/L.

The requested use in sugar beet contains two applications with an interval of 21 days. The hourly PEC_{sw} values were max. 0.0248 µg fenpicoxamid + X642188/L with 10 m NSZ + 10 m VFS + 75% DRN and max. 0.0294 µg fenpicoxamid + X642188/L with 30 m NSZ + 10 m VFS. Please note that the concentration of X642188 was converted to a parent equivalent (x 614.2/514.2) for calculation of the PEC_{sw} values, but not for the measured exposure from the mesocosm study, which makes the comparison a worst case.

The maximum exposure in the mesocosm study is 4.7 to 5.6 times higher than the max. summed PEC_{sw}. However, the number of peaks is higher in the modelled EPAT profiles. Therefore, the chronic daphnia study (Study ID 110216; 21 day NOEC = 0.53 µg fenpicoxamid/L (time weighted average), DAR 2017) is used for additional confirmation on the effect from repeated exposure at concentrations of 0.53 µg fenpicoxamid/L. In this study, the test medium was renewed daily during 21 days, and no effects on survival, reproduction and body length were observed at the NOEC of 0.53 µg fenpicoxamid/L. Therefore, the higher number of peaks at concentrations below 0.03 µg fenpicoxamid in the modelled EPAT profile is not expected to increase effects. The mesocosm RAC of 0.033 µg fenpicoxamid/L can be used for risk assessment

of the two applications in sugar beet.

FOCUS modelling profiles and exposure in mesocosm study

The FOCUS modelling profiles, together with the measured concentrations of fenpicoxamid + X642188 from the mesocosm study (blue peaks) and the RAC from the mesocosm study (red line) are shown Part B8 and are displayed in Figure 1 for each of the proposed risk mitigation measures.

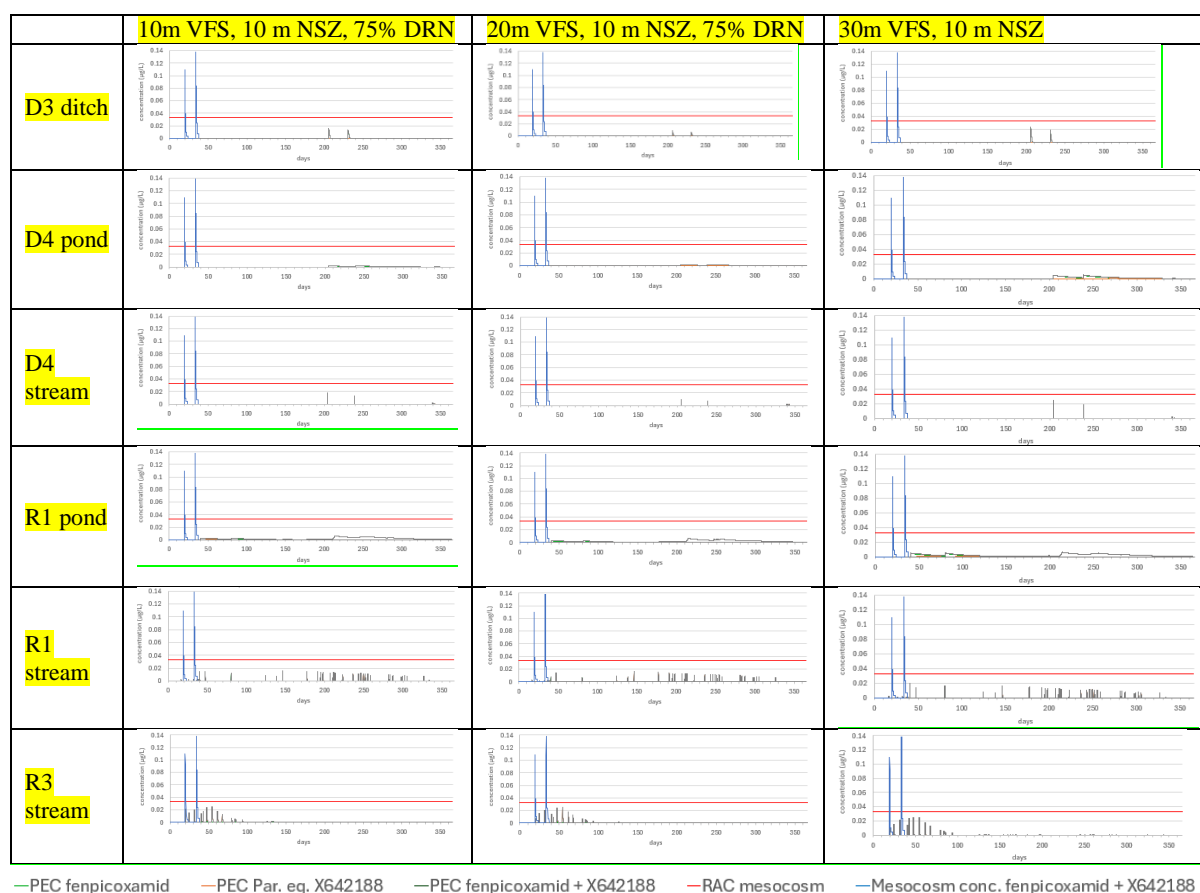


Fig. 1: Focus modelling files for two applications of GF-3307 at 1.5 L/ha with an interval of 21 days in sugar beet

The PEC concentrations of fenpicoxamid + X642188 from the FOCUS modelling profiles is below the RAC from the mesocosm study for all scenarios. There is no major difference between the different risk mitigation measures. The peaks in the stream and ditch scenario are short and drop to zero before the start of the next peak; the concentrations do not add up. The highest peak of 0.029 µg fenpicoxamid + X642188/L is observed in the R3 stream scenario with risk mitigation of 30m VFS and 10m NSZ. The highest density of peaks is observed in the R1 stream scenario between 190 and 310 days; these peaks reached a maximum of 0.014 µg fenpicoxamid + X642188/L.

The peaks in the static pond scenarios are wider/longer than in the flowing stream scenarios. This is due to a DT₅₀ in water of 1000 days was used in modelling for fenpicoxamid and X642188, as is recommended for compounds that are strongly sorbed to sediment (K_{oc} > 2000 mL/g). However, the hydrolysis DT₅₀ at pH 7, 25°C is 0.92 days for fenpicoxamid and 0.22 days for X642188 (EFSA LoEP, 2018) suggests that using 1000 days exaggerates the persistence of fenpicoxamid and X642188 in the water column of a pond. Furthermore, the peaks observed in the ponds from the mesocosm study show the actual degradation in pond systems with a DT₅₀ of less than a day and reflect the degradation expected considering the hydrolysis DT₅₀ of fenpicoxamid and X642188. Therefore, the peaks in the pond scenarios of the FOCUS modelling are not considered to reflect the reality in pond systems.

zRMS comments:

Commenting period process:

PECsw concentrations of fenpicoxamid + X642188 from the FOCUS modelling profiles is below the RAC from the mesocosm study for all scenarios.

Based on the justification presented by the Applicant above in zRMS's opinion the mesocosm can be used for the refinement risk assessment.

Discussion of toxicological independence and minimum number of peaks required in higher tier studies

According to point 9.1.3, toxicological independence of different pulse exposures is shown if either

- a) the life span of sensitive species is not long enough to experience several pulses, or
- b) the internal exposure concentrations in the individual organisms drop below critical threshold levels; or
- c) complete repair of damage occurs between pulses. To demonstrate the toxicological independence of different pulse exposures, either specially designed pulsed exposure tests (section 9.2) or TK/TD models for the relevant organisms and PPP of concern may be used (section 9.2 of EFSA (2013)).

Assessment of toxicological independence of different pulse exposures according to EFSA 2013 GD point 9.1.3

Toxicological independence of different pulse exposures is shown if either

- a) the life span of sensitive species is not long enough to experience several pulses

1. Sensitive species from mesocosm study:

In the mesocosm study (study ID 130984), only 2 species were identified to have clear effects at the next higher test concentration to the NOEC; these species were *Daphnia pulex* and *Cloeon dipterum* (larval stage). For *C. dipterum* larvae, an acute laboratory study (study ID 2025-0642) indicated low sensitivity for *C. dipterum* larvae with an acute EC₅₀ of 405 µg fenpicoxamid/L. That means that the effects observed on *C. dipterum* in the mesocosm study were not direct effects, and therefore, daphnia are the most sensitive species to consider.

2. Life span of sensitive species

Daphnia has been identified as most sensitive species in the mesocosm study; the life span of daphnia considered in the chronic test (OECD 211) is 21 days. During 21 days, several pulses can occur according to the Focus modelling profiles.

- b) the internal exposure concentrations in the individual organisms drop below critical threshold levels

The internal exposure concentrations in the individual organisms have not been measured; however, the potential for bioconcentration in fish is low for fenpicoxamid (predicted value of 18.36 L/kg according to EFSA conclusion, 2018) and for X642188, bioconcentration testing was not triggered since X642188 is unstable in water. Additionally, the concentration of fenpicoxamid and X642188 dropped below the LOQ of 0.05 µg/L within one day at the NOEC test concentration of the mesocosm study. Also in the FOCUS modelling profiles for the ditch and stream scenarios, the concentration drops in the same manner as in the mesocosm study, and concentrations of several peaks do not add up. In the pond scenarios, peaks are wider, but this is an artefact as explained in the discussion of the FOCUS modelling profiles and does not reflect the actual situation, which is reflected by the concentrations in the ponds from the mesocosm study. The quick decline is further confirmed by the DT₅₀ (pH 7, 25°C)

of 0.92 days for fenpicoxamid and 0.22 days for X642188 (EFSA LoEP, 2018). This is an additional line of evidence for the toxicological independence of the pulses.

- c) complete repair of damage occurs between pulses. To demonstrate the toxicological independence of different pulse exposures, either specially designed pulsed exposure tests (section 9.2) or TK/TD models for the relevant organisms and PPP of concern may be used (section 9.2 of EFSA (2013)).

Due to the parameters observed in the daphnia studies, which are mortality in acute studies and size/reproduction at the end of the chronic studies, it is not possible to observe potential repair of damage. However, a 21-day pulsed-dose study with X642188 on the survival of initially neonate individuals has been conducted with 10 individuals per treatment (Goudie 2020, study ID 191071) to check the toxicological independence of pulses. The initial pulse at day 0 was followed by a second pulse at day 3 or 8. Figure 2 shows that the second pulse at nominal concentrations of 23, 28 and 65 µg X642188/L (confirmed by recoveries >100%) caused a slight decrease of survival when it occurred on day 3, but it did not impact survival when it occurred at day eight. It also showed that the nominal concentrations of 10 or 18 µg X642188/L, which did not impact survival after the 1st pulse, did not cause an impact after the second pulse either. Note that these concentrations were just slightly lower than the concentrations that caused effects. Therefore, it is concluded that daphnids are sensitive to additional pulses only during their early development at the tested concentrations but not after 8 days anymore; and only if the pulses are higher than the NOEC.

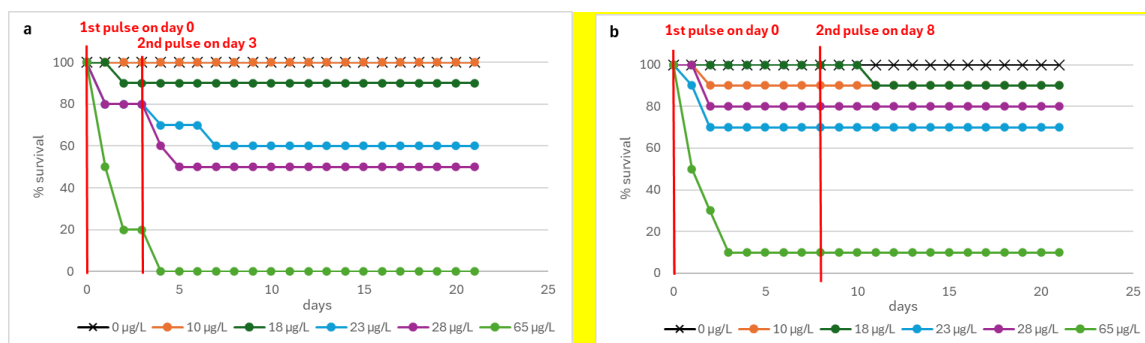


Figure 2: Survival of *D. magna* individuals after two pulses with X642188 at a) 0 and 3 days or b) 0 and 8 days (study ID 191071).

Chronic effects on reproduction and growth were assessed in the chronic *D. magna* study Fournier, 2012, study ID 110216. The zRMS Poland asked that this study “*should not be used for such an analysis, as it was invalidated during the EU process and its results are not considered reliable.*” However, the only point of criticism was that the concentration was below LOQ at 24 hours after each of the 21 daily renewals of test medium, which is due to the intrinsic properties of the active substance fenpicoxamid: “*It was considered by EFSA that as concentrations were not maintained then the study did not provide accurate toxicity endpoints for the tier 1 risk assessment*” Fenpicoxamid DAR, 2017. This means that the study cannot be used for Tier 1 risk assessment, but it is a valid study for the current purpose, which shows that with 21 peaks of nominally 2.4 µg fenpicoxamid/L (confirmed by recoveries ≥86%) during 21 days, no chronic effects are observed.

Conclusions:

- Daphnia are the sensitive species to consider from the mesocosm study; during the life span of 21 days, several pulses might occur according to the Focus modelling profiles.
- Sensitivity to acute effects decreases with the age of the daphnids; at the NOEC of 18 µg X642188/L, no acute effects of the first and additional pulses after 3 or 8 days are observed.
- For chronic exposure, 21 fenpicoxamid pulses during 21 days did not cause effects at the NOEC of 2.4 µg fenpicoxamid/L_(nom)

- Fenpicoxamid as well as X642188 degrade quickly with a Deg DT₅₀ of less than one day. Therefore, the concentrations of different pulses do not add up, which is also shown in the Focus modelling profiles.
- The maximum peak in the focus modelling profiles was 0.029 µg fenpicoxamid + X642188/L (R3 stream with risk mitigation of 30m VFS and 10m NSZ). The highest density of peaks was observed in the R1 stream scenario between 190 and 310 days; these peaks had a maximum height of 0.014 µg fenpicoxamid + X642188/L).

Since the peaks in the Focus modelling profiles are lower than the NOEC (and also lower than the RAC), no effects of repeated peaks are expected. The pulses are considered to be toxicologically independent.

Since it was concluded that the pulses from the FOCUS modelling profiles are toxicologically independent due to their low concentration, the next step (assessment of the minimum number of toxicologically dependent pulse exposures to address in higher tier effect studies according to point 9.1.4) is not needed. However, the following points are addressed for completeness:

According to point 9.1.4, it may not be necessary to incorporate all the toxicologically dependent pulsed exposures in higher tier effect studies if it can be demonstrated that fewer pulse exposures already result in the maximum effects.

The number of applications in the higher tier studies should be as low as possible and is guided by:

- a) the predicted exposure profile and the number and duration of toxicologically dependent pulsed exposures exceeding the lower tier RAC_{sw;ac} and/or RAC_{sw;ch};
- b) the time-course of the responses observed in the available laboratory toxicity tests with sensitive standard and additional test species;
- c) biological information of the species potentially at risk; and
- d) read-across information for compounds with similar toxic modes of action

Assessment of the minimum number of toxicologically dependent pulse exposures to address in higher tier effect studies according to EFSA 2013, point 9.1.4

The number of applications in the higher tier studies should be as low as possible and is guided by:

- a) predicted exposure profile and the number and duration of toxicologically dependent pulsed exposures exceeding the lower tier RAC_{sw;ac} and/or RAC_{sw;ch}

Fenpicoxamid and its metabolite X642188 have a DegDT₅₀ of less than a day in water. Therefore, the ecotoxicological studies were conducted with static-renewal or flow through design, which led to low endpoints because the endpoint was calculated based on the geometric mean measured concentration and/or due to frequent re-dosing of the test item. Since in the Focus modelling profiles the peak concentrations are considered, the measured peak concentrations from the ecotoxicological studies should be used for this comparison. In the following, the RACs used in the Tier 1 risk assessment as well as the RACs based on nominal or measured concentrations (bold letters) from static renewal studies, which are more appropriate for this comparison, are presented.

RAC_{sw;ac} fenpicoxamid: 0.0093 µg fenpicoxamid/L (gm),

0.08 to 0.16 µg fenpicoxamid/L (nom; confirmed by recoveries ≥ 92%)
(study ID 110215)

RAC_{sw;ac} X642188: 0.0079 µg X642188/L (mm, flow-through design with 12 volume replacements per day), (study ID 180562)

0.31 µg X642188/L (initial measured), (study ID 120381)

RAC_{sw;ch} fenpicoxamid: 0.053 µg fenpicoxamid/L_(time weighted average) (chronic daphnia study, study ID 110216) or
0.033 µg fenpicoxamid/L_(initial measured) (invertebrate mesocosm study, study ID 130984)
0.24 µg fenpicoxamid/L_(nom, confirmed by recoveries ≥86%) (chronic daphnia study, study ID 110216)

RAC_{sw;ch} X642188: no chronic risk assessment required for X642188

None of the peaks from the Focus modelling profiles exceed the acute and chronic Tier 1 RAC values based on the initial measured or analytically confirmed nominal concentrations of static-renewal studies (all RACs are higher than the RAC from the mesocosm study of 0.033 µg fenpicoxamid/L, which is displayed as a red line in the diagrams of all Focus modelling profiles in Figure 1.)

b) time-course of the responses observed in the available laboratory toxicity tests with sensitive standard and additional test species

- Acute effects (mortality) are observed on the first and second day for *D. magna*; see Fig. 2
- Chronic effects (size, reproduction) of *D. magna* are assessed at the end of the chronic test according to OECD 211; i.e. after 21 days

c) biological information of the species potentially at risk

Daphnia are the most sensitive species; see above in the assessment for point 9.3.1., a) 1. Daphnia have a life cycle of 21 days; i.e. the chronic daphnia test according to OECD 211 lasts 21 days.

d) read-across information for compounds with similar toxic modes of action

Fenpicoxamid belongs to the FRAC group 21: C4: complex III cytochrome bc1 (ubiquinone reductase) at Qi site # 21 QiI fungicides (Quinone inside Inhibitors) https://efsa.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.2903%2Fj.efsa.2016.4503&file=efs24503-sup-0001-Appendix_A.pdf. Other active substances in this group are amisulbrom, cyazofamid and florylpicoxamid

Information on sensitivity of aquatic invertebrate species:

- Amisulbrom: *D. magna* is the only aquatic invertebrate species with information available in the EFSA LoEP (2014) for amisulbrom, with an EC₅₀ = 0.0368 mg/L (mortality) and a NOEC = 0.0197 mg/L (mortality, reproduction). <file:///C:/Users/kg4217/Downloads/EFSA%20Journal%20-%202014%20-%20-%20-%20Conclusion%20on%20the%20peer%20review%20of%20the%20pesticide%20risk%20assessment%20of%20the%20active%20substance%20amisulbrom.pdf>
- Cyazofamid: *D. magna* is the only aquatic invertebrate species with information available in the EFSA LoEP (2016) for Cyazofamid, with an EC₅₀ >0.107 mg a.s./L (mortality) and a NOEC = 0.011 mg/L (reproduction). https://efsa.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.2903%2Fj.efsa.2016.4503&file=efs24503-sup-0001-Appendix_A.pdf
- Florylpicoxamid: *D. magna* is the only aquatic invertebrate species with information available in the safety data sheet for florylpicoxamid (2022, , with an EC₅₀ = 0.059 mg/L. https://www.30agro.co.kr/up-loaded/board/msds/1_ab57ded53f08ac839b84be029521ee4c0.pdf)

Therefore, for all other compounds with similar toxic modes of action (FRAC group 21), there is no information available on toxicity to aquatic invertebrate species other than *D. magna*.

Conclusion:

- None of the peaks from the Focus modelling profiles exceed the acute and chronic Tier 1 RAC values based on the initial measured or analytically confirmed nominal concentrations of static-renewal studies
- For other active substances from the same FRAC group, no information on toxicity to aquatic invertebrate species other than *D. magna* is available

Based on the of the Focus modelling profiles for two applications of 1.5 L GF3307 in sugar beet, there is no need for additional pulses in higher tier effect studies, if they are conducted at concentrations higher than the expected exposure. The existing aquatic invertebrate mesocosm study (study ID 130984) covers the current Focus modelling profiles.

The “summed” PEC_{sw} values were compared to the invertebrate mesocosm RAC as shown below. ETRs less than 1 indicate acceptable risk.

zRMS comments:

Additional discussion, considering the new studies with the active substance – fepioxamid for species *Daphnia magna* and *Cloeon dipterum* (larval stage) are not evaluated by zRMS at zonal level. These data should be firstly evaluated at EU level.

In zRMS opinion the study chronic effects on reproduction and growth assessed in the chronic *D. magna* study Fournier, 2012, study ID 110216 cannot be used for Tier 1 risk assessment, but can be used as additional information for the current purpose, which shows that with 21 peaks of nominally 2.4 µg fepicoxamid/L (confirmed by recoveries ≥86%) during 21 days, no chronic effects are observed in case of *Daphnia magna*.

Table 9.5-18: Aquatic invertebrates: acceptability of risk (PEC < RAC) for fepicoxamid + X642188 based on FOCUS Step 4 calculations for the use of GF-3307 (S7K-3-3) in sugar beet/fodder beet at 1 to 2 x 75 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	‘summed’ PEC _{gl-max} (µg/L)	ETR = PEC/RAC ETR < 1 is acceptable risk
Step 4 – Sugar beet, 10 m NSZ + 10 m VFS + 75% DRN – summed		
D3 ditch	0.0166*	0.50
D4 pond	0.0029	0.09
D4 stream	0.0188*	0.57
R1 pond	0.009	0.27
R1 stream	0.028	0.85
R3 stream	0.0414	1.25
Step 4 – Sugar beet, 10 m NSZ + 10 m VFS + 75% DRN – summed (hourly PEC_{sw})		
R3 stream	0.0248	0.75
Step 4 – Sugar beet, 20 m NSZ + 10 m VFS + 75% DRN – summed		

D3 ditch	0.0086*	0.26
D4 pond	0.0020	0.06
D4 stream	0.0103*	0.31
R1 pond	0.0081	0.25
R1 stream	0.0220	0.67
R3 stream	0.0329	0.997
Step 4 – Sugar beet, 20 m NSZ + 10 m VFS + 75% DRN – summed (hourly PEC_{sw})		
R3 stream	0.0248	0.75
Step 4 – Sugar beet, 30 m NSZ + 10 m VFS		
D3 ditch	0.0234*	0.71
D4 pond	0.0056	0.17
D4 stream	0.0261*	0.79
R1 pond	0.0115	0.35
R1 stream	0.0324	0.98
R3 stream	0.0491*	1.49
Step 4 – Sugar beet, 30 m NSZ + 10 m VFS – summed (hourly PEC_{sw})		
R3 stream	0.0294	0.89

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.

* Maximum PEC_{sw} value from calculation with single application

Acceptable risk was demonstrated for the FOCUS Step 4 D and R1 scenarios based on summed PEC_{sw} values and for the FOCUS Step 4 R3 scenarios based on summed hourly PEC_{sw} values with a 10 m NSZ + 10 m VFS + 75% DRN or with a 20 m NSZ + 10 m VFS + 75% DRN or with a 30 m NSZ + 10 m VFS.

For all scenarios relevant in this submission, acceptable risk to aquatic invertebrates from fenpicoxamid+X642188 using the ‘summed’ PEC_{sw} values is demonstrated for sugar beets/fodder beets at 1-2 x 75 g a.s./ha with a:

- 10 m NSZ + 10 m VFS + 75% DRN
- 20 m NSZ + 10 m VFS + 75% DRN
- 30 m NSZ + 10 m VFS

zRMS comments:

In Core Dossier for ppp GF-3307 for cereals, the zRMS agreeded 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 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 9.5-18 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-3307 according the Central Zone GAP in scenario R1 provided that 10 meters vegetated filter strip to surface water bodies is respected in combination with 75% drift reduction using appropriate drift reducing techniques (based on summed PEC_{sw}).

The acceptable risk in scenario R3 scenario can be concluded based on summed hourly PEC_{sw} values with a 10 m NSZ + 10 m VFS + 75% DRN or 20 m NSZ + 10 m VFS + 75% DRN with or a 30 m NSZ + 10 m VFS .

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

Due to their low hazard and low exposure, the following metabolites were only required to be analysed at FOCUS Steps 1 and 2.

Table 9.5-19: 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-3307 (S7K-3-3) in sugar beet/fodder beet

Group		Fish acute	Inverteb. acute	Algae		Sediment dwell-ers
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>P. subcapitata</i>		<i>C. riparius</i>
Endpoint		LC ₅₀	EC ₅₀	ErC ₅₀		EC ₁₀
(µg/L)		>9800	>9100	>9000		580 µg/kg dws
AF		100	100	10		10
RAC (µg/L)		98	91	900		58
FOCUS Sce-nario	PEC _{gl-max} (µg/L)	ETR = PEC/RAC; ETR < 1 is acceptable risk			PEC _{sed-max} (µg/L)	
Step 1						
	19.07	0.19	0.21	0.02	16.37	0.28
Step 2 - 1X or 2X (worst case) sugar beet/fodder beet @ 75 g a.s./ha						
N-Europe	0.81	0.01	0.01	<0.01	0.69	0.01
S-Europe	1.4	0.01	0.02	<0.01	1.23	0.02

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 sugar beet/fodder beet, 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 9.5-20: 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-3307 (S7K-3-3) in sugar beet/fodder beet

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 ₅₀	EC ₅₀	E _y C ₅₀		NOEC for X1233572
(µg/L)		>9980	>10000	4440		2200 µg/kg dws
AF		100	100	10		10
RAC (µg/L)		99.8	100	444		220
FOCUS Scenario	PEC _{gl-max} (µg/L)	ETR = PEC/RAC; ETR < 1 is acceptable risk			PEC _{sed-max} (µg/L)	
Step 1						
	17.22	0.17	0.17	0.04	53.12	0.24
Step 2 - 1X or 2X (worst case) sugar beet/fodder beet @ 75 g a.s./ha						
N-Europe	0.74	0.01	0.01	<0.01	2.24	0.01
S-Europe	1.27	0.01	0.01	<0.01	3.88	0.02

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 sugar beet/fodder beet, 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 9.5-21: 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-3307 (S7K-3-3) in sugar beet/fodder beet

Group		Fish acute	Inverteb. acute	Algae		Sediment dwellers
Test species		<i>O. mykiss</i>	<i>D. magna</i>	QSAR		<i>C. riparius</i>
Endpoint		LC ₅₀	EC ₅₀	E _r C ₅₀		NOEC for X1233572
(µg/L)		>10000	>10000	15000		2200 µg /kg dws
AF		100	100	10		10
RAC (µg/L)		100	100	1500		220
FOCUS Scenario	PEC ^{gl-max} (µg/L)	ETR = PEC/RAC; ETR < 1 is acceptable risk				
Step 2 - 1X or 2X (worst case) sugar beet/fodder beet @ 75 g a.s./ha						
	1.94	0.02	0.02	<0.01	12.77 253.3*	0.06 1.15*
Step 2 - 1X or 2X (worst case) sugar beet/fodder beet @ 75 g a.s./ha						
N-Europe	0.09	<0.01	<0.01	<0.01	0.56 21.5*	0.00 0.09*
S-Europe	0.16	<0.01	<0.01	<0.01	1.07	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.

* PEC_{SED,ACCU}, (calculated as PEC_{SED} by multiplication of initial PEC_{SED} by 20, Section 8)

For the intended uses in sugar beet/fodder beet, 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.

Table 9.5-22: 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 GF-3307 (S7K-3-3) in sugar beet/fodder beet

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>O. mykiss</i>	<i>D. magna</i>	Parent
Endpoint		LC ₅₀	EC ₅₀	E _r C ₅₀
(µg/L)		>2000	545	522
AF		100	100	10
RAC (µg/L)		20	5.45	52.2
FOCUS Scenario	PEC _{gl-max} (µg/L)	ETR = PEC/RAC; ETR < 1 is acceptable risk		
Step 1				
	3.28	0.16	0.60	0.06
Step 2 - 1X or 2X (worst case) sugar beet/fodder beet @ 75 g a.s./ha				
N-Europe	0.15	0.01	0.03	<0.01
S-Europe	0.29	0.01	0.05	0.01

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 sugar beet/fodder beet, 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 9.5-23: 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 GF-3307 (S7K-3-3) in sugar beet/fodder beet

Group		Fish acute	Inverteb. acute	Algae		Sediment dwellers
Test species		<i>O. mykiss</i>	<i>D. magna</i>	QSAR		<i>C. riparius</i>
Endpoint		LC ₅₀	EC ₅₀	E _r C ₅₀		NOEC for X1233572
(µg/L)		>10000	>9500	350000		2200 µg/kg dws
AF		100	100	10		10
RAC (µg/L)		100	95	35000		220
FOCUS Scenario	PEC _{gl-max} (µg/L)	ETR = PEC/RAC; ETR < 1 is acceptable risk			PEC _{sed-max} (µg/L)	
Step 1						
	1.48	0.01	0.02	<0.01	110.14 2202.9	0.50 10.01
Step 2 - 1X or 2X (worst case) sugar beet/fodder beet @ 75 g a.s./ha						
N-Europe	0.13	<0.01	<0.01	<0.01	5.07 172.0	0.02 0.8
S-Europe	0.13	<0.01	<0.01	<0.01	8.60	0.04

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 sugar beet/fodder beet, 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.

Table 9.5-24: 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-3307 (S7K-3-3) in sugar beet/fodder beet

Group		Fish acute	Inverteb. acute	Algae		Sediment dwellers
Test species		<i>O. mykiss</i>	<i>D. magna</i>	Parent		<i>C. riparius</i>
Endpoint		LC ₅₀	EC ₅₀	E _r C ₅₀		EC ₁₀
(µg/L)		>1900	>8500	522		580 µg/kg dws
AF		100	100	10		10
RAC (µg/L)		19	85	52.2		58
FOCUS Scenario	PEC _{gl-max} (µg/L)	ETR = PEC/RAC; ETR < 1 is acceptable risk			PEC _{sed-max} (µg/L)	
Step 1						
	8.09	0.43	0.10	0.15	9.29	0.16
Step 2 - 1X or 2X (worst case) sugar beet/fodder beet @ 75 g a.s./ha						
N-Europe	0.11	0.01	<0.01	<0.01	0.11	<0.01
S-Europe	0.19	0.01	<0.01	<0.01	0.22	<0.01

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 sugar beet/fodder beet, 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 9.5-25: 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 GF-3307 (S7K-3-3) in sugar beet/fodder beet

Group		Fish acute	Inverteb. acute	Algae
Test species		QSAR	<i>D. magna</i>	QSAR
Endpoint		LC ₅₀	EC ₅₀	E _r C ₅₀
(µg/L)		568000	>10000	275000
AF		100	100	10
RAC (µg/L)		5680	100	27500
FOCUS Scenario	PEC _{gl-max} (µg/L)	ETR = PEC/RAC; ETR < 1 is acceptable risk		
Step 1				
	0.69	<0.01	<0.01	<0.01
Step 2 - 1X or 2X (worst case) sugar beet/fodder beet @ 75 g a.s./ha				
N-Europe	0.03	<0.01	<0.01	<0.01
S-Europe	0.06	<0.01	<0.01	<0.01

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 sugar beet/fodder beet, 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.

Table 9.5-26: 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-3307 (S7K-3-3) in sugar beet/fodder beet

Group		Fish acute	Inverteb. acute	Algae		Sediment dwellers
Test species		<i>O. mykiss</i>	<i>D. magna</i>	Parent		<i>C. riparius</i>
Endpoint		LC ₅₀	EC ₅₀	ErC ₅₀		EC ₁₀
(µg/L)		>10000	>10000	522		580 µg/kg dws
AF		100	100	10		10
RAC (µg/L)		100	100	52.2		58
FOCUS Scenario	PEC ^{gl-max} (µg/L)	ETR = PEC/RAC; ETR < 1 is acceptable risk			PEC ^{sed-max} (µg/L)	
Step 1						
	3.59	0.04	0.04	0.07	2.40	0.04
Step 2 - 1X or 2X (worst case) sugar beet/fodder beet @ 75 g a.s./ha						
N-Europe	0.07	<0.01	<0.01	<0.01	0.04	<0.01
S-Europe	0.12	<0.01	<0.01	<0.01	0.07	<0.01

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 sugar beet/fodder beet, 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 9.5-27: 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-3307 (S7K-3-3) in sugar beet/fodder beet

3507 (S/R-5-3) in sugar beet/fodder beet						
Group		Fish acute	Inverteb. acute	Algae		Sediment dweller
Test species		QSAR	<i>D. magna</i>	QSAR		<i>C. riparius</i>
Endpoint		LC ₅₀	EC ₅₀	E _r C ₅₀		NOEC
(µg/L)		12700000	> 8600	1100000		2200
AF		100	100	10		10
RAC (µg/L)		127000	86	110000		220
FOCUS Scenario	PEC ^{gl-max} (µg/L)	ETR = PEC/RAC; ETR < 1 is acceptable risk			PEC ^{gl-max} (µg/kg)	
Step 1						
	13.65	<0.01	0.16	<0.01	0.13	<0.01
Step 2 - 1X or 2X (worst case) sugar beet/fodder beet @ 75 g a.s./ha						
N-Europe	0.24	<0.01	<0.01	<0.01	<0.01	<0.01
S-Europe	0.42	<0.01	<0.01	<0.01	<0.01	<0.01

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 sugar beet/fodder beet, 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.

Table 9.5-28: 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-3307 (S7K-3-3) in sugar beet/fodder beet

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>P. subcapitata</i>
Endpoint		LC ₅₀	EC ₅₀	ErC ₅₀ /EyC ₅₀
(µg/L)		7100	11	>10000
AF		100	100	10
RAC (µg/L)		71	0.11	1000
FOCUS Scenario	PEC _{gl-max} (µg/L)	ETR = PEC/RAC; ETR < 1 is acceptable risk		
Step 1				
	1.61	0.02	15	<0.01
Step 2 - 1X or 2X (worst case) sugar beet/fodder beet @ 75 g a.s./ha				
N-Europe	0.02	<0.01	0.18	<0.01
S-Europe	0.04	<0.01	0.36	<0.01

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 sugar beet/fodder beet, 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 9.5-29: 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-3307 (S7K-3-3) in sugar beet/fodder beet

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>O. mykiss</i>	<i>D. magna</i>	parent
Endpoint		LC ₅₀	EC ₅₀	ErC ₅₀
(µg/L)		> 10000	1100	522
AF		100	100	10
RAC (µg/L)		100	11	52.2
FOCUS Scenario	PEC _{gl-max} (µg/L)	ETR = PEC/RAC; ETR < 1 is acceptable risk		
Step 1				
	3.26	0.03	0.30	0.06
Step 2 - 1X or 2X (worst case) sugar beet/fodder beet @ 75 g a.s./ha				
N-Europe	0.12	<0.01	0.01	<0.01
S-Europe	0.16	<0.01	0.01	<0.01

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 sugar beet/fodder beet, 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 LC₅₀, daphnid 48 hr EC₅₀, and green algae 96 hr EC₅₀ values are 81.990, 48.857, and 44.437 mg/l, respectively.

Table 9.5-30: 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-3307 (S7K-3-3) in sugar beet/fodder beet

Group		Fish acute	Inverteb. acute	Algae
Test species		QSAR	QSAR	QSAR
Endpoint		LC ₅₀	EC ₅₀	E _r C ₅₀
(µg/L)		81990	48857	44437
AF		100	100	10
RAC (µg/L)		819.9	488.57	4443.7
FOCUS Scenario	PEC _{gl-max} (µg/L)	ETR = PEC/RAC; ETR < 1 is acceptable risk		
Step 1				
	8.77	0.01	0.02	<0.01
Step 2 - 1X or 2X (worst case) sugar beet/fodder beet @ 75 g a.s./ha				
N-Europe	0.32	<0.01	<0.01	<0.01
S-Europe	0.44	<0.01	<0.01	<0.01

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 sugar beet/fodder beet, 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.

It should be pointed out that in aquatic system metabolism studies no decline of two sediment metabolites (X12313581 and X696476) was observed indicating their high persistence and for this reason the risk assessment performed with consideration of the maximum annual PEC_{SED} will not cover situation after multiple years of use of GF-3307. Therefore, the annual PEC_{SED} multiplied by 20 (to account for application over 20 years) was used by zRMS for calculation of ETR for these two compounds.

Overall, acceptable risk from fenpicoxamid metabolites could be concluded following the application of GF-3307 according to the Central Zone GAP with no need for risk mitigation measures.

Risk assessment for prothioconazole at 1 to 2 x 150 g a.s./ha

In the following table, the ratios between PEC_{SW} and/or PEC_{SED} and RAC for aquatic organisms are given per intended use for each FOCUS scenario and each organism group.

The GAP modelled in sugar beet/fodder beet at FOCUS Step 1 and 2 was aligned to the current GAP of 1 - 2 x 150 g a.s./ha. ETRs below 1 indicate acceptable risk.

Table 9.5-31: Aquatic organisms: acceptability of risk (PEC < RAC) for prothioconazole for each organism group based on FOCUS Step 1 and 2 calculations for the use of GF-3307 (S7K-3-3) in sugar beet/fodder beet

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged		Algae	Higher plant
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>C. riparius</i>	<i>P. subcapitata</i>	<i>Lemna gibba</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	NOEC	NOEC	E _r C ₅₀	E _r C ₅₀
(µg/L)		1830	308	1300	560	9140	2180	177.6
AF		100	10	100	10	10	10	10
RAC (µg/L)		18.3	30.8	13	56	914	218	17.76
FOCUS Scenario	PEC _{gl-max} (µg/L)	ETR = PEC/RAC; ETR < 1 is acceptable risk						
Step 1 – 1X or 2X (worst case) 150 g as/ha								
	32.5801	1.78	1.06	3	0.58	0.04	0.15	1.83
Step 2 – 1X or 2X (worst case) sugar beet/fodder beet @ 150 g a.s./ha								
N-Europe	1.3795	0.08	0.04	0.11	0.02	<0.01	0.01	0.08
S-Europe	1.3795	0.08	0.04	0.11	0.02	<0.01	0.01	0.08

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 use of prothioconazole in sugar beet/fodder beet, calculated ETRs did indicate an acceptable risk for all groups of aquatic organisms in FOCUS Step 2. Therefore, no further assessment is necessary.

Risk assessment for JAU 6476-desthio at 1 to 2 x 150 g prothioconazole/ha

In the following tables, the ratios between PEC_{SW} and/or PEC_{SED} and RAC for aquatic organisms are given per intended use for each FOCUS scenario and each organism group.

The GAP modelled in sugar beet/fodder beet at FOCUS Step 1 and 2 was aligned to the current GAP of 1-2 x 150 g a.s./ha from BBCH 30. ETRs below 1 indicate acceptable risk.

Table 9.5-32: Aquatic organisms: acceptability of risk (PEC < RAC) for metabolite JAU 6476-desthio for each organism group based on FOCUS Step 1, 2 and 3 calculations for the use of GF-3307 (S7K-3-3) in sugar beet/fodder beet

calculations for the use of G1 5507 (51X 5 5) in sugar beet/fodder beet										
Group		Fish acute	Fish pro- longed	Inverteb. acute	Inverteb. prolonged		Algae	Higher plant		Sed. dwell. pro- longed
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>C. riparius</i>	<i>P. subcapitata</i>	<i>L. gibba</i>		<i>C. riparius</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	NOEC	NOEC	ErC ₅₀	ErC ₅₀		NOEC
(µg/L)		6630	3.34	10000	100	2000	550	80.9		50000
AF		100	10	100	10	10	10	10		10
RAC (µg/L)		66.3	0.334	100	10	200	55	8.09		5000
FOCUS Sce- nario	PEC ^{gl-max} (µg/L)	ETR = PEC/RAC; ETR < 1 is acceptable risk							PEC ^{gl-max} (µg/kg)	
Step 1 – 1X or 2X (worst case) 150 g as/ha										
	58.5727	0.88	175	0.59	6	0.29	1.06	7	333.3964	0.07
Step 2 – 1X or 2X (worst case) sugar beet/fodder beet @ 150 g a.s./ha										
N-Europe	2.745	0.04	8	0.03	0.27	0.01	0.05	0.34	15.4097	<0.01
S-Europe	5.0409	0.08	15	0.05	0.50	0.03	0.09	0.62	28.6114	0.01
Step 3 - 1X or 2X (worst case) sugar beet/fodder beet @ 150 g a.s./ha										
D3 ditch	0.0798	<0.01	0.24	<0.01	0.01	<0.01	<0.01	0.01	0.1065	<0.01
D4 pond	0.026	<0.01	0.08	<0.01	<0.01	<0.01	<0.01	<0.01	0.3527	<0.01
D4 stream	0.0424	<0.01	0.13	<0.01	<0.01	<0.01	<0.01	0.01	0.0224	<0.01
R1 pond	0.0529	<0.01	0.16	<0.01	0.01	<0.01	<0.01	0.01	0.7994	<0.01
R1 stream	0.2225	<0.01	0.67	<0.01	0.02	<0.01	<0.01	0.03	0.375	<0.01

R3 stream	0.3583	0.01	1.07	<0.01	0.04	<0.01	0.01	0.04	1.472	<0.01
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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.

For the intended uses in sugar beet/fodder beet, calculated PEC/RAC ratios for JAU 6476-desthio did indicate an acceptable risk for acute fish, acute and chronic invertebrates, algae, higher plants, and sediment dwellers in FOCUS Step 2. For chronic fish, acceptable risk was not shown at FOCUS Step 3. Therefore, Step 4 assessment has been conducted for chronic fish.

Table 9.5-33: Aquatic organisms: PEC calculation and acceptability of risk (PEC < RAC) for metabolite JAU 6476-desthio based on FOCUS Step 4 calculations and toxicity data for chronic fish with mitigation options for the use of GF-3307 (S7K-3-3) in sugar beet/fodder beet

Intended use	sugar beet/fodder beet			
Active substance	Metabolite JAU 6476-desthio			
Application rate (g/ha)	1-2 x 150			
No-spray buffer	30 m	10 m	20 m	10 m
Vegetated filter strip	10 m	10 m	10 m-	10 m-
Nozzle reduction	-	-	75%	75%
Step 4 - @ 1X or 2X (worst case) sugar beet/fodder beet @ 150 g a.s./ha				
D3 ditch	0.0049	0.0138	0.0018	0.0035
D4 pond	0.0095	0.0162	0.0067	0.0074
D4 stream	0.0357	0.0357	0.0357	0.0357
R1 pond	0.0211	0.0245	0.0189	0.0195
R1 stream	0.101	0.101	0.1010	0.101
R3 stream	0.1633	0.1633	0.1633	0.1633
RAC (µg/L), chronic fish	ETR = PEC/RAC, ETR < 1 is acceptable risk			
0.334				
No-spray buffer	30 m	10 m	20 m	10 m
Vegetated filter strip	10 m	10 m	10 m-	10 m-
Nozzle reduction	-	-	75%	75%
Step 4 - @ 1X or 2X (worst case) sugar beet/fodder beet @ 150 g a.s./ha				
D3 ditch	0.01	0.04	0.01	0.01
D4 pond	0.03	0.05	0.02	0.02
D4 stream	0.11	0.11	0.11	0.11
R1 pond	0.06	0.07	0.06	0.06
R1 stream	0.30	0.30	0.30	0.30
R3 stream	0.49	0.49	0.49	0.49

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 all taxa, acceptable risk for for metabolite JAU 6476-desthio is demonstrated for sugar beet/fodder beet at 1 to 2 x 150 g a.s./ha using a:

- 30 m NSZ + 10 m VFS
- 10 m NSZ + 10 m VFS
- **20 m NSZ + 10 m VFS + 75% DRN**
- 10 m NSZ + 10 m VFS + 75% DRN

Risk assessment for prothioconazole relevant metabolites – Other metabolites at 1 to 2 x 150 g a.s./ha

Due to their low hazard and low exposure, the following metabolites were only required to be analysed at FOCUS Steps 1 and 2.

Table 9.5-34: Aquatic organisms: acceptability of risk (PEC < RAC) for metabolite JAU 6476-S-methyl for each organism group based on FOCUS Step 1 and 2 calculations for the use of GF-3307 (S7K-3-3) in sugar beet/fodder beet

Group		Fish acute	Inverteb. acute	Sed-dwellers – pro-longed	Algae
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>C. riparius</i>	<i>P. subcapitata</i>
Endpoint		LC ₅₀	EC ₅₀	EC₁₀	E _r C ₅₀
(µg/L)		1800	2800	71	47400
AF		100	100	10	10
RAC (µg/L)		18	28	7.1	4740
FOCUS Scenario	PEC _{gl-max} (µg/L)	ETR = PEC/RAC; ETR < 1 is acceptable risk			
Step 1					
	6.8092	0.38	0.24	0.96	<0.01
Step 2 @ 2 x 150 g a.s./ha, sugar beet/fodder beet					
N-Europe	0.311	0.02	0.01	0.04	<0.01
S-Europe	0.5659	0.03	0.02	0.08	<0.01

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 use of JAU 6476-S-methyl in sugar beet/fodder beet, calculated ETRs did indicate an acceptable risk for all groups of aquatic organisms at FOCUS Step 1 and 2. Therefore, no further assessment is necessary.

Table 9.5-35: Aquatic organisms: acceptability of risk (PEC < RAC) for metabolite 1, 2, 4-triazole for each organism group based on FOCUS Step 1 and 2 calculations for the use of GF-3307 (S7K-3-3) in sugar beet/fodder beet

Group		Fish acute	Fish chronic	Inverteb. acute	Algae
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>P. subcapitata</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	E _c C ₅₀
(µg/L)		498000	3200	900000	22500
AF		100	10	100	10
RAC (µg/L)		4980	320	9000	2250
FOCUS Scenario	PEC _{gl-max} (µg/L)	ETR = PEC/RAC; ETR < 1 is acceptable risk			
Step 1					
	7.7309	<0.01	0.02	<0.01	<0.01
Step 2 @ 2 x 150 g a.s./ha, sugar beet/fodder beet					
N-Europe	0.2100	<0.01	<0.01	<0.01	<0.01
S-Europe	0.2323	<0.01	<0.01	<0.01	<0.01

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 use of 1,2,4-triazole in sugar beet/fodder beet, calculated ETRs did indicate an acceptable risk for all groups of aquatic organisms at FOCUS Step 1 and 2. Therefore, no further assessment is necessary.

zRMS comments:

The aquatic risk assessment presented above has been amended accordingly with consideration of the surface water exposure agreed in the course of evaluation in area of Section 8.

Based on the performed calculations following conclusions may be derived:

1.Sugar beet/fodder beet:

- Prothioconazole: acceptable risk with no need for risk mitigation measures
- JU 6476-desthio:
 - Scenarios: D3, D4, R1 and R3: risk acceptable with
 - 30 m NSZ + 10 m VFS
 - 10 m NSZ + 10 m VFS
 - 10 m NSZ + 10 m VFS + 75% DRN
 - 20 m NSZ + 10 m VFS + 75% DRN**

Risk assessment for GF-3307 at 1.5 L/ha

Acute aquatic organism mixture risk assessment

As mentioned previously in Section 9.5.1, fenpicoxamid drives the acute risk assessment for fish and invertebrates as it accounts for greater than ≥99.8% of the toxicity, therefore the aquatic risk assessment for fish and invertebrates exposed to GF-3307 (S7K-3-3) should be based on that of fenpicoxamid (see Tables 9.5-14 and 15). Acceptable risk is demonstrated at the proposed GAP (1 to 2 x 75 g fenpicoxamid/ha) with appropriate mitigations.

However, as previously demonstrated, the TU approach does not hold for algae, so a mixtures risk assessment is provided below using the measured GF-3307 (S7K-3-3) algal endpoint.

In order to directly compare the PEC_{mix} (EFSA Aquatic GD, 2013, Eq.16)— derived by adding the FOCUS Step 2 maximum PEC_{sw} for each active substance—the EC_{50-PPP} was adjusted using the Finney equation to only account for concentrations of the actives ($ErC_{50-PPP} = 8000 \mu\text{g prep/L} \times 14.2\% \text{ a.s.} = 1136 \mu\text{g/L}$). Then, the exposure toxicity ratio (ETR) was calculated (Eq. 17) and if the ETR is < 0.1 for algae (1/trigger of 10), the mixture risk is acceptable.

$$PEC_{mix} = \sum_{i=1}^n PEC_i$$

Where: n = number of components

i = individual components (per default: a.s.)

$$ETR_{ppp} = \frac{PEC_{mix}}{ECx_{ppp}}$$

Table 9.5-36: Mixtures risk assessment for algae at FOCUS Steps 1-3 exposed to GF-3307 (S7K-3-3) in sugar beet_measured ErC_{50}

Group				Algae
Test species				<i>P. subcapitata</i>
Endpoint				ErC_{50}
($\mu\text{g/L}$)				1136
FOCUS Scenario	Fenpicoxamid PEC_{gl-max} ($\mu\text{g/L}$)	Prothioconazole PEC_{gl-max} ($\mu\text{g/L}$)	Fenpicoxamid + Prothioconazole PEC_{mix} ($\mu\text{g/L}$)	ETR = PEC/EC_{50-PPP} ETRs <0.1 are acceptable
Step 1 @ 1-2 x 75 g fenpicoxamid + 150 g prothioconazole/ha				
	7.1257	32.5801	39.7058	0.035
Step 2 @ 1-2 x 75 g fenpicoxamid + 150 g prothioconazole/ha				
N-Europe	0.6898	1.3795	2.0693	0.002
S-Europe	0.6898	1.3795	2.0693	0.002
Step 3 - 1-2 x sugar beet @ 75 g fenpicoxamid + 150 g prothioconazole/ha				
D3 ditch	0.3863	0.7849	1.1712	0.001
D4 pond	0.016	0.0317	0.0477	0.000
D4 stream	0.3211	0.6529	0.974	0.001
R1 pond	0.0156	0.0317	0.0473	0.000
R1 stream	0.2654	0.54	0.8054	0.001
R3 stream	0.3775	0.7672	1.1447	0.001

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; $PEC/EC_{50-PPP} = ETR$, ratios above the relevant trigger of 0.1 are shown in **bold** and indicate unacceptable risk.

The ETR values for algae are < 0.1 for all FOCUS Step 1-3 scenarios indicating acceptable risk from GF-3307 (S7K-3-3) without the need for mitigation.

Chronic aquatic organism mixture risk assessment

As mentioned previously in Section 9.5.1, fenpicoxamid drives the chronic risk assessment for fish and invertebrates as it accounts for greater than ≥99.4% of the toxicity, therefore the aquatic risk assessment for fish and invertebrates exposed to GF-3307 (S7K-3-3) should be based on that of fenpicoxamid (see Tables 9.5-14 and 15). Acceptable risk is demonstrated at the proposed GAP (1-2 x 75 g fenpicoxamid/ha) with appropriate mitigations.

However, as previously noted, the chronic fish endpoint for JAU 6476-desthio is two orders of magnitude lower than that of prothioconazole (3.34 versus 308 µg/L, respectively). And while the TU approach held for chronic fish using fenpicoxamid + prothioconazole, when JAU 6476-desthio is considered there is no apparent driver. Therefore it was necessary to derive a predicted chronic fish NOEC for the risk assessment.

While a predicted NOEC can be derived based on the formulation ratios ‘as is’ (0.34 FNP and 0.66 JAU 6476-desthio, the latter being a substitute for the parent), given the high toxicity of fenpicoxamid and JAU 6476-prothio to fish, it was apparent that refinements and mitigations would be needed to achieve a safe use. Therefore a predicted GF-3307 (S7K-3-3) fish NOEC is derived for each FOCUS Step 1-3 based on the proportion of fenpicoxamid and JAU 6476-desthio resulting from the FOCUS PEC_{sw} values and their respective chronic fish NOECs (0.91 µg fenpicoxamid/L and 3.34 µg JAU 6476-desthio/L). Then, the ETR was calculated as shown below and if the ETR is < 0.1 for chronic fish (1/trigger of 10), the mixture risk is acceptable.

$$ETR_{ppp} = \frac{PEC_{mix}}{NOEC_{ppp}}$$

Table 9.5-37: Mixtures risk assessment for prolonged fish at FOCUS Steps 1-3 exposed to GF-3307 (S7K-3-3) in sugar beet/fodder beet_predicted NOEC

FOCUS Scenario	FNP PEC _{gl-max} (µg/L)	PTZ-desthio PEC _{gl-max} (µg/L)	FNP + PTZ-desthio PEC _{mix} (µg/L)	Ratio of FNP in PEC _{mix}	Ratio of PTZ- desthio in PEC _{mix}	FNP + PTZ- desthio ra- tios	Prolonged Fish NOE- C _{PPP} based on FO- CUS PEC ratio	ETR _{mix}
Step 1 @ 1 - 2 (worst case) x 75 g fenpicoxamid + 150 g prothioconazole/ha								
	7.1257	58.5727	65.6984	0.11	0.89	1	2.59	25.37
Step 2 @ 1 – 2 (worst case) x 75 g fenpicoxamid + 150 g prothioconazole/ha								
N-Europe	0.6898	2.745	3.4348	0.20	0.80	1	2.17	1.58
S-Europe	0.6898	5.0409	5.7307	0.12	0.88	1	2.53	2.27
Step 3 – 1 – 2 x (worst case) sugar beet @ 75 g fenpicoxamid + 150 g prothioconazole/ha								
D3 ditch	0.3863	0.0798	0.4661	0.83	0.17	1	1.04	0.45
D4 pond	0.016	0.026	0.042	0.38	0.62	1	1.66	0.03
D4 stream	0.3211	0.0424	0.3635	0.88	0.12	1	0.99	0.37
R1 pond	0.0156	0.0529	0.0685	0.23	0.77	1	2.08	0.03
R1 stream	0.2654	0.2225	0.4879	0.54	0.46	1	1.36	0.36
R3 stream	0.3775	0.3583	0.7358	0.51	0.49	1	1.41	0.52

FNP: fenpicoxamid; PTZ-desthio: JAU 6476-desthio, or prothioconazole-desthio; PEC: Predicted environmental concentration; PEC/NOEC_{PPP} = ETR, ratios above the relevant trigger of 0.1 are shown in **bold** and indicate unacceptable risk.

Table 9.5-38: Mixtures risk assessment for prolonged fish at FOCUS Step 4 exposed to GF-3307 (S7K-3-3) in sugar beet/fodder beet predicted NOEC

[illegible]

D3 ditch	0.0234	0.0049	0.0234	0.83	0.17	1	1.04	0.03
D4 pond	0.005	0.0095	0.005	0.34	0.66	1	1.74	0.01
D4 stream	0.025	0.0357	0.025	0.41	0.59	1	1.59	0.04
R1 pond	0.005	0.0211	0.005	0.19	0.81	1	2.21	0.01
R1 stream	0.0206	0.101	0.0206	0.17	0.83	1	2.30	0.05
R3 stream	0.0294	0.1633	0.0294	0.15	0.85	1	2.37	0.08

FNP: fenpicoxamid; PTZ-desthio: JAU 6476-desthio, or prothioconazole-desthio; PEC: Predicted environmental concentration; PEC/NOEC_{PPP} = ETR, ratios above the relevant trigger of 0.1 are shown in **bold** and indicate unacceptable risk

zRMS comments:

The mixture toxicity assessment has been validated by zRMS.

As mentioned previously in Section 9.5.1, fenpicoxamid drives the acute risk assessment for fish and invertebrates as it accounts for greater than $\geq 99.8\%$ of the toxicity, therefore the aquatic risk assessment for fish and invertebrates exposed to GF-3307 (S7K-3-3) is based on that of fenpicoxamid (see Tables 9.5-14 and 15). Acceptable risk is demonstrated at the proposed GAP (1 to 2 x 75 g fenpicoxamid/ha) with appropriate mitigations.

However, as previously demonstrated, the TU approach does not hold for algae, so a mixtures risk assessment is provided the measured GF-3307 (S7K-3-3) algal endpoint.

As mentioned previously in Section 9.5.1, fenpicoxamid drives the chronic risk assessment for fish and invertebrates as it accounts for greater than $\geq 99.4\%$ of the toxicity, therefore the aquatic risk assessment for fish and invertebrates exposed to GF-3307 (S7K-3-3) should be based on that of fenpicoxamid (see Tables 9.5-14 and 15). Acceptable risk is demonstrated at the proposed GAP (1-2 x 75 g fenpicoxamid/ha) with appropriate mitigations.

In the same time it should be taken into account that the chronic fish endpoint for JAU 6476-desthio is two orders of magnitude lower than that of prothioconazole (3.34 versus 308 $\mu\text{g/L}$, respectively). And while the TU approach held for chronic fish using fenpicoxamid + prothioconazole, when JAU 6476-desthio is considered there is no apparent driver. Therefore it was necessary to derive a predicted **chronic fish NOEC for the risk assessment**.

The ETR values for the GF-3307 mixtures assessment for chronic fish using the predicted NOEC values are below the trigger of 0.1 indicating acceptable risk at 1 x 1.5 L GF-3307/ha when:

- 10 m NSZ + 10 m VFS + 75% DRN or
- **20 m NSZ + 10 m VFS + 75% DRN** or
- 30 m NSZ + 10 m VFS are applied to surface water bodies

Additionally, to the risk assessment following the toxic driver approach based on the content of the active substances in the formulated product, during commenting period process a risk assessment based on the ratio of the substances during the actual expected exposure in surface water has been conducted using the AGD_Aqua-mix_v122.xlsm Excel Aquatic MixTox tool, calculations for the following mixture assessments have been submitted

- 75g fenpicoxamid +150g prothioconazole _acute risk assessment
- 75g fenpicoxamid +150g prothioconazole _chronic risk assessment
- 75g fenpicoxamid +150g prothioconazole - desthio _chronic risk assessment

The result tables have been copied into Appendix 3 of the Core dossier.

In step 5 of the calculations, fenpicoxamid has been confirmed as toxic driver for

- Acute risk to fish and aquatic invertebrates from fenpicoxamid + prothioconazole
- Chronic risk to fish and aquatic invertebrates from fenpicoxamid + prothioconazole
- Chronic risk to aquatic invertebrates from fenpicoxamid + prothioconazole-desthio

There is no clear driver for

- Chronic risk to fish from fenpicoxamid + prothioconazole-desthio
- Risk to algae

That means the outcome for the toxic driver assessment was identical when the ratio of the substances in the formulated product versus the ratio during the actual expected exposure in surface water was used.

Nevertheless, a complete mixture assessment for all scenarios has been calculated in step 8b and presented in Appendix 3 of the core dossier. It shows acceptable risk for all scenarios with the following risk mitigation.

- 30 m NSZ + 10 m NSZ or
- 10 m NSZ + 10 m VFS + 75% DRN .or
- 20 m NSZ + 10 m VFS + 75% DRN or

The ETR values for the GF-3307 (S7K-3-3) mixtures assessment for chronic fish using the predicted NOEC values are below the trigger of 0.1 indicating acceptable risk at 1 x 1.5 L GF-3307/ha with a:

- 10 m NSZ + 10 m VFS + 75% DRN.
- 20 m NSZ + 10 m VFS + 75% DRN
- 30 m NSZ + 10 m VFS

Aquatic Mixtures Assessment using the AGD MixTox Tool

Aquatic mixture toxicity has been assessed with the AGD_Aquamix_v122.xlsm Excel Aquatic MixTox tool, which can be found at <https://zenodo.org/records/7788826>. The input parameters and the output are presented in Annex 3 for:

- acute effects from fenpicoxamid + prothioconazole
- chronic effects from fenpicoxamid + prothioconazole
- chronic effects from fenpicoxamid + JAU 6476-desthio (since the metabolite JAU 6476-desthio is more toxic to fish (chronic) than the parent Prothioconazole)

The output from the mixture risk assessment using the AGD MixTox Tool indicates acceptable acute and chronic risk with

- 30 m NSZ + 10 m NSZ and
- 20 m NSZ + 10 m VFS + 75% DRN and
- 10 m NSZ + 10 m VFS + 75% DRN.

zRMS comments :

The mixture toxicity assessment has been validated by zRMS.

Based on acute and chronic mixture risk assessment using the AGD MixTox Tool indicates acceptable acute and chronic risk when :

-10 m• 20 m NSZ + 10 m VFS + 75% DRN or
-20 m NSZ + 10 m VFS + 75% DRN or
-30 m NSZ + 10 m VFS are applied to surface water bodies

9.5.3 Overall conclusions

Acceptable risk is demonstrated for fenpicoxamid, prothioconazole, relevant metabolites, and GF-3307 (S7K-3-3) in sugar beet/fodder beet at 1 to 2 applications of 1.5 L GF-3307/ha (1 to 2 x 75 g fenpicoxamid/ha + 150 g prothioconazole/ha) with a:

- 30 m NSZ + 10 m NSZ and
- 20 m NSZ + 10 m VFS + 75% DRN and
- 10 m NSZ + 10 m VFS + 75% DRN.

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, prothioconazole, and relevant metabolites. Full details of these studies are provided in the respective EU DAR (fenpicoxamid: United Kingdom, 2017; prothioconazole: United Kingdom, 2007) and related documents as well as in Appendix 2 of this document (new studies).

Effects on bees of GF-3307 (S7K-3-3) 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 ₅₀ >303.0 µg/bee*	EFSA, 2018
<i>Bombus terrestris</i>	Fenpicoxamid	Oral	LD ₅₀ >272 µg/bee*	Cornement/2022/Study No. 201076
<i>Apis mellifera</i>	Fenpicoxamid	Contact	LD ₅₀ >202.4 µg/bee*	EFSA, 2018
<i>Bombus terrestris</i>	Fenpicoxamid	Contact	LD ₅₀ >300 µg/bee*	Cornement/2022/Study No. 201076
Fenpicoxamid metabolites				
<i>Apis mellifera</i>	X642188	Oral	LD ₅₀ >101.9 µg/bee*	EFSA, 2018
<i>Apis mellifera</i>	X696476	Oral	LD ₅₀ >14.2 µg/bee*	EFSA, 2018
<i>Apis mellifera</i>	X12019520	Oral	LD ₅₀ = 132.6 µg/bee	EFSA, 2018

*highest dose tested

zRMS comments:

Endpoints presented in Table 9.6-1 for bees are in line with the EU agreed endpoints reported in EFSA Journal 2018;16(1):5146.

In addition to bee studies, also acute studies on effects of fenpicoxamid to bumblebees were performed.

The studies were not evaluated by the zRMS and their endpoints will be not used in the risk assessment since currently there is not data requirement in this area at the product authorisation. The study has been struck through in tables above.

Table 9.6-2: Endpoints and effect values relevant for the risk assessment for bees – prothioconazole and JAU 6476-desthio

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i>	Prothioconazole	Oral, 48 hr	LD ₅₀ >71 µg/bee	EFSA, 2007
<i>Apis mellifera</i>	Prothioconazole	Contact, 48 hr	LD ₅₀ >200 µg/bee	EFSA, 2007
<i>Bombus terrestris</i>	Prothioconazole	Oral	LD ₅₀ >214.32 µg/bee	RAR LoEP Dec 2020
<i>Bombus terrestris</i>	Prothioconazole	Contact	LD ₅₀ >100 µg/bee	RAR LoEP Dec 2020
<i>Apis mellifera</i>	JAU 6476-desthio	Oral	LD ₅₀ >106.5 µg/bee	Sekine/2015/Report# 100071035
<i>Apis mellifera</i>	JAU 6476-desthio	Contact	LD ₅₀ >100 µg/bee	Sekine/2015/Report# 100071035

When more than one endpoint exists for a species, the value in bold is used in the risk assessment.

zRMS comments:

Endpoints presented in Table 9.6-2 are in line with the EU agreed endpoints reported in EFSA Scientific Report (2007) 106, 1-98.

For prothioconazole additional endpoints derived for acute toxicity studies for prothioconazole metabolite JAU 6476-desthio are reported in Table 9.6-2. Although the Applicant have not provided respective summaries of the studies, they were already evaluated and agreed during the ongoing EU renewal process of prothioconazole. Reported endpoints may be confirmed based on information available in the updated DRAR (2020), which is available on the EFSA DMS. Although the renewal process is not finalised yet, these endpoints were already peer-reviewed

and will not change at next stages of the renewal. Please note that access to these studies was granted to Polish authorities by owner of prothioconazole via LoA and they cannot be disclosed the Applicant for GF-3307. Taking this into account no study summaries may be presented in this Core Assessment.

Table 9.6-3: Endpoints and effect values relevant for the risk assessment for bees - GF-3307 (S7K-3-3)

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i>	GF-3307	Oral	LD ₅₀ = 212.5 µg/bee	Schmitzer/2014/DAS# 140220 & 140213
<i>Apis mellifera</i>	GF-3307	Oral	LD₅₀ = 55.46 µg/bee	Noel/2015/DAS# 150736
<i>Bombus terrestris</i>	GF-3307	Oral	LD ₅₀ >453 µg/bee*	Cornement/2022/Study No. 201075
<i>Apis mellifera</i>	GF-3307	Contact	LD₅₀ = 92.3 µg/bee	Schmitzer/2014/DAS# 140220 & 140213
<i>Apis mellifera</i>	GF-3307	Contact	LD ₅₀ = 199.9 µg/bee	Noel/2015/DAS# 150737
<i>Bombus terrestris</i>	GF-3307	Contact	LD ₅₀ >500 µg/bee*	Cornement/2022/Study No. 201075
<i>Apis mellifera</i>	GF-3307	Chronic, adult, 10 d	LDD ₅₀ >0.418 µg prep/bee/day NOEDD >0.418 µg prep/bee/day NOEC >15 mg prep/kg-diet*	Verge/2018/DAS# 170077
<i>Apis mellifera</i>	GF-3307	22 d, larval (OECD 239)	NOED = 6.62 µg prep/larva per developmental period NOEC = 43 mg prep/kg-diet	Oberrauch/2018/DAS# 171043
Higher-tier studies (tunnel test, field studies)				
<i>Apis mellifera</i>	GF-3307	Semi-field, OECD 75/EPPO 170 in flowering Phacelia tanacetifolia T1 = 1 L prep/ha, 50 g XDE-777/ha + 100 g prothioconazole/ha T2 = 2 L prep/ha, 100 g XDE-777/ha + 200 g prothioconazole/ha	NOER _{adult mortality, larval/pupae mortality} = 2 L GF-3307/ha Had no effect on foraging activity. Had an effect on worker behavior. NOER _{colony size, brood cells} = 2 L/ha NOAER _{nectar cells, pollen storage} = 2 L/ha NOER _{brood index, compensation index, termination rate of 1st brood} = 2 L/ha NOER _{brood index, compensation index, termination rate of 2nd brood} < 1 L/ha	Kleinhenz/2018/DAS# 170673
<i>Apis mellifera</i>	GF-3307	Colony feeding test in the field, OECD 75/EPPO Bulletin No. 22 (Oomen et. al, 1992) T1, T2, T3, T4, T5 = 4, 20, 50, 100, 500 mg GF-3307/kg sugar solution	Up to the highest rate tested (500 mg GF-3307/kg sugar solution): No effects on • mortality of worker bees, pupae and larvae • behavior of worker bees • development of eggs, young larvae, old larvae • development of individual brood cells • colony size • mean weight of pupae during 2 brood cycles No effects on • Total amount of brood or certain	Gonsior/2021/Corteva #200660

Species	Substance	Exposure System	Results	Reference
			brood stages • Mean number of food cells Residues in pollen, nectar, honey, larvae, pupae, worker jelly, and feeding solution were determined	
<i>Apis mellifera</i> / oilseed rape	GF-3307	Residues in nectar, pollen and plants of oilseed rape after application of 2 L/ha GF-3307 (Guideline 7029/VL/95 (rev. 5))	<p><u>Max. residues in plants:</u> Fenpicoxamid 1.75 mg/kg to 2.33 mg/kg Prothioconazole 0.319 mg/kg to 1.17 mg/kg Prothioconazole-desthio 1.57 mg/kg to 2.65 mg/kg</p> <p><u>Max residues in nectar:</u> Fenpicoxamid 0.0435 mg/kg to 0.283 mg/kg Prothioconazole 0.0312 mg/kg to 0.232 mg/kg Prothioconazole-desthio 0.0108 mg/kg to 0.136 mg/kg</p> <p><u>Max residues in pollen:</u> Fenpicoxamid 5.31 mg/kg to 17.3 mg/kg Prothioconazole 9.21 mg/kg to 36.6 mg/kg Prothioconazole-desthio 0.900 mg/kg to 4.98 mg/kg</p>	Appeltauer/2021/Dow AgroSciences #200670

*highest dose tested

zRMS comments:

The laboratory studies on toxicity of GF-3307 to bees were evaluated in course of zonal authorisation of the product GF-3307 in cereals and is relevant for the current evaluation of GF-3307 for extended uses. For details of the evaluation please refer to Appendix 2.

In addition, to bee studies, also acute studies on effects of GF-3307 to bumblebees were performed. The studies were evaluated by the zRMS, but their endpoints will be not used in the risk assessment since currently there is not data requirement in this area. For summaries of the studies and details of evaluation, please refer to Appendix 2.

In support of evaluation of GF-3307 the Applicant submitted a tunnel study performed on flowering *Phacelia tanacetifolia*, which addressed to chronic effects of GF-3307 on adult bees and larvae.

In addition, the colony feeding study for bees with GF-3307 was conducted with free-flying honey bee colonies under field conditions. The bee colonies were fed for 9 consecutive days with the test substance diluted in sugar solution at rates; 4, 20, 50, 100, 500 mg GF-3307/L sugar. The magnitude of residues in pollen, nectar, honey, pupae, larvae and worker jelly under field conditions were determined.

The Applicant submitted the residue study for application of GF 3307 in oilseed rape during flowering (Residue study in oilseed rape by Appeltauer, 2021). This study was not considered in the risk assessment for sugar beet/fodder. No sufficient information are conducted (please see in Appendix 2). The study has been struck through in tables above.

9.6.1.1 Justification for new endpoints

Not applicable.

9.6.2 Risk assessment

9.6.2.1 Hazard quotients for bees

SANCO acute risk assessment for honeybees

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).

The results of the risk assessment for fenpicoxamid, prothioconazole, relevant metabolites, and GF-3307 (S7K-3-3) are summarised in the following tables.

Table 9.6-4: First-tier assessment of the risk for bees due to the use of fenpicoxamid in sugar beet/fodder beet

Intended use	Sugar beet/ fodder beet		
Active substance	Fenpicoxamid		
Application rate (g/ha)	2 x 75, interval 21 days		
Test design	LD ₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	>303.3	75	< 0.25
Contact toxicity	>202.4		< 0.37
Intended use	Sugar beet/ fodder beet		
Metabolite	X642188		
Application rate (g/ha)	2 x 75; molecular wt. conversion = 0.837; = 62.8 g/ha, interval 21 days		
Test design	LD ₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	>101.9 µg/bee	62.8	< 0.62
Intended use	Sugar beet/ fodder beet		
Metabolite	X696476		
Application rate (g/ha)	2 x 75; molecular wt. conversion = 0.275; = 20.6 g/ha, interval 21 days		
Test design	LD ₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	>14.2 µg/bee	20.6	< 1.5
Intended use	Sugar beet/ fodder beet		
Metabolite	X12019520		
Application rate (g/ha)	2x 75; molecular wt. conversion = 0.306; = 23.0 g/ha, interval 21 days		
Test design	LD ₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	132.6 µg/bee	23.0	0.17

Q_{HO}, Q_{Hc}: Hazard quotients for oral and contact exposure. Q_H values shown in **bold** breach the relevant trigger.

Table 9.6-5: First-tier assessment of the risk for bees due to the use of prothioconazole and relevant metabolites in sugar beet/ fodder beet

JAU 6476-desthio			
Intended use	Sugar beet/ fodder beet		
Active substance	Prothioconazole		
Application rate (g/ha)	2 x 150, interval 21 days		
Test design	LD ₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	>71	150	< 2.1
Contact toxicity	>200		< 0.75
Intended use	Sugar beet/ fodder beet		
Metabolite	JAU 6476-desthio		
Application rate (g/ha)	2 x 150; molecular wt. conversion = 0.907; = 2 x 136 g JAU 6476-desthio/ha, interval 21 days		
Test design	LD ₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	>106.5	136	< 1.3
Contact toxicity	>100		< 1.4

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in **bold** breach the relevant trigger.

Table 9.6-6: First-tier assessment of the risk for bees due to the use of GF-3307 (S7K-3-3) in sugar beet/fodder beet

Intended use	Sugar beet/ fodder beet		
Product	GF-3307		
Application rate (g/ha)	2 x 1566 (= 2 x 1.5 L/ha) , interval 21 days		
Test design	LD₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q_{HO}, Q_{HC} criterion: Q_H ≤ 50
Oral toxicity	55.46	1566	28
Contact toxicity	92.3		17

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in **bold** breach the relevant trigger.

The Q_{HC} and Q_{HO} values for fenpicoxamid, prothioconazole, relevant metabolites, and GF-3307 (S7K-3-3) in honeybee are below the Annex VI trigger of 50; therefore, the acute oral and contact risk to honey bees is acceptable.

zRMS comments:

Acute risk assessment to bees:

The risk assessment performed in line with SANCO/10329/2002 rev 2 final presented in Tables from 9.6-3 to 9.6-6 is agreed by the zRMS.

On the basis of calculated HQ values acceptable acute risk to bees may be concluded from all intended Central Zone uses of GF-3307.

EFSA acute risk assessment for honeybees

The oral and contact hazard quotients for fenpicoxamid, prothioconazole, relevant metabolites, and GF-3307 (S7K-3-3) provided above show acceptable acute risk to bees. Guidance has been provided by EFSA [Guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees), European Food Safety Authority (EFSA), EFSA Journal 2013: 11(7):3295], but it has not been noted by the member states and is therefore provided as a courtesy.

Table 9.6-7: EFSA screening level acute risk assessment for honeybees exposed to fenpicoxamid and relevant metabolites in sugar beet/ fodder beet

Intended use		Sugar beet/fodder beet		
Active substance		Fenpicoxamid		
Application rate (g/ha)		2 x 75, interval 21 days		
Test design	LD ₅₀ (lab.)	Single application rate	Q _{HC} ≤ 42	
	(µg/bee)	(g/ha)		
Contact toxicity, acute	>202.4	75	< 0.37	
Test design	LD ₅₀ (lab.)	Single application rate	SV, downward	ETR ≤ 0.2
	(µg/bee)	(g/ha)		
Oral toxicity, acute	>303.3	75	7.6	< 0.0019
Intended use		Sugar beet/fodder beet		
Active substance		X642188		
Application rate (g/ha)		2 x 75; molecular wt. conversion = 0.837; = 2 x 62.8 g/ha, interval 21 days		
Test design	LD ₅₀ (lab.)	Single application rate	SV, downward	ETR ≤ 0.2
	(µg/bee)	(g/ha)		
Oral toxicity, acute	>101.9	62.8	7.6	0.0047
Intended use		Sugar beet/fodder beet		
Active substance		X696476		
Application rate (g/ha)		2 x 75; molecular wt. conversion = 0.275; = 2 x 20.6 g/ha, interval 21 days		
Test design	LD ₅₀ (lab.)	Single application rate	SV, downward	ETR ≤ 0.2
	(µg/bee)	(g/ha)		
Oral toxicity, acute	>14.2	20.6	7.6	0.011
Intended use		Sugar beet/fodder beet		
Active substance		X12019520		
Application rate (g/ha)		2 x 75; molecular wt. conversion = 0.306; = 2 x 23.0 g/ha, interval 21 days		
Test design	LD ₅₀ (lab.)	Single application rate	SV, downward	ETR ≤ 0.2
	(µg/bee)	(g/ha)		
Oral toxicity, acute	132.6	23.0	7.6	0.0013

Q_{HC}: Hazard quotient for contact exposure. Q_H values shown in **bold** breach the relevant trigger.

ETR: Exposure-Toxicity Ratio. ETR values shown in **bold** breach the relevant trigger.

The acute contact hazard quotient (Q_{HC}) for fenpicoxamid as well as the acute oral ETR values for fenpicoxamid and relevant metabolites are below the triggers indicating acceptable risk to honeybees.

Table 9.6-8: EFSA screening level acute risk assessment for honeybees exposed to prothioconazole and prothioconazole-desthio in sugar beet/ fodder beet

Intended use		Sugar beet/ fodder beet		
Active substance		Prothioconazole		
Application rate (g/ha)		2 x 150, interval 21 days		
Test design	LD ₅₀ (lab.)	Single application rate	Q _{HC} ≤ 42	
	(µg/bee)	(g/ha)		
Contact toxicity, acute	>200	150	< 0.75	
Test design	LD ₅₀ (lab.)	Single application rate	SV, downward	ETR ≤ 0.2
	(µg/bee)	(g/ha)		
Oral toxicity, acute	>71	150	7.6	< 0.016
Intended use		Sugar beet/ fodder beet		
Active substance		Prothioconazole-desthio (JAU 6476-desthio)		
Application rate (g/ha)		2 x 150; molecular wt. conversion = 0.907; = 2 x 136 g JAU 6476-desthio/ha, interval 21 days		
Test design	LD ₅₀ (lab.)	Single application rate	Q _{HC} ≤ 42	
	(µg/bee)	(g/ha)		
Contact toxicity, acute	>100	136	< 1.4	
Test design	LD ₅₀ (lab.)	Single application rate	SV, downward	ETR ≤ 0.2
	(µg/bee)	(g/ha)		
Oral toxicity, acute	>106.5	136	7.6	< 0.010

Q_{HC} : Hazard quotient for contact exposure. Q_H values shown in **bold** breach the relevant trigger.

ETR: Exposure-Toxicity Ratio. ETR values shown in **bold** breach the relevant trigger.

The Q_{HC} values and acute oral ETR values for prothioconazole and prothioconazole-desthio are below the triggers indicating acceptable risk to honeybees.

zRMS comments:

Acute risk assessment to bees:

The risk assessment performed in line with EFSA 2013 (not noted at EU level) presented in Table 9.6-7 and 9.6-8 performed for both active substances and Prothioconazole-desthio (JAU 6476-desthio is agreed by the zRMS). On the basis of calculated HQ values acceptable acute risk to bees may be concluded from all intended Central Zone uses of GF-3307.

Table 9.6-9: EFSA screening level acute risk assessment for honeybees exposed to GF-3307 (S7K-3-3) in sugar beet/fodder beet

Intended use		Sugar beet/ fodder beet		
Active substance		GF-3307		
Application rate (g/ha)		2 x 1566, interval 21 days		
Test design	LD ₅₀ (lab.)	Single application rate	Q _{HC} ≤ 42	
	(µg/bee)	(g/ha)		
Contact toxicity, acute	92.3	1566	< 17	
Test design	LD ₅₀ (lab.)	Single application rate		ETR ≤ 0.2

	(µg/bee)	(g/ha)	SV, down-ward	
Oral toxicity, acute	55.46	1566	7.6	0.21

Q_{HC}: Hazard quotient for contact exposure. Q_H values shown in **bold** breach the relevant trigger.

ETR: Exposure-Toxicity Ratio. ETR values shown in **bold** breach the relevant trigger.

The Q_{HC} for GF-3307 (S7K-3-3) is below the trigger of 42 indicating acceptable risk for contact.

The acute oral ETR for GF-3307 (S7K-3-3) is slightly above the trigger (0.21 vs 0.20); therefore, the Tier 1 risk assessment is conducted.

Table 9.6-10: EFSA 1st tier acute oral risk assessment for honeybees exposed to GF-3307 (S7K-3-3) in sugar beet/fodder beet

Crop	category	scenario	BBCH	Honeybee	
				ETR	trigger
Sugar beet	acute	treated crop	10 - 39	0.21	0.2
Sugar beet	acute	treated crop	40 - 69	0.21	0.2
Sugar beet	acute	weeds	10 - 39	0.10	0.2
Sugar beet	acute	weeds	40 - 69	0.03	0.2
Sugar beet	acute	field margin	10 - 39	<0.01	0.2
Sugar beet	acute	field margin	40 - 69	<0.01	0.2
Sugar beet	acute	adjacent crop	10 - 39	<0.01	0.2
Sugar beet	acute	adjacent crop	40 - 69	<0.01	0.2

The acute oral tier 1 risk assessment indicates that the risk from weeds, field margins and adjacent crops is low. For the treated crop, the trigger is slightly exceeded (0.21 vs 0.20). Since sugar beets/fodder beets are not attractive to bees, this exceedance is considered acceptable. However, higher tier studies and a higher tier risk assessment are presented in section 9.6.2.2.

zRMS comments:

Acute risk assessment to bees from exposure to GF-3307:

The risk assessment performed in line with EFSA 2013 (not noted at EU level) presented in Tables 9.6-9 and 9.6-10 performed for formulation GF-3307 is agreed by the zRMS.

On the basis of calculated ETR values the risk from weeds, field margins and adjacent crops is low. For the treated crop, the trigger is slightly exceeded (0.21 vs 0.20). zRMS agreed that since sugar beets/fodder beets are not attractive to bees and taking into account the results from semi-field studies for bees summarised in the Point 9.6.2.2, this exceedance is considered acceptable.

Chronic Toxicity to Bees

Corteva recognizes the need to review the bee pollinator risk assessment based on scientific progress. However, the EFSA Bee Guidance Document (EFSA Journal 2013; 11(7): 3295) hasn't been noted and the new draft EFSA bee GD 2023 is not yet ready for implementation. In the interim, Corteva has provided below adult and larval risk assessments conducted following the EPPO 2010¹ 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. Additionally Corteva has provided risk assessments utilizing the non-noted 2013 EFSA GD.

¹ 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.

EPPO chronic risk assessment for larvae

Worst case data from Rortais *et al.*, 2005² 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:

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

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 max application rate of GF 3307 (S7K 3-3) is $2 \times 1.5 \text{ L product/ha}$ with an interval of 21 days, equivalent to $2 \times 0.075 \text{ kg fenpicoxamid/ha} + 0.150 \text{ kg prothioconazole/ha}$. The proposed crops on the label are sugar and fodder beet. None of these crops are considered to be attractive to foraging bees and therefore the exposure to applications in the treated crop will be negligible. Exposure via flowering weeds is also negligible since the mean total ground coverage of attractive flowering weeds in sugar beet/fodder beet fields was determined to be 0.0 % when untreated control plots from 45 sugar beet/fodder beet field trials were evaluated for the occurrence and soil covering of flowering weeds³.

Nectar concentration: $1 \text{ application} \times 1.566 \text{ kg/ha} \times 2.9 \text{ mg/kg/kg/ha} = 4.54 \text{ mg/kg nectar}$

Pollen concentration: $1 \text{ application} \times 1.566 \text{ kg/ha} \times 6.1 \text{ mg/kg/kg/ha} = 9.55 \text{ mg/kg pollen}$

Nectar dose over 5 days of consumption by larvae: $4.54 \text{ mg/kg nectar} \times 198 \text{ mg nectar} \times 10^{-6} \text{ kg nectar/larvae} = 0.899 \times 10^{-3} \text{ mg GF 3307/larvae} = 0.899 \text{ } \mu\text{g GF 3307/larvae}$.

Pollen dose over 5 days of consumption by larvae: $9.55 \text{ mg/kg pollen} \times 2 \text{ mg pollen} \times 10^{-6} \text{ kg pollen/larvae} = 1.91 \times 10^{-5} \text{ mg GF 3307/larvae} = 0.0191 \text{ } \mu\text{g GF 3307/larvae}$.

Total dose over 5 days = Nectar dose + Pollen dose = $0.899 + 0.0191 \text{ } \mu\text{g GF 3307/larvae} = 0.918 \text{ } \mu\text{g GF 3307/larvae}$

The total dose of pollen and nectar over 5 days can be compared to the GF 3307 larval NOED of $6.62 \text{ } \mu\text{g GF 3307/larva}$ from the chronic larval study (Oberrauch, 2017) to derive a TER.

$$\text{TER} = \text{Toxicity/Exposure} = 6.62 \text{ } \mu\text{g GF 3307/larvae} / 0.918 \text{ } \mu\text{g GF 3307/larvae} = 7.21$$

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 7.21 there is a safety margin, indicating that the proposed use of GF 3307 (S7K-3-3) poses an acceptable risk to bee larval development.**

² 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

³ Last G, Lewis G, Pap G (2019) Regulatory report on the occurrence of flowering weeds in agricultural fields. ERM report Nr. 0482579. ERM, Harrogate, United Kingdom.

EFSA chronic risk assessment for larvae

A chronic larval bee assessment has been provided below in accordance with EFSA Journal 2013: 11(7):3295 as a courtesy.

Table 9.6-11: EFSA sceening level chronic larval risk assessment for honeybees exposed to GF-3307 (S7K-3-3) in sugar beet/fodder beet

Intended use		Sugar beet/fodder beet		
Active substance		GF-3307		
Application rate (g/ha)		2 x 1566, interval 21 days		
Test design	NOED (lab.)	Single application rate	SV, down-ward	ETR ≤ 0.2
	(µg/larva/development period)	(g/ha)		
Larva, chronic	6.62	1566	4.4	1.0

ETR: Exposure-Toxicity Ratio. ETR values shown in **bold** breach the relevant trigger.

Table 9.6-12: EFSA Tier 1 chronic larval risk assessment for honeybees exposed to GF-3307 (S7K-3-3) in sugar beet/fodder beet

Intended use		Sugar beet/fodder beet		
Active substance		GF-3307		
Application rate (g/ha)		2 x 1566, interval 21 days		
NOED (lab.) (µg/larva/development period)			6.62	
category	scenario	BBCH	Honeybee	
			ETR	trigger
larva	treated crop	10 - 39	0.88	0.2
larva	treated crop	40 - 69	0.88	0.2
larva	weeds	10 - 39	0.44	0.2
larva	weeds	40 - 69	0.11	0.2
larva	field margin	10 - 39	0.00	0.2
larva	field margin	40 - 69	0.00	0.2
larva	adjacent crop	10 - 39	0.00	0.2
larva	adjacent crop	40 - 69	0.00	0.2

ETR: Exposure-Toxicity Ratio. ETR values shown in **bold** breach the relevant trigger.

The chronic larval ETR for GF-3307 (S7K-3-3) was above the trigger of 0.2 in the EFSA (2013) screening assessment and in the Tier 1 assessment for the treated crop and for weeds at BBCH 10 – 39 indicating further assessment is needed. It has to be considered that sugar beets/fodder beets are harvested before they form flowering parts in the second year. Sugar beets/fodder beets that form flowers prematurely in the first year of cultivation are removed by the farmer because their seeds would cause serious weed problems in the following crop. For the weed scenario, it needs to be considered that a risk was identified only for BBCH 10 – 39, meanwhile the application window is from BBCH 39 – 49. Furthermore, exposure via flowering weeds is also negligible since the mean total ground coverage of attractive flowering weeds in sugar beet/fodder beet fields was determined to be 0.0 % when untreated control plots from 45 sugar beet/fodder beet field trials were evaluated for the occurrence and soil covering of flowering weeds. Additionally, the higher tier risk assessment presented below in section 9.6.2.2 can be used for refinement.

EPPO chronic risk assessment for adult bees

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 LDD₅₀ 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:

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

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:

$$\text{Daily dose } (\mu\text{g GF-3307/bee}) = \text{A.R.} \times (0.128 \text{ g}/0.3) \times \text{RUD}$$

where: A.R. = application rate in kg prep/ha

RUD = residue per unit dose from the EFSA bee guidance. Mean $\text{RUD}_{\text{nectar}} = 2.9 \text{ mg/kg}$ (foliar sprays), Mean $\text{RUD}_{\text{nectar}} = 0.0458 \text{ mg/kg}$ (seed treatment).

$$\text{Daily dose} = 1 \text{ application} \times 1.566 \text{ kg/ha} \times 0.427 \times 2.9 = 1.94 \mu\text{g GF-3307/bee}$$

The daily dose can be compared to the GF-3307 adult NOED of $> 0.418 \mu\text{g GF-3307/bee/day}$ from the chronic adult oral study (Verge, 2018) to derive a TER.

$$\text{TER} = \text{NOEDD/daily dose} = 0.418 \mu\text{g GF-3307/bee/day} / 1.94 \mu\text{g GF-3307/bee} = 0.22$$

The EPPO 2010 scheme proposes a trigger of 1 for assessment of the risk to honey bees when a NOEDD is used in this assessment. The TER value of 0.22 is less than the trigger, indicating that further higher tier work is needed to further evaluate the risk. Therefore an OECD 75 tunnel study, a colony feeding study and pollen and nectar residue trials have been conducted to further investigate the acute and chronic risk of GF-3307 to both adult honeybees and the developing brood.

zRMS comments:

Chronic risk assessment to larvae bees from exposure to GF-3307:

The risk assessment performed in line with EFSA 2013 (not noted at EU level) presented in Table 9.6-11 and 9.6-12 performed for formulation GF-3307 is agreed by the zRMS.

On the basis of calculated ETR value trigger of 0.2 in the EFSA (2013) screening assessment and in the Tier 1 assessment for the treated crop and for weeds at BBCH 10 – 39 indicating further assessment is needed.

It has to be considered that sugar beets/fodder beets are harvested before they form flowering parts in the second year. Sugar beets/fodder beets that form flowers prematurely in the first year of cultivation are removed by the farmer because their seeds would cause serious weed problems in the following crop. For the weed scenario, it needs to be considered that a risk was identified only for BBCH 10 – 39, meanwhile the application window is from BBCH 39 – 49.

In support of evaluation of GF-3307 the Applicant submitted a tunnel study performed on flowering Phacelia tanacetifolia, which addressed to chronic effects of GF-3307 on adult bees and larvae summarised below.

EFSA chronic risk assessment for adult bees

A chronic adult bee assessment has been provided below in accordance with EFSA Journal 2013: 11(7):3295 as a courtesy.

Table 9.6-13: EFSA sceening level chronic adult risk assessment for honybees exposed to GF-3307 (S7K-3-3) in sugar beet/fodder beet

Intended use		Sugar beet/fodder beet		
Active substance		GF-3307		
Application rate (g/ha)		2 x 1566, interval 21 days		
Test design	LD50 (lab.)	Single application rate	SV, downward	ETR ≤ 0.03
	($\mu\text{g/bee/day}$)			
Oral toxicity, chronic	>0.418	1566	7.6	< 28

ETR: Exposure-Toxicity Ratio. ETR values shown in **bold** breach the relevant trigger.

Table 9.6-14: EFSA Tier 1 chronic adult risk assessment for honeybees exposed to GF-3307 (S7K-3-3) in sugar beet/fodder beet

Intended use		Sugar beet/fodder beet		
Active substance		GF-3307		
Application rate (g/ha)		2 x 1566, interval 21 days		
LD50 (lab.) (µg/bee/day)			> 0.481	
category	scenario	BBCH	Honeybee	
			ETR	trigger
Chronic adult	treated crop	10 - 39	15.65	0.03
Chronic adult	treated crop	40 - 69	15.65	0.03
Chronic adult	weeds	10 - 39	7.82	0.03
Chronic adult	weeds	40 - 69	1.96	0.03
Chronic adult	field margin	10 - 39	0.07	0.03
Chronic adult	field margin	40 - 69	0.07	0.03
Chronic adult	adjacent crop	10 - 39	0.05	0.03
Chronic adult	adjacent crop	40 - 69	0.05	0.03

ETR: Exposure-Toxicity Ratio. ETR values shown in **bold** breach the relevant trigger.

The chronic adult ETR for **GF-3307** was above the trigger of 0.03 at screening level as well as at Tier 1 for all scenarios indicating further assessment is needed. See section 9.6.2.2 regarding higher tier testing.

zRMS comments:

Chronic risk assessment to adult bees from exposure to GF-3307:

The risk assessment performed in line with EFSA 2013 (not noted at EU level) presented in Table 9.6-13 and 9.6-14 performed for formulation GF-3307 is agreed by the zRMS.

On the basis of calculated ETR value was above the trigger of 0.03 at screening level as well as at Tier 1 for all scenarios indicating further assessment is needed.

In support of evaluation of GF-3307 the Applicant submitted a tunnel study evaluated in Core Dossier, 2023 performed on flowering *Phacelia tanacetifolia*, which addressed to chronic effects of GF-3307 on adult bees and larvae summarised below.

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

OECD 75 tunnel study (Kleinhenz/2018/DAS# 170673)

To further evaluate the risk of GF-3307 applications to foraging bees, an OECD 75 tunnel study was conducted in Germany in 2017. The study consisted of four treatment groups: two test item groups T1 and T2 (GF-3307 (S7K-3-3), a.i.: fenpicoxamid + prothioconazole), 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 64-65. The application rates of GF-3307 were 50 g fenpicoxamid a.i./ha plus 100 g prothioconazole a.i./ha (2 L GF-3307/ha) in treatment group T1 and 100 g fenpicoxamid a.i./ha plus 200 g prothioconazole a.i./ha (4 L GF-3307/ha) in the treatment group T2. Commercial bee colonies were placed in the tunnel tents at beginning of flowering (BBCH 62-63). 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 start of overwintering of the colonies. Additionally, the weight and malformations of pupae collected from combs was evaluated.

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 whole *Phacelia*

plants and forager bees (for preparation of pollen and nectar) were taken once before and three times after application in Cs, T1s and T2s for subsequent residue analysis.

There was no adverse effect of the test item treatments T1 and T2 on the mortality of adult worker bees, worker bee pupae, male adult bees and male pupae, foraging activity, colony size, amount of brood (total number of brood cells or number of cells with eggs, larvae or pupae) and storage of nectar and pollen. There was a slight adverse effect on the behaviour of worker bees in test item treatment T1 during the monitoring period outside tunnels and a slight transient effect on honeybee behaviour in T2 on the day of application (0DAA).

There was no effect of the test item treatment on the termination rates, brood indices and compensation indices of eggs during the 1st brood cycle (1DBA to 21DAA) and no effect on the young larvae and old larvae during the 1st and 2nd brood cycle (1DBA to 42DAA). There was a slight effect on the termination rates, brood index and compensation index of eggs on BFD+17 and BFD+21 of the 2nd brood cycle. There was no effect of the test item treatments T1 or T2 on the weight or malformations of pupae sampled from combs during the 1st brood cycle (17DAA).

The results demonstrate an acceptable risk to foraging honeybees following applications of GF-3307 (S7K-3-3) to the proposed crops on the label. When applied in worst-case experimental conditions to a highly attractive crop contained inside a tunnel to avoid foraging in untreated areas, there was no effect on the mortality of honeybees and only a slight effect on the termination rates in the second brood cycle, which had no impact on colony level.

It should be noted that the OECD 75 methodology is currently under review by the International Commission for Plant-Pollinator Relationships (ICPPR) as brood development is affected by placing the colonies in confined tunnels which make results difficult to interpret. For this reason, a colony feeding study was conducted with GF-3307 (S7K-3-3) where bees are exposed to the test chemical in the open field thus brood development follows a more consistent and natural pattern. Residue trials have also been conducted to more accurately determine exposure from pollen and nectar following application during flowering. These exposure values can then be used to interpret the results of the colony feeding study by comparing the likely exposure to effects seen on brood development. This approach to higher tier risk refinement has been conducted by the United States Environmental Protection Agency to evaluate thiamethoxam and clothianidin (EPA, 2020⁴). The results from these studies and the risk assessment are presented below.

Oilseed rape residue study (Appeltauer/2021/DAS #200670)

This oilseed rape residue study has been conducted for a submission for application of GF 3307 on oilseed rape during flowering. Residues in nectar, pollen and plants were determined after application of 2L GF-3307/ha on oilseed rape (c.f. Residue section KCA 6.10, 6.10.1). The residues observed in this study are compared to the endpoints from a colony feeding study. The current submission is for application of 1.5 L GF-3307/ha on sugar beet/fodder beet, which is not considered attractive to bees. Therefore, the risk assessment for oilseed rape based on field studies conducted at an application rate of 2 L GF-3307/ha is worst case and can be considered protective of the lower rate of 1.5 L GF-3307/ha on sugar beet/fodder beet.

The study was conducted as five separate field tunnel trials in Germany and Spain in 2020. Three trials were located in Germany and two trials in Spain. The study consisted of one treatment group per trial: one application of 2L GF-3307/ha was sprayed during flowering at BBCH 61-64; there were three replicates. Control samples were taken as pre-application samples in the treatment tunnels. Winter oilseed rape plants, forager bees for residue analysis and for determination of sugar content and pollen from winter oilseed rape were collected once before application and six times after application. Sampling of plants for residue analysis after application was conducted first at 0DAA and last sampling 5-8DAA. Sampling of pollen and

⁴ EPA (2020). Final Bee Risk Assessment to Support the Registration Review of Clothianidin and Thiamethoxam. United States Environmental Protection Agency Office of Chemical Safety and Pollution Prevention. PC Codes: 044309, 060109, DP Barcode: 455645.

forager bees for preparation of nectar for residue analysis after application was conducted first at 0DAA and last sampling 7–10DAA.

In plant specimen maximum residues of fenpicoxamid over all trials were in a range of 1.75 mg/kg to 2.33 mg/kg and maximum residues of prothioconazole were in a range of 0.319 mg/kg to 1.17 mg/kg over all trials. Maximum residues of prothioconazole desthio were in a range of 1.57 mg/kg to 2.65 mg/kg over all trials. In nectar specimen maximum residues of fenpicoxamid over all trials were in a range of 0.0435 mg/kg to 0.283 mg/kg and maximum residues of prothioconazole were in a range of 0.0312 mg/kg to 0.232 mg/kg over all trials. Maximum residues of prothioconazole desthio were in a range of 0.0108 mg/kg to 0.136 mg/kg over all trials. In pollen specimen maximum residues of fenpicoxamid over all trials were in a range of 5.31 mg/kg to 17.3 mg/kg and maximum residues of prothioconazole were in a range of 9.21 mg/kg to 36.6 mg/kg over all trials. Maximum residues of prothioconazole desthio were in a range of 0.900 mg/kg to 4.98 mg/kg over all trials. Overall there was a decline of residues of fenpicoxamid and prothioconazole for plants, pollen and nectar from the peak concentration to the last sampling observed for all trials. For the honey bee exposure assessment, the mean concentration in pollen and nectar on the day of application was calculated.

Residues in pollen and nectar at the day of application

Active substance	Mean residues in nectar at day of appl. (mg/kg)	Mean residues in pollen at day of appl. (mg/kg)
Fenpicoxamid	0.065	9.72
Prothioconazole	0.083	15.64
Prothioconazole desthio	0.032	2.48
Total active substance	0.18	27.84

The mean concentration of total active substance in pollen and nectar was 27.84 mg total a.s./kg and 0.18 mg total a.s./kg, respectively.

Colony feeding study (Gonsior/2021/Corteva #200660)

The aim of the study was to determine potential effects of GF-3307 (S7K-3-3) (active substance: Fenpicoxamid and Prothioconazole) on the honey bee mortality and behaviour, condition of the colonies, hive weight, honey bee brood development over two brood cycles, overwintering success, morphological abnormalities and weight of pupae and *Varroa* infestation of the colonies. Furthermore, the magnitude of residues in pollen, nectar, honey, pupae, larvae and worker jelly under field conditions were determined.

The study was conducted with free-flying honey bee colonies under field conditions. The bee colonies were fed for 9 consecutive days with the test substance diluted in sugar solution. Five test concentrations of 4, 20, 50, 100, 500 mg GF-3307/L, one control and two toxic reference substances (dimethoate and fenoxycarb) were evaluated with 5 replicates (GF-3307 concentrations and control) or 2 replicates (reference substances) each. Assessments were carried out for up to 358 days after beginning of feeding; the development of brood in individually marked cells was evaluated over two brood cycles.

No effect on mortality of worker bees, pupae and larvae, behaviour of worker bees, development on eggs, young larvae and old larvae in individual cells, mean weight of pupae, mean number of food cells, total amount of brood and colony size were observed for GF-3307 (S7K-3-3) up to the highest concentration of 500 mg GF-3307/L sugar solution.

The toxic reference substance dimethoate had significant effects on mortality of worker bees, pupae and larvae, on brood and compensation index and termination rate of young larvae, mean weight of pupae, mean number of food cells, total amount of brood and colony size. The toxic reference substance fenoxycarb had significant effects on mortality of worker pupae and larvae, on brood and compensation indices as well as termination rates for eggs and old larvae, mean number of food cells, total amount of brood and colony

size. The clear and significant multiple effects of the toxic reference substances confirm the suitability of the test design and exposure of the bee colonies to the treated feeding solution.

Slight, but significant effects on adult mortality and mean brood termination rate at 50 mg GF-3307/L were observed, but they were assumed to be caused by high variability of data since they were not dose related. The loss of queens observed in several colonies was assumed to be caused by disturbance of the colonies during assessment.

The residues determined in pollen, nectar honey, worker jelly, larvae and pupae declined rapidly in all matrices inside the colony.

Risk assessment

The residue values determined in pollen and nectar following application during flowering provide a more realistic exposure value to refine the risk assessment. Using pollen and nectar values to refine risk is outlined in the EFSA (2013) guidance document which states any assessment of the risk to organisms has to be based on concentrations that are most relevant for the effect (called the ecotoxicologically relevant concentrations, abbreviated ERCs). Risk to colonies of honey bees results from consumption of nectar and pollen in the hive. This consumption is considered an important driver for possible effects on colonies. Such effects are likely to be related not to concentrations in nectar and pollen collected by an individual bee but to the average concentration in the nectar and pollen entering the hive. Therefore, EFSA (2013⁵) proposes to assess the maximum average concentration entering the hive over time as the basis for the exposure assessment. This approach is supported by the US Environmental Protection Agency who recommend using residue measurements from pollen and nectar samples taken from bees that have collected the samples to quantify exposure to the colony in their risk assessment guidance (EPA, 2019⁶).

The mean exposure in pollen and nectar on the day of application (and therefore worst case) of 2 L GF-3307/ha on flowering oilseed rape was calculated across all trials in Germany and Spain from the oilseed rape residue study (Appeltauer/2021/DAS #200670):

Exposure via pollen:

- Sum of residues in pollen: 9.72 mg fenpicoxamid/kg + 15.64 mg prothioconazole/kg + 2.48 mg prothioconazole-desthio/kg = 27.84 mg total a.s./kg
- Maximum pollen consumption: 12 mg/bee/day by nurse bees (Rortais et al. 2005)
- Maximum exposure via pollen: 0.02784 µg a.s./mg * 12 mg/bee/day = 0.33 µg total a.s./bee

Exposure via nectar:

- Sum of residues in nectar: 0.065 mg fenpicoxamid/kg + 0.083 mg prothioconazole/kg + 0.032 mg prothioconazole-desthio/kg = 0.18 mg total a.s./kg
- Maximum nectar consumption: 427 mg/bee/day by forager bees (Rortais et al. 2005, assuming 30% sugar content of nectar from US EPA (2012))
- Maximum exposure via nectar: 0.00018 µg a.s./mg * 427 mg/bee/day = 0.077 µg total a.s./bee

Exposure via pollen + nectar

0.33 µg total a.s./bee + 0.077 µg total a.s./bee = 0.407 µg total a.s./bee

The exposure from pollen and nectar measured in the oilseed rape residue study is compared to the toxicity of GF 3307 (S7K 3-3) to honeybees in the laboratory studies. In the acute oral test (Noel/2015/DAS# 150736), a LD₅₀ of 55 µg GF 3307/bee (equals 7.9 µg total a.s./bee) was calculated. In the 10 day chronic adult study (Verge/2018/DAS# 170077), the NOEC was 15 mg GF 3307/kg diet (equals 2.2 mg total a.s./kg diet) and in the 22 day chronic larvae study (Oberrauch/2018/DAS# 171043), the NOEC was 43 mg GF 3307/kg diet (equals 6.2 mg total a.s./kg diet). These laboratory studies indicate a margin of acute and

⁵ European Food Safety Authority, 2013. EFSA Guidance Document on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees). EFSA Journal 2013;11(7):3295, 268 pp., doi:10.2903/j.efsa.2013.3295

⁶ EPA (2019). Guidance for Assessing Pesticide Risks to Bees. Office of Pesticide Programs United States Environmental Protection Agency.

chronic safety from exposure of bees to residues in nectar and a potential chronic risk from residues in pollen. However, honey bees consume far more nectar than pollen as indicated above (e.g. forager bees consume no pollen).

In the OECD 75 tunnel study (Kleinhenz/2018/DAS# 170673), 64.7 mg total a.s./kg pollen and 1.2 mg total a.s./kg nectar have been measured at an application rate of 2 L GF 3307/ha in *P. tanacetifolia*. These residues are 2.3 times higher for pollen and 6.7 times higher for nectar compared to the residues measured in oilseed rape after application of the same rate. Due to the higher exposure, the OECD 75 tunnel study conducted in *P. tanacetifolia* can also be used to estimate the risk of an application in oilseed rape during flowering. No effect on mortality, foraging activity, total number of brood cells or presence of the different brood stages or colony size had been observed in this study when 2 L/ha GF 3307 was sprayed directly onto foraging bees, but there were slight effects on development in brood cells initially containing eggs. However, there were no effects on development in brood cells containing young or old larvae and no effect on colony strength. Due to the shortcomings with this study (based on the confinement of colonies in tunnels and the effect this has on brood development) emphasis has been placed on the comparison of exposure values with endpoints from the colony feeding study.

Endpoints from a colony feeding study can be compared to the residue levels in pollen and nectar sampled from bees entering the hive as demonstrated in the thiamethoxam and clothianidin assessment by the EPA (EPA, 2020). In the GF 3307 (S7K 3-3) colony feeding study (Gonsior/2021/Corteva #200660), the maximum concentration of 500 mg GF 3307/kg feeding solution (equals 75 mg total a.s./kg) was higher than the concentration in pollen (27.84 mg total a.s./kg) and nectar (0.18 mg total a.s./kg) after application of 2 L GF 3307 onto flowering oilseed rape. This is considered to be the most valid comparison of exposure and effects in the risk assessment (EPA, 2020), as it compares the highest concentration for exposure from bees bringing residues into the colony in pollen and nectar (from the residue trials in oilseed rape) to the highest concentration bees will be exposed to in the colony feeding study which resulted in no effect. Moreover, the higher exposure in the colony feeding study was also confirmed by the concentration in nectar of 11.13 mg total a.s./kg nectar sampled from the combs at the end of the feeding period (10 DAF) compared to 0.18 mg total a.s./kg nectar sampled from the honeybee stomach in the residue study at the day of application. This shows that the free flying bees took up the feeding solution and stored it throughout the combs in the colony, which inevitably resulted in some dilution of the feeding solution. Exposure via pollen was with 0.68 mg total a.s./kg pollen collected at the end of the feeding period (10 DAF) from the combs much lower in the colony feeding study, but when the daily consumption of pollen of 12 mg/bee/day by nurse bees pollen and the daily consumption of nectar of 427 mg/bee/day by forager bees (Rortais *et al.* 2005) is considered, the daily exposure from pollen and nectar is 4.76 µg total a.s./bee in the colony feeding study, compared to 0.407 µg total a.s./bee in the oilseed rape residue study. Therefore, the colony feeding study and oilseed rape residue trials represent a worst case scenario in the risk assessment with a higher application rate (2 L GF 3307/ha in the residue trials compared to 1.5 L/ha in the current submission) and a 11.7 times higher uptake of GF 3307 in the colony feeding study. No effects of GF 3307 on adult bee mortality, brood development, colony size and overwintering survival were observed in the colony feeding study compared to the control. A low risk from application of 2 L GF 3307/ha on flowering oil seed rape has been demonstrated. Therefore, a low risk to honey bees from GF 3307 (S7K 3-3) applied at the lower rate of 1.5 L/ha on sugar beet/ fodder beet, which are not attractive to bees, can be concluded.

zRMS comments:

The risk assessment based comparison of residue analysis in pollen, nectar after application of GF-3307 in bee attractive crops and oil seed rape is not evaluated by zRMS for current application of GF-3307 for sugar beet/fodder beet.

zRMS comments:

In support of evaluation of GF-3307 the Applicant submitted a tunnel study performed on flowering *Phacelia tanacetifolia*, which addressed to chronic effects of GF-3307 on adult bees and larvae.

This tunnel study by Kleinhenz, M.; 2018 was conducted in bee attractive crop (*Phacelia tanacetifolia*,) in which application rates of GF-3307 were as follows: 50 g fenpicoxamid a.i./ha plus 100 g prothioconazole a.s./ha (corresponding to 1 L GF-3307/ha) in treatment group T1 and 100 g fenpicoxamid a.i./ha plus 200 g prothioconazole a.s./ha (corresponding to 2 L GF-3307/ha) in the treatment group T2.

The results of this study with regard to the brood development of the honey bees did not indicate unacceptable adverse effects on adult bees or bee brood development at a single application at up to 2 L GF-3307/ha equivalent to 100 g fenpicoxamid plus 200 g prothioconazole/ha. The critical GAP for the product is a single application rate of 1 x 1.5 L GF-3307/ha.

Therefore, the results from semi-field study with a NOAER = 2 L GF-3307/ha covers the application rate proposed in the GAP.

zRMS considers that exposure to bees from field flowering weeds and flowering plants in field margins, adjacent crop and the next crop will be negligible compared to the exposure in the tunnel study with the bee attractive crop *Phacelia*.

The colony feeding study (Gonsior/2021/Corteva #200660) with GF-3307 was conducted with free-flying honey bee colonies under field conditions. The bee colonies were fed for 9 consecutive days with the test substance diluted in sugar solution at rates; 4, 20, 50, 100, 500 mg GF-3307/L sugar. The magnitude of residues in pollen, nectar, honey, pupae, larvae and worker jelly under field conditions were determined.

Assessments were carried out for up to 358 days after beginning of feeding; the development of brood in individually marked cells was evaluated over two brood cycles

No effect on mortality of worker bees, pupae and larvae, behaviour of worker bees, development on eggs, young larvae and old larvae in individual cells, mean weight of pupae, mean number of food cells, total amount of brood and colony size were observed for GF-3307 up to the highest concentration of 500 mg GF-3307/L sugar solution. Slight, but significant effects on adult mortality and mean brood termination rate at 50 mg GF-3307/L were observed, but they were assumed to be caused by high variability of data since they were not dose related. The loss of queens observed in several colonies was assumed by the study author to be caused by disturbance of the colonies during assessment. The residues determined in pollen, nectar honey, worker jelly, larvae and pupae declined rapidly in all matrices inside the colony.

In conclusion:

In zRMS's opinion a low risk from application of 2 L GF-3307/ha on bee attractive crop (*Phacelia tanacetifolia*,) has been demonstrated. Therefore, a low risk to honey bees from GF-3307 (S7K-3-3) applied at the lower rate of 1.5 L/ha on sugar beet/ fodder beet, which are not attractive to bees, can be concluded.

However, the final decision of using higher tier studies to refine the risk according to EFSA GD 2013 is left at MS level according to recommendation given during 7 th CZHM in Warsaw, 2023.

9.6.3 Effects on bumble bees

SANCO acute risk assessment for bumble bees

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).

Table 9.6-15: First-tier assessment of the risk for bumble bees due to the use of fenpicoxamid in sugar beet/fodder beet

Intended use	Sugar beet/fodder beet		
Active substance	Fenpicoxamid		
Application rate (g/ha)	2 x 75, interval 21 days		
Test design	LD ₅₀ (lab.) (µg/bumble bee)	Single application rate (g/ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	≥272	75	≤0.28
Contact toxicity	≥300		≤0.25

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in **bold** breach the relevant trigger.

Table 9.6-16: First-tier assessment of the risk for bumble bees due to the use of prothioconazole in sugar beet/ fodder beet

Intended use	Sugar beet/ fodder beet		
Active substance	Prothioconazole		
Application rate (g/ha)	2 x 150, interval 21 days		
Test design	LD ₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	≥214.32	150	≤0.70
Contact toxicity	≥100		≤1.5

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in **bold** breach the relevant trigger.

Table 9.6-17: First-tier assessment of the risk for bumble bees due to the use of GF-3307 (S7K-3-3) in sugar beet/fodder beet

Intended use	Sugar beet/fodder beet		
Product	GF-3307		
Application rate (g/ha)	2 x 1566 (= 2 x 1.5 L/ha) , interval 21 days		
Test design	LD ₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	>453	1566	< 3.46
Contact toxicity	>500		< 3.13

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in **bold** breach the relevant trigger.

The Q_{HC} and Q_{HO} values for fenpicoxamid and GF-3307 (S7K-3-3) in bumble bee are below the Annex VI trigger of 50; therefore, the acute oral and contact risk to bumble bees is acceptable.

zRMS comments:

The acute risk assessment performed in line with SANCO/10329/2002 rev 2 presented in Table 9.6-17 performed for GF-3307 is agreed by the zRMS.

EFSA acute risk assessment for bumble bees

The oral and contact hazard quotients for fenpicoxamid and GF-3307 (S7K-3-3) provided above show acceptable acute risk to bees. Guidance has been provided by EFSA [Guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees), European Food Safety Authority (EFSA), EFSA Journal 2013: 11(7):3295], but it has not been noted by the member states and is therefore provided as a courtesy.

Table 9.6-18: EFSA screening level acute risk assessment for bumble bees exposed to fenpicoxamid in sugar beet/fodder beet

Intended use		Sugar beet/fodder beet		
Active substance		Fenpicoxamid		
Application rate (g/ha)		2 x 75, interval 21 days		
Test design	LD ₅₀ (lab.)	Single application rate	Q_{HC} ≤ 7	
	(µg/bee)	(g/ha)		
Contact toxicity, acute	>300	75	<0.25	
Test design	LD ₅₀ (lab.)	Single application rate	SV, downward	ETR ≤ 0.036
	(µg/bee)	(g/ha)		
Oral toxicity, acute	>272	75	11.2	<0.0034

Q_{HC}: Hazard quotient for contact exposure. Q_H values shown in **bold** breach the relevant trigger.

ETR: Exposure-Toxicity Ratio. ETR values shown in **bold** breach the relevant trigger.

The Q_{HC} and oral ETR values for fenpicoxamid in bumble bee are below the EFSA trigger values; therefore, the acute oral and contact risk to bumble bees is acceptable.

Table 9.6-19: EFSA screening level acute risk assessment for bumble bees exposed to prothioconazole in sugar beet/fodder beet

Intended use		Sugar beet/fodder beet		
Active substance		Prothioconazole		
Application rate (g/ha)		2 x 150, interval 21 days		
Test design	LD ₅₀ (lab.)	Single application rate	Q_{HC} ≤ 7	
	(µg/bee)	(g/ha)		
Contact toxicity, acute	>100	150	<1.5	
Test design	LD ₅₀ (lab.)	Single application rate	SV, downward	ETR ≤ 0.036
	(µg/bee)	(g/ha)		
Oral toxicity, acute	>214.3	150	11.2	<0.0078

Q_{HC}: Hazard quotient for contact exposure. Q_H values shown in **bold** breach the relevant trigger.

ETR: Exposure-Toxicity Ratio. ETR values shown in **bold** breach the relevant trigger.

The Q_{HC} and oral ETR values for prothioconazole in bumble bee are below the EFSA trigger values; therefore, the acute oral and contact risk to bumble bees is acceptable.

Table 9.6-20: EFSA screening level acute risk assessment for bumble bees exposed to GF-3307 (S7K-3-3) in sugar beet/fodder beet

Intended use		Sugar beet/fodder beet		
Active substance		GF-3307		
Application rate (g/ha)		2 x 1566, interval 21 days		
Test design	LD ₅₀ (lab.)	Single application rate	Q_{HC} ≤ 7	
	(µg/bee)	(g/ha)		
Contact toxicity, acute	>500	1566	< 3.1	
Test design	LD ₅₀ (lab.)	Single application rate	SV, downward	ETR ≤ 0.036
	(µg/bee)	(g/ha)		
Oral toxicity, acute	>453	1566	11.2	< 0.039

Q_{HC}: Hazard quotient for contact exposure. Q_H values shown in **bold** breach the relevant trigger.

ETR: Exposure-Toxicity Ratio. ETR values shown in **bold** breach the relevant trigger.

Table 9.6-21: EFSA Tier 1 acute oral risk assessment for bumble bees exposed to GF-3307 (S7K-3-3) in sugar beet/fodder beet

Intended use		Sugar beet/fodder beet		
Active substance		GF-3307		
Application rate (g/ha)		2 x 1566, interval 21 days		
LD50 (lab.) (µg/bee)			>453	
category	scenario	BBCH	Bumble bee	
			ETR	trigger
Acute oral	treated crop	10 - 39	<0.039	0.036
Acute oral	treated crop	40 - 69	<0.039	0.036
Acute oral	weeds	10 - 39	<0.02	0.036
Acute oral	weeds	40 - 69	<0.01	0.036
Acute oral	field margin	10 - 39	<0.00	0.036
Acute oral	field margin	40 - 69	<0.00	0.036
Acute oral	adjacent crop	10 - 39	<0.00	0.036
Acute oral	adjacent crop	40 - 69	<0.00	0.036

ETR: Exposure-Toxicity Ratio. ETR values shown in **bold** breach the relevant trigger.

The Q_{HC} value for GF-3307 (S7K-3-3) in bumble bee is below the EFSA trigger value of 7; therefore, the acute risk to bumble bees is acceptable.

The oral ETR for GF-3307 (S7K-3-3) in bumble bee is slightly above the EFSA trigger value (<0.039 vs 0.036) in the screening assessment and at Tier 1 in the treated crop scenario indicating potential risk from application to the crop. However, the acute oral toxicity test had 0% mortality at 48 hours in the top concentration tested (500 µg/bee nominal, 453 µg/bee based on consumption) suggesting that risk to bumble bees from GF-3307 (S7K-3-3) is low. Furthermore, sugar beet/fodder beet is not considered to be attractive to foraging bees and therefore the exposure to applications in the treated crop will be negligible. Exposure via flowering weeds is also negligible since the foliage of sugar beet/fodder beet is fully grown at BBCH 39-49 and the mean total ground coverage of attractive flowering weeds in sugar beet/fodder beet fields was determined to be 0.0 % when untreated control plots from 45 sugar beet/fodder beet field trials were evaluated for the occurrence and soil covering of flowering weeds. Furthermore, it has to be considered that sugar beets/fodder beets are harvested before they form flowering parts in the second year. Sugar beets/Fodder beets that form flowers prematurely in the first year of cultivation are removed by the farmer because their seeds would cause serious weed problems in the following crop.

zRMS comments:

Acute risk assessment to bumble bees:

The risk assessment performed in line with EFSA 2013 (not noted at EU level) presented in Table 9.6-20 and 9.6-21 performed for formulation GF-is agreed by the zRMS.

The oral ETR for GF-3307 (S7K-3-3) in bumble bee is slightly above the EFSA trigger value (<0.039 vs 0.036) in the screening assessment and at Tier 1 in the treated crop scenario indicating potential risk from application to the crop.

Sugar beet/fodder beet is not considered to be attractive to foraging bees and therefore the exposure to applications in the treated crop will be negligible.

In support of evaluation of GF-3307 the Applicant submitted a tunnel study performed on flowering *Phacelia tanacetifolia*, which addressed to chronic effects of GF-3307 on adult bees and larvae.

This tunnel study by Kleinhenz, M.; 2018 was conducted in bee attractive crop (*Phacelia tanacetifolia*,) in which application rates of GF-3307 were as follows: 50 g fenpicoxamid a.i./ha plus 100 g prothioconazole a.s./ha (corresponding to 1 L GF-3307/ha) in treatment group T1 and 100 g fenpicoxamid a.i./ha plus 200 g prothioconazole a.s./ha (corresponding to 2 L GF-3307/ha) in the treatment group T2.

The results of this study with regard to the brood development of the honey bees did not indicate unacceptable adverse effects on adult bees or bee brood development at a single application at up to 2 L GF-3307/ha equivalent to 100 g fenpicoxamid plus 200 g prothioconazole/ha. The critical GAP for the product is a single application rate

of 1 x 1.5 L GF-3307/ha.

Therefore, the results from semi-field study with a NOAER = 2 L GF-3307/ha covers the application rate proposed in the GAP.

9.6.4 Effects on solitary bees

Studies not required.

9.6.5 Overall conclusions

Acute assessment

The SANCO Q_H values for fenpicoxamid, prothioconazole, relevant metabolites, and GF-3307 (S7K-3-3) in honeybee and bumble bee are below the Annex VI trigger of 50; therefore, the acute oral and contact risk to honeybees and bumble bees is acceptable. The EFSA acute contact Q_{HC} values and oral ETRs for fenpicoxamid, prothioconazole, and relevant metabolites for honeybees and bumble bees, where available, are below the trigger values indicating acceptable risk to bees. The EFSA Q_{HC} values for GF-3307 (S7K-3-3) for honeybees and bumble bees are below the trigger value indicating acceptable risk. The EFSA acute oral ETRs for GF-3307 (S7K-3-3) for honeybees and bumble bees only slightly exceeded the trigger values indicating low risk.

Chronic assessment

The TER value for honeybee larvae is greater than the EPPO 2010 trigger of 1 indicating acceptable risk from GF-3307 (S7K-3-3). The chronic risk for adult honey bees and the risk assessment based on the EFSA 2013 bee guidance assessment were refined with an OECD 75 tunnel study and a colony feeding study in combination with pollen and nectar residue trials. These colony feeding and residue studies show that there is no effect at 2 L GF-3307/ha applied during flowering on oilseed rape on adult bee mortality, brood development, colony size and overwintering survival. Risk assessment for application of 2 L GF-3307/ha on flowering oilseed rape is worst-case and protective for application of 1.5 L GF-3307/ha on sugar beet and fodder beet. Therefore, the risk is acceptable.

In conclusion: A low risk from application of 2 L GF-3307/ha on bee attractive crop (*Phacelia tanacetifolia*,) has been demonstrated. Therefore, a low risk to honey bees from GF-3307 (S7K-3-3) applied at the lower rate of 1.5 L/ha on sugar beet/ fodder beet, which are not attractive to bees, can be concluded.

However, the final decision of using higher tier studies to refine the risk according to EFSA GD 2013 is left at MS level according to recommendation given during 7 th CZHM in Warsaw, 2023.

Commenting period process:

Some of MSs indicated that although the crop is not attractive to bees considering nectar and/or pollen, the crop may be attractive due to honeydew production.

Therefore this may indeed be an additional point on MS level.

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, prothioconazole, and their relevant metabolites. Full details of these studies are provided in the respective EU DAR (fenpicoxamid: United Kingdom, 2017; prothioconazole: United Kingdom, 2007) and related documents as well as in Appendix 2 of this document (new studies).

Effects on non-target arthropods of GF-3307 (S7K-3-3) 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 ₅₀ > 400 g/ha	EFSA, 2018
<i>Aphidius rhopalosiphi</i> (adults)	Fenpicoxamid	Laboratory test glass plates (2D)	LR ₅₀ = 129 g/ha	EFSA, 2018

zRMS comments:

Endpoints presented in Table 9.7-1 are in line with the EU agreed endpoints reported in EFSA Journal 2018;16(1):5146.

Table 9.7-2: Endpoints and effect values relevant for the risk assessment for non-target arthropods – prothioconazole

Species	Substance	Exposure System	Results	Reference
<i>Typhlodromus pyri</i> (protonymphs)	Prothioconazole (EC 250)	Laboratory test glass plates (2D)	LR ₅₀ = 18.7 g a.s./ha	EFSA, 2007
<i>Aphidius rhopalosiphi</i> (adults)	Prothioconazole (EC 250)	Laboratory test glass plates (2D)	LR ₅₀ = 139.9 g a.s./ha	
<i>Coccinella septempunctata</i>	Prothioconazole (EC 250)	Laboratory test glass plates (2D)	LR ₅₀ = 229.8 g a.s./ha Effects on reproduction are not considered to be treatment related	
<i>Chrysoperla carnea</i>	Prothioconazole (EC 250)	Laboratory test glass plates (2D)	LR ₅₀ > 600 g a.s./ha No adverse effects on reproduction	
<i>Poecilius cupreus</i>	Prothioconazole (EC 250)	Laboratory test quartz sand (2D)	LR ₅₀ > 600 g a.s./ha No adverse effect on feeding rate	
<i>Aleochara bilineata</i>	Prothioconazole (EC 250)	Laboratory test quartz sand (2D)	LR ₅₀ > 400 g a.s./ha	
<i>Typhlodromus pyri</i> (protonymphs)	Prothioconazole (EC 250)	Extended laboratory test bean leaves (2D)	LR ₅₀ = 445.5 g a.s./ha Mortality: -2.3 % at 100 g/ha 1.5 % at 157 g/ha 9.1 % at 245 g/ha 45.1 % at 380 g/ha* 67.8 % at 600 g/ha* Eff. on reproduction: 14 % at 100 g/ha 9 % at 157 g/ha 47 % at 245 g/ha* 40 % at 380 g/ha*	
<i>Aphidius rhopalosiphi</i> (adults)	Prothioconazole (EC 250)	Extended laboratory test	LR ₅₀ > 600 g a.s./ha No significant effect on	

Species	Substance	Exposure System	Results	Reference
		wheat plants (3D)	reproduction in any treatment	
<i>Typhlodromus pyri</i> (protonymphs)	Prothioconazole (EC 250)	Aged-residue test bean leaves	Mortality at 300 g a.s./ha: 14.5 % at 1 DA(L)T 6.4 % at 15 DA(L)T Eff. on repro.: 7.5 % at 1 DA(L)T -28.8 % at 15 DA(L)T	

zRMS comments:

Endpoints presented in Table 9.7-2 are in line with the EU agreed endpoints reported in EFSA Scientific Report (2007) 106, 1-98.

Table 9.7-3: Endpoints and effect values relevant for the risk assessment for non-target arthropods - GF-3307 (S7K-3-3)

Species	Substance	Exposure System	Results	Reference
<i>Typhlodromus pyri</i> (protonymphs)	GF-3307	Laboratory test glass plates (2D)	LR ₅₀ = 3230 ml/ha	Moll/2014/DAS# 140226
<i>Aphidius rhopalosiphi</i> (adults)	GF-3307	Laboratory test glass plates (2D)	LR ₅₀ = 89.5 ml/ha	Moll/2014/DAS# 140224
<i>Aleochara bilineata</i>	GF-3307	Laboratory test quartz sand (2D)	ER ₅₀ >4000 ml/ha Red. of hatch rate: 7.4% at 4000 ml/ha	Tew/2020/DAS# 200609
<i>Aphidius rhopalosiphi</i> (adults)	GF-3307	Extended laboratory test barley plants (3D)	LR ₅₀ = 769 ml/ha Red. of fecundity: -4.1 % at 22.7 ml/ha -22.0 % at 79.3 ml/ha 18.5% at 278 ml/ha	Moll/2014/DAS# 140947
<i>Coccinella septempunctata</i>	GF-3307	Extended laboratory test bean leaves (2D)	LR ₅₀ = 1520 ml/ha ER₅₀ = 209 ml/ha	Kimmel/2016/DAS# 150923
<i>Chrysoperla carnea</i> (larvae)	GF-3307	Extended laboratory test bean leaves (2D)	LR ₅₀ >405 ml/ha ER ₅₀ >405 ml/ha	Moll/2014/DAS# 140948
<i>Aleochara bilineata</i>	GF-3307	Extended laboratory test LUFA 2.1 soil (2D)	ER ₅₀ >4000 ml/ha	Kimmel/2016/DAS# 150926
<i>Aleochara bilineata</i>	GF-3307	Extended laboratory test LUFA 2.1 soil (2D)	ER ₅₀ >4000 ml/ha NOER = 4000 ml/ha	Tew/2020/DAS#200610
<i>Pardosa</i> spp. (adults and sub-adults)	GF-3307	Extended laboratory test LUFA 2.1 soil (2D)	LR ₅₀ >4000 ml/ha ER ₅₀ >4000 ml/ha	Schmitzer/2015/DAS# 150927
<i>Aphidius rhopalosiphi</i> (adults)	GF-3307	Aged-residue test bean plants (3D), assay uses bean leaves	Mortality _{corr} at 2 L/ha x 2 applications with a 14 day interval: 100 % at 0 DALT	Stevens/2016/DAS# 150924

Species	Substance	Exposure System	Results	Reference
			24.3 % at 14 DALT 7.7 % at 28 DALT Red. of parasitism rate at 2 L/ha: +1.9 % at 14 DALT 17.7 % at 28 DALT	
<i>Chrysoperla carnea</i> (larvae)	GF-3307	Aged-residue test bean plants (3D), assay uses bean leaves	Mortality _{corr} at 2 L/ha x 2 applications with a 14 day interval: 24.2 % at 0 DALT 0 % at 14 DALT No adverse effect on reproductive capacity	Vaughan/2015/DAS# 150925
<i>Coccinella septempunctata</i>	GF-3307	Aged-residue test, bean plants (3D), assay used bean leaves	Mortality _{corr} at 2 L/ha x 2 applications with a 14 day interval: 43.3 % at 0 DALT 5.0 % at 14 DALT 20.5 % at 28 DALT Effects on reproduction: 31.6 % at 0 DALT 9.1 % at 14 DALT +54.0 % at 28 DALT	Vaughan/2017/DAS# 170778

zRMS comments:

Studies on toxicity of GF-3307 to non-target arthropods were evaluated by the zRMS and are considered acceptable in course of zonal authorisation of the product GF-3307 (Core Dossier 2023) in cereals and is relevant for the current evaluation of GF-3307 for extended uses.
For details of evaluation, please refer to Appendix 2. Endpoints reported in Table 9.7-3 are confirmed to be correct.

Studies on toxicity of GF-3307 to non-target arthropods were evaluated by the zRMS and are considered acceptable. For details of evaluation, please refer to Appendix 2. Endpoints reported in Table 9.7-1 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, prothioconazole, and GF-3307 (S7K-3-3) are summarised in the following tables. The assessments are done at an application rate of 2 x 75 g fenpicoxamid/ha, 2 x 150 g prothioconazole/ha, and 2 x 1.5 L GF-3307/ha.

Per the recommendation in the EFSA Peer review meeting 185 “Outcome of the pesticide peer review on general recurring issues in ecotoxicology,” (EFSA/EN-1673/2019), a VDF of 5 was used for all tiers of the assessment.

Table 9.7.4: First and higher tier assessment of the in-field risk for non-target arthropods due to the use of fenpicoxamid in sugar beet/fodder beet

Intended use	Sugar beet/fodder beet		
Active substance	Fenpicoxamid		
Application rate (g/ha)	2 x 75, interval 21 days		
MAF	1.7 (foliage)		
Test species Tier 1	LR ₅₀ (lab.) (g/ha)	PER _{in-field} (g/ha)	HQ _{in-field} criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	≥400	127.5	≤0.32
<i>Aphidius rhopalosiphi</i>	129		0.99

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 at tier 1 is demonstrated when fenpicoxamid is applied according to the proposed GAP.

Table 9.7.5: First and higher tier assessment of the in-field risk for non-target arthropods due to the use of prothioconazole in sugar beet/fodder beet

Intended use	Sugar beet/fodder beet		
Active substance	Prothioconazole		
Application rate (g/ha)	2 x 150, interval 21 days		
MAF	1.7 (foliage), 1.9 (soil)		
Test species Tier 1	LR ₅₀ (lab.) (g/ha)	PER _{in-field} (g/ha)	HQ _{in-field} criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	18.7	255	13.6
<i>Aphidius rhopalosiphi</i>	139.9		1.8
<i>Coccinella septempunctata</i>	229.8		1.11
<i>Chrysoperla carnea</i>	≥600		≤0.43
<i>Poecilus cupreus</i>	≥600	285	≤0.48
<i>Aleochara bilineata</i>	≥400		≤0.71
Test species Tier 2	Rate with ≤ 50 % effect*	PER _{in-field} (g/ha)	PER _{in-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	445.5	255	yes
<i>Aphidius rhopalosiphi</i>	≥ 600		yes

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 LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Acceptable in-field risk at tier 2 is demonstrated when prothioconazole is applied according to the proposed GAP.

Table 9.7-6: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of GF-3307 (S7K-3-3) in sugar beet/fodder beet

Intended use	Sugar beet/fodder beet		
Product	GF-3307		
Application rate (ml/ha)	2 x 1500, interval 21 days		
MAF	1.7 (foliage), 1.9 (soil)		
Test species Tier 1	LR₅₀ (lab.) (ml/ha)	PER_{in-field} (ml/ha)	HQ_{in-field} criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	3230	2550	0.79
<i>Aphidius rhopalosiphi</i>	89.5		28.5
<i>Aleochara bilineata</i>	>4000	2850	0.71
Test species Tier 2	Rate with ≤ 50 % effect* (ml/ha)	PER_{in-field} (ml/ha)	PER_{in-field} below rate with ≤ 50 % effect?
<i>Aphidius rhopalosiphi</i>	769	2550	no
<i>Coccinella septempunctata</i>	209		no
<i>Chrysoperla carnea</i>	>405		no
<i>Pardosa spp.</i>	>4000	2850	yes
<i>Aleochara bilineata</i>	>4000		yes
Test species Aged residues	Rate with ≤ 50 % effect (ml/ha) at DALT	PER_{in-field} (ml/ha)	PER_{in-field} below rate with ≤ 50 % effect?
<i>Aphidius rhopalosiphi</i>	2000 ml/ha x 2 applications 24.3 % at 14 DALT	2 x 1500	No effect >50% at the 2 L/ha x 2 apps at 14 DALT.
<i>Chrysoperla carnea</i>	2000 ml/ha x 2 applications: 24.2 % at 0 DALT		No effect >50% at the 2 L/ha x 2 apps at 0 DALT.
<i>Coccinella septempunctata</i>	2000 ml/ha x 2 applications: 5.0 % at 14 DALT		No effect >50% at the 2 L/ha x 2 apps at 14 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 LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Acceptable in-field risk to soil-dwelling organisms is demonstrated for GF-3307 (S7K-3-3) at the proposed GAP. In-field risk to foliar-dwelling organisms (*Aphidius*, *Chrysoperla*, and *Coccinella*) is acceptable at 14, 0 and 0 days post-application, respectively, when exposed to an exaggerated rate (i.e. 2 x 2 L GF-3307/L).

zRMS comments:

The in-field risk assessment performed for the formulation is agreed by the zRMS.
The exposure calculated for particular active substances was not validated as not necessary since the risk assessment for this group of species is performed for formulation for which authorisation is sought.

The calculations of in-field risk assessment for GF-3307 was performed according to relevant guidance. For *A. rhopalosiphi*, the Tier 1 in-field HQ exceeded the trigger value of 2, therefore further consideration of the in-field risk was required. According to ESCORT 2, when a risk relating to in-field exposure is identified for one or both indicator species, higher-tier data on one additional species is required in addition to higher-tier data for the indicator species that exceeded the trigger value. The additional three species *Pardosa* sp. *Septempuncta* and *Chrysoperla carnea* were tested and the risk assessment for them was provided.
At Tier-2 the PER in-field was below the ER₅₀ for *Pardosa* sp and *Aleochara bilineata* but above the ER₅₀ for *A. rhopalosiphi* and *C. septempunctata* and *Chrysoperla carnea* requiring higher-tier studies for the three species.

In an aged-residue study to determine the effects of GF-3307 on the parasitic wasp, *Aphidius rhopalosiphi*, a series of extended laboratory tests were performed on both fresh and outdoor-aged foliar residues. Following two applications to French bean plants at a rate of 2000 mL product/ha, with a 14-day interval, both 14- and 28-day old foliar

residues of GF-3307 had no unacceptable effects on either wasp survival or re-production (i.e. < 50% effects, relative to the control).

The effects of both fresh and field-aged residues of GF-3307 on the green lacewing *Chrysoperla carnea* were evaluated under extended laboratory test conditions. Following application at a rate of 2.0 L product/ha on two separate occasions, with a 14-day interval in between, both fresh (0-day-old) and field-aged (14-day-old) residues of GF-3307 had no adverse effect on either the survival of larvae or the subsequent reproductive capacity of the adult lacewings.

The effects of both freshly-dried and field-aged residues of GF-3307 on the ladybird beetle, *Coccinella septempunctata*, were evaluated under extended laboratory test conditions. Following two applications of GF-3307 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 re-productive capacity of the ladybirds were observed in bioassays initiated 14 and 28 days after the second treatment application.

In conclusion for the affected in-field non-target arthropods there is a potential for recolonisation / recovery within 14 days after the application of GF-3307. According to EU Guidance Document on Terrestrial Ecotoxicology (SANCO/ 10329/2002 rev 2) it has to be demonstrated that there is a potential for recolonisation /recovery at least within one year but preferably in a shorter period de-pending on the biology (seasonal pattern) of the species.

9.7.2.2 Risk assessment for off-field exposure

Table 9.7.7: First and higher tier assessment of the off-field risk for non-target arthropods due to the use of fenpicoxamid in sugar beet/fodder beet

Intended-use		Sugar beet/fodder beet			
Active substance		Fenpicoxamid			
Application rate (g/ha)		2 x 75, interval 21 days			
MAF		1.7 (foliage)			
vdf		5			
Test species	LR ₅₀ (lab.)	Drift rate	PER _{off-field}	CF	HQ _{off-field}
Tier-1	(g/ha)		(g/ha)		criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	>400	2.38%	0.607	10	< 0.015
<i>Aphidius rhopalosiphi</i>	129				0.047

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 at tier 1 is demonstrated when fenpicoxamid is applied according to the proposed GAP.

Table 9.7.8: First and higher tier assessment of the off-field risk for non-target arthropods due to the use of prothioconazole in sugar beet/fodder beet

Intended-use		Sugar beet/fodder beet			
Active substance/product		Prothioconazole			
Application rate (g/ha)		2 x 150, interval 21 days			
MAF		1.7 (foliage)			
vdf		5			
Test species	LR ₅₀ (lab.)	Drift rate	PER _{off-field}	CF	HQ _{off-field}
Tier-I	(g/ha)		(g/ha)		criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	18.7	2.38%	1.21	10	0.65
<i>Aphidius rhopalosiphi</i>	139.9				0.087
<i>Coccinella septempunctata</i>	229.8				0.053

<i>Chrysoperla carnea</i>	>600				<0.020
<i>Poecilus cupreus</i>	>600				<0.020
<i>Aleochara bilineata</i>	>400				<0.030
Test species Tier 2	Rate with $\leq 50\%$ ef- fect* (g/ha)	Drift rate	PER_{off-field} (g/ha)	CF	corr. PER_{off-field} below rate with $\leq 50\%$ ef- fect?
<i>Typhlodromus pyri</i>	445.5	2.38%	1.21	10	yes
<i>Aphidius rhopalosiphi</i>	> 600	2.38%	1.21	10	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 LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with $\leq 50\%$ effect.

Acceptable off field risk at tier 1 and tier 2 is demonstrated when prothioconazole is applied according to the proposed GAP.

Table 9.7-9: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of GF-3307 (S7K-3-3) in sugar beet/fodder beet

Intended use	Sugar beet/fodder beet				
Product	GF-3307				
Application rate (ml/ha)	2 x 1500, interval 21 days				
MAF	1.7 (foliage)				
vdf	5				
Test species Tier 1	LR₅₀ (lab.) (ml/ha)	Drift rate	PER_{off-field} (ml/ha)	CF	HQ_{off-field} criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	3230	2.38%	12.1	10	0.038
<i>Aphidius rhopalosiphi</i>	89.5				1.36
<i>Aleochara bilineata</i>	>4000				<0.03
Test species Tier 2	Rate with $\leq 50\%$ ef- fect* (ml/ha)	Drift rate	PER_{off-field} (ml/ha)	CF	corr. PER_{off-field} below rate with $\leq 50\%$ ef- fect?
<i>Aphidius rhopalosiphi</i>	769	2.38%	60.5	5	yes
<i>Coccinella septempunctata</i>	209	2.38%	12.1	5	yes
<i>Chrysoperla carnea</i>	>405	2.38%		5	yes
<i>Pardosa</i>	>4000	2.38%		5	yes
<i>Aleochara bilineata</i>	>4000	2.38%		5	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 LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with $\leq 50\%$ effect.

Acceptable off-field risk at tier 1 and tier 2 is demonstrated when GF-3307 (S7K-3-3) is applied according to the proposed GAP.

zRMS comments:

The off-field risk assessment performed for the formulation is agreed by the zRMS. Acceptable risk could be concluded with no need for risk mitigation measures.
As already mentioned above, the risk assessment for particular active compounds was not validated a not relevant for authorisation of GF-3307.

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 prothioconazole, the tier 1 in-field HQ values are below the trigger of 2 for five of the six species tested, and the off-field HQ values are below the trigger of 2 for all six species tested. *A. rhopalosiphi* and *T. pyri* tier 2 in- and off-field HQ values are below the trigger of 1 indicating low risk to non-target arthropods in sugar beet/fodder beet at the proposed GAP.

Acceptable off-field risk is demonstrated for GF-3307 (S7K-3-3) when used according to proposed GAP. In-field risk to foliar-dwelling organisms (*Aphidius*, *Chrysoperla* and *Coccinella*) is acceptable 14 days post second application when used according to proposed GAP with no need for risk mitigation.

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, prothioconazole, and relevant metabolites. Full details of these studies are provided in the respective EU DAR (fenpicoxamid: United Kingdom, 2017; prothioconazole: United Kingdom, 2007) 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-3307 (S7K-3-3) 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 10 % peat content	NOEC = 19.85 mg/kg dw NOEC _{corr} = 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 _{corr} = 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 _{corr} = 19.85 mg/kg dw*	EFSA, 2018
Fenpicoxamid metabolites				
<i>Eisenia fetida</i>	X642188	Mixed into substrate 56 d,	NOEC = 5.6 mg/kg dw	EFSA, 2018

Species	Substance	Exposure System	Results	Reference
		chronic 10 % peat content	NOEC _{corr} = 2.8 mg/kg dw*	
<i>Folsomia candida</i>	X642188	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 10 mg/kg dw NOEC _{corr} = 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 _{corr} = 10 mg/kg dw*	EFSA, 2018
<i>Eisenia fetida</i>	X11963422	Mixed into substrate 56 d, chronic 10 % 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 10 % 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 _{corr} = 0.99 mg/kg dw*	EFSA, 2018
<i>Folsomia candida</i>	X12313581		NOEC _{corr} = 0.397 mg/kg dw*	EFSA, 2018
<i>Hypoaspis aculeifer</i>	X12313581		NOEC _{corr} = 1.985 mg/kg dw*	EFSA, 2018
<i>Eisenia fetida</i>	X6964872	Est. Assumes 10X parent (including 2X Kow factor)	NOEC _{corr} = 0.99 mg/kg dw*	EFSA, 2018
<i>Folsomia candida</i>	X6964872		NOEC _{corr} = 0.397 mg/kg dw*	EFSA, 2018
<i>Hypoaspis aculeifer</i>	X6964872		NOEC _{corr} = 1.985 mg/kg dw*	EFSA, 2018
<i>Eisenia fetida</i>	X696476	Mixed into substrate 56 d, chronic 10 % 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 _{corr} = 0.99 mg/kg dw*	EFSA, 2018
<i>Folsomia candida</i>	X12314005		NOEC _{corr} = 0.397 mg/kg dw*	EFSA, 2018

Species	Substance	Exposure System	Results	Reference
<i>Hypoaspis aculeifer</i>	X12314005		NOEC _{corr} = 1.985 mg/kg dw*	EFSA, 2018
<i>Eisenia fetida</i>	X763024	Est. Assumes 10X parent (including 2X Kow factor)	NOEC _{corr} = 0.99 mg/kg dw*	EFSA, 2018
<i>Folsomia candida</i>	X763024		NOEC _{corr} = 0.397 mg/kg dw*	EFSA, 2018
<i>Hypoaspis aculeifer</i>	X763024		NOEC _{corr} = 1.985 mg/kg dw*	EFSA, 2018
<i>Eisenia fetida</i>	X12019520	Est. Assumes 10X parent (including 2X Kow factor).	NOEC _{corr} = 0.99 mg/kg dw*	EFSA, 2018
<i>Folsomia candida</i>	X12019520		NOEC _{corr} = 0.397 mg/kg dw*	EFSA, 2018
<i>Hypoaspis aculeifer</i>	X12019520		NOEC _{corr} = 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 _{corr} = 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 _{corr} = 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 _{corr} = 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) – prothioconazole and JAU 6476-desthio

Species	Substance	Exposure System	Results*	Reference
<i>Eisenia fetida</i>	Prothioconazole	acute 10 % peat content	LC ₅₀ >1000 mg/kg dw LC _{50-corr} = 500 mg/kg dw	EFSA, 2007
<i>Eisenia fetida</i>	Prothioconazole (EC 250)	chronic 10 % peat content	NOEC = 1.33 mg/kg dw (1000 g a.s./ha) NOEC _{corr} = 0.665 mg/kg dw	EFSA, 2007
<i>Folsomia candida</i>		chronic 10 % peat content	NOEC = 64 mg a.s./kg dw NOEC _{corr} = 32 mg/kg dw	EFSA, 2007
<i>Hypoaspis aculeifer</i>		chronic Lufa 2.1 soil, 0.9% organic carbon	NOEC = 100 mg a.s./kg dw	EFSA, 2007
Prothioconazole metabolites				
<i>Eisenia fetida</i>	JAU 6476-desthio	28 d, acute 10 % peat content	LC ₅₀ > 1000 mg/kg dw LC _{50-corr} = 500 mg/kg dw	EFSA, 2007
<i>Eisenia fetida</i>		Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 1 mg/kg NOEC _{corr} = 0.5 mg/kg dw	EFSA, 2007

Species	Substance	Exposure System	Results*	Reference
<i>Folsomia candida</i>		Mixed into substrate 28 d, chronic 10 % peat content	NOEC = 62.5 mg/kg dw NOEC _{corr} = 31.25 mg/kg dw	EFSA, 2007
<i>Hypoaspis aculeifer</i>		Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 100 mg/kg dw EC ₁₀ >100 mg/kg NOEC _{corr} =50 mg/kg dw	Schulz/2014/Report# 141048102S
<i>Eisenia fetida</i>	JAU-6476-s-methyl	28 d, acute 10 % peat content	NOEC = 1000 mg/kg NOEC _{corr} = 500 mg/kg dw	EFSA, 2007
<i>Eisenia fetida</i>		Mixed into substrate 56 d, chronic	NOEC = 100 mg/kg NOEC _{corr} = 50 mg/kg dw	EFSA, 2007
<i>Folsomia candida</i>		Mixed into substrate 28 d, chronic 10 % peat content	NOEC = 31.6 mg/kg NOEC _{corr} = 15.3 mg/kg dw	EFSA, 2007
<i>Hypoaspis aculeifer</i>		Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 100 mg/kg EC ₁₀ >100 mg/kg NOEC _{corr} = 50 mg/kg	Schulz/2014/Report# 141048103S

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

zRMS comments:

Endpoints presented in Table 9.8-1 are in line with the EU agreed endpoints reported in EFSA Journal 2018;16(1):5146.

Table 9.8-3: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) – GF-3307

Species	Substance	Exposure System	Results*	Reference
<i>Eisenia fetida</i>	GF-3307	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 120 mg/kg dw NOEC _{corr} = 60 mg/kg dw	Ganßmann/2014/DAS# 140234
<i>Folsomia candida</i>	GF-3307	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 38 mg/kg dw NOEC _{corr} = 19 mg/kg dw	Ganßmann/2014/DAS# 140227
<i>Hypoaspis aculeifer</i>	GF-3307	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 150 mg/kg dw NOEC _{corr} = 75 mg/kg dw	Ganßmann/2014/DAS# 140230

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

zRMS comments:

Studies on toxicity of GF-3307 to earthworms and other non-target soil organisms were evaluated by the zRMS in course of zonal authorisation of the product GF-3307 in cereals and is relevant for the current evaluation of GF-3307 for extended uses.
For details of evaluation, please refer to Appendix 2. Endpoints reported in Table 9.8-3 are confirmed to be correct.

9.8.1.1 Justification for new endpoints

Not applicable.

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_{soil} for risk assessments covering the proposed use pattern are taken from Section 8.7. According to the assessment of environmental-fate data, multi-annual accumulation in soil does not need to be considered for fenpicoxamid or prothioconazole.

The GAP modelled for the active substances and the formulation was aligned to the current GAP of 2 x 1.5 L GF-3307/ha.

Table 9.8-4: PEC_{soil} for fenpicoxamid, prothioconazole, relevant metabolites, and GF-3307 (S7K-3-3) in sugar beet/fodder beet

Product/metabolite/active substance	Initial PEC_{soil} (mg/kg dw)
Fenpicoxamid	0.0411
X642188	0.0178
X11963422	0.0086
X12264475	0.0114
X12313581	0.0027
X696872	0.0055
X696476	0.0077
X12314005	0.0007
X763024	0.001
X12019520	0.001
X12255349	0.0018
Prothioconazole	0.0603
JAU 6476-desthio	0.0565
JAU 6476-S-methyl	0.0157
GF-3307	1.253

Note: X696476 has a PEC accumulation value of 0.0539 mg/kg.

The results of the first-tier risk assessment for fenpicoxamid and GF-3307 are summarised in the following table.

Table 9.8-5: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of GF-3307 (S7K-3-3) in sugar beet/fodder beet

Sugar beet/fodder beet			
Intended use	Sugar beet/fodder beet		
Acute effects on earthworms			
Product/active substance	LC ₅₀ or LC _{50-corr} [†] (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _a (criterion TER ≥ 10)
Prothioconazole	500	0.0603	8292
JAU 6476-desthio	500	0.0565	8850
JAU 6476-S-methyl	500	0.0157	31847
Chronic effects on earthworms			

Product/active substance	NOEC or NOEC _{corr} [†] (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{it} (criterion TER ≥ 5)
Fenpicoxamid	9.925	0.0411	241
X642188	2.8	0.0178	157
X11963422	10	0.0086	1163
X12264475	10	0.0114	877
X12313581	0.99	0.0027	367
X696872	0.99	0.0055	180
X696476	10	0.0077	1299
X12314005	0.99	0.0007	1414
X763024	0.99	0.001	990
X12019520	0.99	0.001	990
X12255349	5	0.0018	2778
Prothioconazole	0.665	0.0603	11
JAU 6476-desthio	0.5	0.0565	9
JAU 6476-S-methyl	50	0.0157	3185
GF-3307	60	1.253	48
Chronic effects on other soil macro- and mesofauna: <i>Folsomia candida</i>			
Product/active substance	NOEC or NOEC _{corr} [†] (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{it} (criterion TER ≥ 5)
Fenpicoxamid	3.97	0.0411	97
X642188	5	0.0178	281
X11963422	5	0.0086	581
X12264475	10	0.0114	877
X12313581	0.397	0.0027	147
X696872	0.397	0.0055	72
X696476	10	0.0077	1299
X12314005	0.397	0.0007	567
X763024	0.397	0.001	397
X12019520	0.397	0.001	397
X12255349	5	0.0018	2778
Prothioconazole	32	0.0603	531
JAU 6476-desthio	31.25	0.0565	553
JAU 6476-S-methyl	15.5	0.0157	975
GF-3307	19	1.253	15
Chronic effects on other soil macro- and mesofauna: <i>Hypoaspis aculeifer</i>			
Product/active substance	NOEC or NOEC _{corr} [†] (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{it} (criterion TER ≥ 5)
Fenpicoxamid	19.85	0.0411	483
X642188	10	0.0178	562
X11963422	10	0.0086	1163
X12264475	10	0.0114	877
X12313581	1.985	0.0027	735

X696872	1.985	0.0055	361
X696476	10	0.0077	1299
X12314005	1.985	0.0007	2836
X763024	1.985	0.001	1985
X12019520	1.985	0.001	1985
X12255349	5	0.0018	2778
Prothioconazole	100	0.0603	1658
JAU 6476-desthio	50	0.0565	885
JAU 6476-S-methyl	50	0.0157	3185
GF-3307	75	1.253	60

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

†Corrected values (i.e. divided by 2) are shown for endpoints where the log K_{ow} is > 2.

TER_A values for prothioconazole and relevant metabolites are above the Annex VI trigger value of 10 indicating there is low acute risk to earthworms. TER_{LT} values for fenpicoxamid, prothioconazole, relevant metabolites, and GF-3307 (S7K-3-3) are above the Annex VI trigger value of 5 indicating there is acceptable chronic risk to earthworms, meso-, and macrofauna at the proposed GAP.

As mentioned previously, X646476 has the potential to accumulate in soil, therefore an additional assessment using the PEC_{acc} is shown below.

Table 9.8-6: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of GF-3307 (S7K-3-3) in sugar beet/fodder beet_X696476 using PEC_{soil-acc}

Intended use	Sugar beet/fodder beet		
Metabolite	X646476		
Species	NOEC _{corr} (mg/kg dw)	PEC _{soil-acc} (mg/kg dw)	TER _{it} (criterion TER ≥ 5)
<i>Eisenia fetida</i>	10	0.1645	61
<i>Folsomia candida</i>	10		61
<i>Hypoaspis aculeifer</i>	10		61

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

TER_{LT} 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 9.8-4 is confirmed to be in line with PEC_{SOIL} values agreed by the zRMS in area of Section 8. The risk assessment presented in Table 9.8-5 for both active substances and their metabolites is in general agreed by the zRMS.

For metabolite X696476 the PEC_{SOIL,ACCU} separate risk assessment based on accumulated PEC_{SOIL} has been used.

Overall, acceptable risk to soil macro- and meso-fauna may be concluded from the intended Central Zone uses of GF-3307.

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

TER_A values for prothioconazole and relevant metabolites are above the Annex VI trigger value of 10 indicating there is low acute risk to earthworms. TER_{LT} values for fenpicoxamid, prothioconazole, relevant metabolites, and GF-3307 (S7K-3-3) are above the Annex VI trigger value of 5 indicating there is acceptable chronic risk to earthworms, meso-, and macrofauna at the proposed GAP.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

Studies on effects on soil microorganisms have been carried out with fenpicoxamid, prothioconazole, and relevant metabolites. Full details of these studies are provided in the respective EU DAR (fenpicoxamid: United Kingdom, 2017; prothioconazole: United Kingdom, 2007) and related documents as well as in Appendix 2 of this document (new studies).

Effects on soil microorganisms of GF-3307 (S7K-3-3) 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	Nitrate formation rate 2.187 mg/kg soil dw + 8.50 %	EFSA, 2018
C-mineralisation	Fenpicoxamid	28 d, aerobic soil type	CO ₂ formation 2.187 mg/kg soil dw - 4.39 %	Not required
Fenpicoxamid metabolites				
N-mineralisation	X642188	28 d, aerobic soil type	Nitrate formation rate 0.438 mg/kg soil dw + 2.6 %	EFSA, 2018
C-mineralisation	X642188	28 d, aerobic soil type	CO ₂ formation 0.438 mg/kg soil dw - 1.10 %	Not required
N-mineralisation	X11963422	28 d, aerobic soil type	Nitrate formation rate 0.600 mg/kg soil dw - 3.6 %	EFSA, 2018
N-mineralisation	X12264475	28 d, aerobic soil type	Nitrate formation rate 0.733 mg/kg soil dw + 5.04 %	EFSA, 2018
N-mineralisation	X12313581		Not available*	EFSA, 2018
N-mineralisation	X696872		Not available*	EFSA, 2018
N-mineralisation	X696476	28 d, aerobic soil type	Nitrate formation rate 0.533 mg/kg soil dw + 1.92 %	EFSA, 2018
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	Nitrate formation rate 1.467 mg/kg soil dw + 8.91 %	EFSA, 2018

zRMS comments:

Endpoints presented in Table 9.9-1 are in line with the EU agreed endpoints reported in EFSA Journal 2018;16(1):5146.

Table 9.9-2: Endpoints and effect values relevant for the risk assessment for soil microorganisms – prothioconazole and relevant metabolites

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Prothioconazole	28 d, aerobic	No effects up to 2.0 kg as/ha (2.667 mg/kg dsw)	EFSA, 2007
C-mineralisation	Prothioconazole	28 d, aerobic	No effects up to 2.0 kg as/ha (2.67 mg/kg dsw)	EFSA, 2007
Prothioconazole metabolites				
N-mineralisation	JAU 6476-desthio	28 d, aerobic	No effects up to 1.0 kg as/ha (1.33 mg/kg dsw)	EFSA, 2007
N-mineralisation	JAU 6476-S-methyl	28 d, aerobic	No effects up to 2.0 kg/ha (2.69 mg/kg dsw)	EFSA, 2007

zRMS comments:

Endpoints presented in Table 9.9-2 are in line with the EU agreed endpoints reported in EFSA Scientific Report (2007) 106, 1-98.

Table 9.9-3: Endpoints and effect values relevant for the risk assessment for soil microorganisms – GF-3307 (S7K-3-3)

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	GF-3307	28 d, aerobic soil type	Nitrate formation rate 13.92 mg/kg soil dw -11.23%	Hammesfahr/2014/DAS# 140237
C-mineralisation	GF-3307	28 d, aerobic soil type	CO ₂ formation 13.92 mg/kg soil dw -5.08 %	Hammesfahr/2014/DAS# 140237

zRMS comments:

The study on effects of GF-3307 on soil nitrogen transformation was evaluated by the zRMS in course of zonal authorisation of the product GF-3307 in cereals (Core Dossier 2023) and is relevant for the current evaluation of GF-3307 for extended uses.

For details of evaluation, please refer to Appendix 2. The endpoint reported in Table 9.9-3 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 from 9.9-1 and 9.9-3.

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_{soil} 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).

The GAP modelled for the active substances and for the formulation was aligned to the current GAP of 2 x 1.5 L GF-3307/ha.

The results of the risk assessment for fenpicoxamid, prothioconazole, relevant metabolites and GF-3307 (S7K-3-3) are summarised in the following table.

Table 9.9-4: Assessment of the risk for effects on soil micro-organisms due to the use of GF-3307 (S7K-3-3) in sugar beet/fodder beet

Intended use		Sugar beet/fodder beet	
N-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
Fenpicoxamid	2.187 (at 14-28 d)	0.0411	yes
X642188	0.438 (at 14-28 d)	0.0178	yes
X11963422	0.600 (at 14-28 d)	0.0086	yes
X12264475	0.733 (at 14-28 d)	0.0114	yes
X696476	0.533 (at 14-28 d)	0.1645	yes
X12255349	1.467 (at 14-28 d)	0.0018	yes
X12313581	0.219 *	0.0027	yes
X696872	0.219 *	0.0055	yes
X12314005	0.219 *	0.0007	yes
X763024	0.219 *	0.001	yes
X12019520	0.219 *	0.001	yes
Prothioconazole	2.667 (at 14-28 d)	0.0603	yes
JAU 6476-desthio	1.333 (at 14-28 d)	0.0565	yes
JAU 6476-S-methyl	2.69	0.0157	yes
GF-3307	13.92 (at 14-28 d)	1.253	yes
C-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
Fenpicoxamid	2.187 (at 28 d)	0.0411	yes
X642188	0.438 (at 28 d)	0.0178	yes
Prothioconazole	2.667 (at 28 d)	0.0603	yes
GF-3307	13.92 (at 28 d)	1.253	yes

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

Acceptable risk is demonstrated when GF-3307 (S7K-3-3) is used according to the proposed GAP.

zRMS comments:

The risk assessment presented in Table 9.9-4 is in general agreed by the zRMS. For metabolite X696476 the PEC_{SOIL,ACCU} 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 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-3307.

9.9.3 Overall conclusions

The maximum concentrations with less than 25% effects for fenpicoxamid, prothioconazole, 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 GF-2925) and prothioconazole. Full details of these studies are provided in the respective EU DAR (fenpicoxamid: United Kingdom, 2017; prothioconazole: United Kingdom, 2007) and related documents as well as in Appendix 2 of this document (new studies).

Effects on non-target terrestrial plants of GF-3307 (S7K-3-3) 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
Seedling emergence				
Sunflower	GF-2925 (fenpicoxamid)	21 d Seedling emergence	ER ₅₀ >2 L prep/ha (>260 g a.s./ha)	EFSA, 2018
<i>Amaranthus retroflexus</i>	Prothioconazole	21 d Seedling emergence	ER ₅₀ >200 g a.s./ha	EFSA, 2007
<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-3307	21 d Seedling emergence	ER ₅₀ >4 L prep/ha	Brockmann/2014/D AS# 140707
Vegetative vigour				
Oat	GF-2925 (fenpicoxamid)	21 d Vegetative vigour	ER ₅₀ >2 L prep/ha (>260 g a.s./ha)	EFSA, 2018
<i>Amaranthus retroflexus</i>	Prothioconazole	21 d Vegetative vigour	ER ₅₀ >250 g a.s./ha	EFSA, 2007
<i>Lolium perenne</i> , <i>Avena sativa</i> , <i>Allium cepa</i> ,	GF-3307	21 d Vegetative vigour	ER ₅₀ >4 L prep/ha	Brockmann/2014/D AS# 140555

Species	Substance	Exposure System	Results	Reference
<i>Glycine max, Brassica napus, Beta vulgaris, Daucus carota, Cucumis sativa, Helianthus annuus, Lycopersicon esculentum</i>				

m: monocotyledonous; d: dicotyledonous

zRMS comments:

Endpoints presented in Table 9.10-1 are in line with the EU agreed endpoints reported in EFSA Journal 2018;16(1):5146 and EFSA Scientific Report (2007) 106, 1-98.

Studies on toxicity of GF-3307 to non-target terrestrial plants were evaluated in course of zonal authorisation of the product GF-3308, GF-3307 in cereals and is relevant for the current evaluation of GF-3307 for extended uses.

For details of evaluation, please refer to 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-3307 (S7K-3-3) 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 sugar beet/fodder beet and are thus considered an indicator for an acceptable risk.**

$PER_{\text{off-field}} = \text{Application rate} \times \text{MAF} \times \text{drift factor}$

Table 9.10-2: Assessment of the risk for non-target plants due to the use of GF-3307 (S7K-3-3) in sugar beet/fodder beet

Intended use	Sugar beet			
Active substance/product	GF-3307			
Application rate	2 × 1.5 L/ha, interval of 21 days			
MAF	1.7			
Test species	ER ₅₀	Drift rate	PER _{off-field} (L/ha)	TER criterion: TER ≥ 5
<i>Lolium perenne, Avena</i>	ER ₅₀ >4 L prep/ha	2.38%	0.0607 (VV)	>66

<i>sativa, Allium cepa, Glycine max, Brassica napus, Beta vulgaris, Daucus carota, Cucumis sativa, Helianthus annuus, Lycopersicon esculentum</i>			0.0607 (SE)	>66
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MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.

zRMS comments:

Based on performed calculations acceptable risk to NTTP_s may be concluded for application of GF-3307 at 1.5 L/ha without the need of any specific risk mitigations.

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-3307 (S7K-3-3) in sugar beet/fodder beet according to good agricultural practice is acceptable.

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 is H410; see Part C for full explanation

zRMS comments:


Endpoints from studies on acute toxicity of GF-3307 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 addition, the active substance is considered as not readily biodegradable.

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-3307 of 6.39 % (based on pure active ingredient) multiplied by M factor of 100 gives 639 %, 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-3307:

Hazard pictograms:	GHS09 
Signal word:	Warning
Hazard statement(s):	H410 - Very toxic to aquatic life with long lasting effects
Precautionary statement(s):	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.

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: EN-924.

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.

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

EFSA, 2019. Technical report: Outcome of the pesticide peer review meeting on general recurring issues in ecotoxicology (PPRM 185). EFSA Journal 2019: EN-1673.

Appendix 1 Lists of data considered in support of the evaluation

Please sort by data points and within one data point by names of authors

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	██████	2014	GF-3307: An Acute Oral Toxicity Study with the Northern Bobwhite using a Sequential Testing Procedure DAS Report No.140447 ██████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
KCP 10.2.1/1	██████	2014, revised 2017	GF-3307: Acute Toxicity to the Rainbow Trout, Oncorhynchus mykiss, Determined Under Static-Renewal Test Conditions DAS Report No.140479 ██████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
KCP 10.2.1/2	Hadsell, R. L., Hoover, E.	2014, revised 2018	GF-3307: Acute Toxicity to the Cladoceran, Daphnia magna, 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	No	Corteva Agriscience
KCP 10.2.1/3	Hicks, S	2014, Final report addendum 2020	GF-3307: Growth Inhibition Test with the Unicellular Green Alga, Pseudokirchneriella subcapitata DAS Report No.140491 ABC Laboratories, Inc., 7200 E. ABC Lane Columbia, Missouri 65202, USA GLP/GEP (Y/N): Yes Published (Y/N): No	No	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	No	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 10.2.1/5	██████	2018a	X12019520 (a metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, Oncorhynchus mykiss, Determined Under Static-Renewal Test Conditions DAS# 180560 ██████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
KCP 10.2.1/6	██████	2018b	X12446477 (metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, Oncorhynchus mykiss, Determined Under Static-Renewal Test Conditions DAS# 180561 ██████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
KCP 10.2.1/7	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	No	Corteva Agriscience
KCP 10.2.1/8	██████	2018	GF-3307: Acute Toxicity to the Rainbow Trout, Oncorhynchus mykiss, Determined Under Flow-Through Test Conditions DAS Report No.180975 ██████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
KCP 10.2.1/9	Goudie, O.J	2020a	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	No	Corteva Agriscience
KCP 10.2.1/10	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	No	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 10.2.1/1	Gossion, G.	2025	Fenpicoxamid: Acute Immobilisation Toxicity Test with the Mayfly (<i>Cloeon-dipterum</i>) under Semi-Static Conditions Corteva Report No. 2025-0642 Lab Report No. G25-10 GG BioTech Design GmbH, Homburg (Ohm), Germany GLP/GEP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience
KCP 10.2.1/2	Goudie, O.J.	2020	X642188 (a metabolite of XDE-777): Toxicity Test with the Cladoceran, <i>Daphnia magna</i> , Conducted Under Pulse-Dosed, Static-Renewal Conditions Corteva Report No. 191071 Lab Report No. 88456 Eurofins EAG Agrosience LLC, Columbia, Missouri, USA GLP/GEP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience
KCP 10.2.2/1	Beasley, J.	2018	X1642188 (a metabolite of XDE-777): Chronic Toxicity in Whole Sediment to Freshwater Midge, <i>Chironomus riparius</i> , Using Spiked Sediment DAS# 180563 ABC Laboratories, Inc., Columbia, Missouri, USA GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
KCP 10.2.2/2	Leak, T.	2018	X12335723 (a metabolite of XDE-777): Chronic Toxicity in Whole Sediment to Freshwater Midge, <i>Chironomus riparius</i> , Using Spiked Sediment DAS# 180564 ABC Laboratories, Inc., Columbia, Missouri, USA GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
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 Agrosiences, 9330 Zionsville Rd, Indianapolis, IN 46268 GLP/GEP (Y/N): No Published (Y/N): No	No	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 10.2.3/2	Hicks, S.	2016	GF-3308: Population Effects Study in an Indoor Aquatic Microcosm with <i>Daphnia magna</i> DAS# 160126 ABC Laboratories, Inc., 7200 E. ABC Lane Columbia, Missouri 65202, USA GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
KCP 10.2.3/3	Hicks, S.	2017	XDE-777: Population Effects Study in an Indoor Aquatic Microcosm with <i>Daphnia magna</i> DAS# 160125 ABC Laboratories, Inc., 7200 E. ABC Lane Columbia, Missouri 65202, USA GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
KCP 10.2.3/4	Lamichhane, K.	2015	X642188 (a metabolite of XDE-777): Population Effects Study in an Indoor Aquatic Microcosm with <i>Daphnia magna</i> DAS# 131295 ABC Laboratories, Inc., Columbia, Missouri, USA GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
KCP 10.2.3/5	Mathieson, T.	2018	Efficacy of XDE-777 metabolites to <i>Septoria tritici</i> on wheat DAS# NA Dow AgroSciences, LLC, Zionsville, Indiana, USA GLP/GEP (Y/N): No Published (Y/N): No	No	Corteva Agriscience
KCP 10.2.3/6	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	No	Corteva Agriscience
KCP 10.2.3/7	Brüggemann, M., Böhmer, W., Kosak, L	2020	GF-3307: Population Effects Study in an Indoor Aquatic Microcosm with <i>Daphnia magna</i> DAS Study No. 181382 Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Schmallenberg, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
KCP	Noël, E.	2015a	GF-3307: A laboratory study to determine the acute oral toxicity on the honey bees <i>Apis mellifera</i> L.	No	Corteva

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
10.3.1.1.1/1			(Hymenoptera: Apidae). DAS Report No.150736 SynTech Research France S.A.S., La Chapelle de Guinchay, France GLP/GEP (Y/N): Yes Published (Y/N): No		Agriscience
KCP 10.3.1.1.1/2 KCP 10.3.1.1.2/1	Schmitzer, S	2014	GF-3307: Acute contact and oral effects on honeybees (<i>Apis mellifera</i> L.) in the laboratory DAS Report No.140220 & 140213 Institut für Biologische Analytik und Consulting IBACON GmbHArheilger Weg 17, 64380 Rossdorf, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
KCP 10.3.1.1.1/3	Cornement, M., Morgenthal, K.	2022	XDE-777 TGAI - Acute Oral and Contact Toxicity to Bumble Bees (<i>Bombus terrestris</i>) under Laboratory Conditions Corteva Report No. 201076 IES GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
KCP 10.3.1.1.1/4	Cornement, M., Morgenthal, K.	2022	GF-3307 - Acute Oral and Contact Toxicity to Bumble Bees (<i>Bombus terrestris</i>) under Laboratory Conditions Corteva Report No. 201075 IES GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
KCP 10.3.1.1.2/2	Noël, E.	2015b	GF-3307: A laboratory study to determine the acute contact toxicity on the honey bees <i>Apis mellifera</i> L. (Hymenoptera: Apidae). DAS Report No.150737 SynTech Research France S.A.S., La Chapelle de Guinchay, France GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
KCP 10.3.1.2/1	Oberrauch, S.	2018	GF-3307 - Honey Bee (<i>Apis mellifera</i> L.) 22 Day Larval Toxicity Test (Repeated Exposure) DAS# 171043 Institut für Biologische Analytik und Consulting IBACON GmbH, 64380 Rossdorf, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
KCP 10.3.1.2/2	Verge, E., Kastel, A.	2018	GF-3307 - Assessment of Effects on the Adult Honey Bee, <i>Apis mellifera</i> L., in a 10 Day Chronic Feeding Test under Laboratory Conditions	No	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
			DAS# 170077 Eurofins Agroscience Services EcoChem / Eurofins Agroscience Services Ecotox GmbH GLP/GEP (Y/N): Yes Published (Y/N): No		
KCP 10.3.1.5	Kleinhenz, M.	2018	GF-3307 (Fenpicoxamid + Prothioconazole): Brood Development of the Honeybee (<i>Apis mellifera</i> L.) in a Semi-Field Tunnel Study in <i>Phacelia tanacetifolia</i> in Germany 2017 DAS Report No. 170673 Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH, Niefern-Öschelbronn, Germany GLP: Yes Published: No	No	Corteva Agriscience
KCP 10.3.1.6/1	Appeltauer, A.	2021	Determination of Residues of Fenpicoxamid and Prothioconazole in Nectar, Pollen and Plants of Winter Oilseed Rape after One Application of GF-3307 in a Semi-Field Residue Study in Central and Southern Europe in 2020 DAS Study No. 200670 Eurofins Agroscience Services Ecotox GmbH, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
KCP 10.3.1.6/2	Gonsoir, G.	2021	Assessment of Side-Effects on the GF-3307 (Fenpicoxamid and Prothioconazole): Brood Development of the Honey Bee (<i>Apis mellifera</i> L.) in a Colony Feeding Test in Germany 2020 DAS Report No. 200660 Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH, Niefern-Öschelbronn, Germany GLP: Yes Published: No	No	Corteva Agriscience
KCP 10.3.1.6/4	United States Environmental Protection Agency (US EPA)	2019	EPA (2019). Guidance for Assessing Pesticide Risks to Bees. Office of Pesticide Programs United States Environmental Protection Agency.	No	US EPA
KCP 10.3.1.6/5	US EPA	2020	EPA (2020). Final Bee Risk Assessment to Support the Registration Review of Clothianidin and Thiamethoxam. United States Environmental Protection Agency Office of Chemical Safety and Pollution Prevention. PC Codes: 044309, 060109, DP Barcode: 455645	No	US EPA

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 10.3.1.6/6	Last, G. et al.	2019	Last G, Lewis G, Pap G (2019) Regulatory report on the occurrence of flowering weeds in agricultural fields. ERM report Nr. 0482579. ERM, Harrogate, United Kingdom	No	ERM
KCP 10.3.2.1/2	Moll, M.	2014a	GF-3307: Effects on the Predatory Mite Typhlodromus pyri in the Laboratory (Tier I) - Dose Response Test - DAS Report No.140226 Institut für Biologische Analytik und Consulting IBACON GmbH, 64380 Rossdorf, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
KCP 10.3.2.1/1	Moll, M.	2014b	GF-3307: Effects on the Parasitoid Aphidius rhopalosiphi in the Laboratory (Tier I) - Dose Response Test DAS Report No.140224 Institut für Biologische Analytik und Consulting IBACON GmbH, 64380 Rossdorf, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
KCP 10.3.2.1/3	Tew, G.	2020	GF-3307: A laboratory Study of the Effects of Freshly Treated Substrate on the Rove Beetle, Aleochara bilineata (Coleoptera, Staphylinidae) DAS Report No.200609 Mambo Tox, Southampton, UK GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
KCP 10.3.2.2/1	Kimmel, S.	2016a	GF-3307: Effects on mortality and reproduction to Coccinella septempunctata L (Coleoptera:Coccinellidae) under extended Laboratory Conditions DAS Report No. 150923 Innovative Enivironmental Services (IES) Ltd, Witterswil, Switzerland GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
KCP 10.3.2.2/2	Kimmel, S.	2016b	GF-3307: Effects to the Parasitoid Rove Beetle Aleochara bilineata (Coleoptera: Staphylinidae) under extended Laboratory Conditions DAS Report No. 150926 Innovative Enivironmental Services (IES) Ltd, Witterswil, Switzerland GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 10.3.2.2/3	Moll, M.	2014c	GF-3307: Effects on the Lacewing Chrysoperla carnea under Extended Laboratory Conditions (Tier II) DAS Report No.140948 Institut für Biologische Analytikund Consulting IBACON GmbH, Arheilger Weg 1764380 Rossdorf Germany GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
KCP 10.3.2.2/4	Moll, M.	2014d	GF-3307: Effects on the Parasitoid Aphidius rhopalosiphi, Extended Laboratory Study (Tier II) - Dose Response Test DAS Report No.140947 Institut für Biologische Analytikund Consulting IBACON GmbH, Arheilger Weg 1764380 Rossdorf Germany GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
KCP 10.3.2.2/5	Schmitzer, S.	2015	GF-3307: Effects on the Wolf Spider Pardosa spec. in the Laboratory – Extended Laboratory Study (Tier II) DAS Report No.150927 Institut für Biologische Analytikund Consulting IBACON GmbH, Arheilger Weg 1764380 Rossdorf Germany GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
KCP 10.3.2.2/6	Tew, G.	2020	GF-3307: A Rate-Response Extended Laboratory Study of the Effects of Freshly Treated Substrate on the Rove Beetle, Aleochara bilineata (Coleoptera, Staphylinidae) DAS Report No. 200610 Mambo-Tox, Southhampton, UK GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
KCP 10.3.2.3/1	Stevens, J.	2016	GF-3307: An aged residue extended laboratory study on the parasitic wasp Aphidius rhopalosiphi (Hymenoptera, Braconidae) DAS Report No.150924 Mambo-Tox Ltd., 2 Venture Road, University Science Park, Southampton SO16 7NP, UK GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
KCP 10.3.2.3/2	Vaughan, R.	2015	GF-3307: An aged-residue extended laboratory study with the green lacewing Chrysoperla carnea (Neuroptera, Chrysopidae) DAS Report No.150925 Mambo-Tox Ltd.Southampton SO16 7NP, UK GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 10.3.2.3/3	Vaughan, R.	2018	GF-3307: An aged-residue extended laboratory tests to determine effects on the ladybird beetle, Coccinella septempunctata (Coleoptera, Coccinellidae DAS Report No.170778 Mambo-Tox Ltd.Southampton SO16 7NP, UK GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
KCP 10.4.1.1/1	Ganßmann, M.	2014a	GF-3307: Effects on Reproduction and Growth of Earthworms Eisenia fetida in Artificial Soil with 10% Peat DAS Report No.140234 Institut für Biologische Analytikund Consulting IBACON GmbH, Arheilger Weg 1764380 Rossdorf Germany GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
KCP 10.4.2.1/1	Ganßmann, M.	2015	GF-3307: Effects on Reproduction of the Collembola Folsomia candida in Artificial Soil with 5% Peat DAS Report No.140227 Institut für Biologische Analytik und Consulting IBACON GmbH Arheilger Weg 17 64380 Rossdorf Germany GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
KCP 10.4.2.1/2	Ganßmann, M.	2014b	GF-3307: Effects on Reproduction of the Predatory Mite Hypoaspis aculeifer in Artificial Soil with 5% Peat DAS Report No.140230 Institut für Biologische Analytikund Consulting IBACON GmbH, Arheilger Weg 1764380 Rossdorf Germany GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
KCP 10.5/1	Hammesfahr, U.	2014	GF-3307: Effects on the Activity of the Soil Microflora in the Laboratory DAS Report No.140237 Institut für Biologische Analytik, und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
KCP 10.6.2/1	Brockmann, A.	2014a	GF-3307 (XDE-777 + prothioconazole 50 + 100 g as/L, EC): A Vegetative Vigour Test with ten Non Target Plant Species, GLP DAS Report No.140555 agro-check Dr. Teresiak & Erdmann GbR, Dorfstr.15D-16833 Lentzke, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
KCP 10.6.2/2	Brockmann, A., Teresiak, H..	2014b	GF-3307 (XDE-777 + prothioconazole 50 + 100 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	No	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
			208) – Europe 2014 DAS Report No.140707 agro-check Dr. Teresiak & Erdmann GbR, Dorfstr.15 D-16833 Lentzke, Germany GLP/GEP (Y/N): Yes Published (Y/N): No		

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

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 8.1.1.1 /1	██████	2012	XR-777: An Acute Oral Toxicity Study with the Northern Bobwhite Using a Sequential testing Procedure DAS Report No.: 110247 ██████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
CA 8.1.1.3 /1	██████	2013	XDE-777 TGAI: A Reproduction Study with the Northern Bobwhite DAS Report No.: 120384 ██████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
CA 8.1.1.3/2	██████	2015	XDE-777: Reproductive Toxicity Test with the Northern Bobwhite (<i>Colinus virginianus</i>) (Amended report) DAS Report No.: 140424 ██████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
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>). DAS Study No. 120384A Lab: Wildlife International; Dow AgroSciences; GLP/GEP (Y/N): N Published (Y/N): N	No	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 8.1.1.3/4	Valverde P	2016	XDE-777: Comparative analysis of historical control data in the reproductive toxicity tests with the northern bobwhite (<i>Colinus virginianus</i>). DAS Study No. 140424B Lab: Smithers Viscient; Dow AgroSciences; GLP/GEP (Y/N): N Published (Y/N): N	No	Corteva Agriscience
CA 8.2.1 /1	██████	2012	XR-777 - Acute Toxicity to Rainbow Trout (<i>Oncorhynchus mykiss</i>) Under Flow-Through Conditions, Following OECD Guideline #203 DAS Report No.: 110213 ██████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
CA 8.2.1 /2	██████	2012	XDE-777 Technical: Acute Toxicity to the Common Carp, <i>Cyprinus carpio</i> , Determined Under Flow-Through Test Conditions DAS Report No.: 120392 ██████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
CA 8.2.1 /3	██████	2012	X642188 Metabolite: Acute Toxicity Test with the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Flow-Through Test Conditions DAS Report No.: 120382 ██████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
CA 8.2.1/4	██████	2014	X11963422 (a metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions (Revision) DAS Report No.: 130361 ██████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	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	████	2014	X12264475 (a metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 130360 ████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
CA 8.2.1/6	████	2014	X12313581 (a metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 130362 ████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
CA 8.2.1/7	████	2014	X696872 (a metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 130363 ████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
CA 8.2.1/8	████	2014	X696476 (a metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 130364 ████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
CA 8.2.1/9	████	2014	X12314005 (a metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 130365 ████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
CA 8.2.1/10	████	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 ████	Yes	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
			GLP/GEP (Y/N): Yes Published (Y/N): No		
CA 8.2.1	██████	2016	XDE-777: Acute Toxicity to the Zebra Fish, <i>Danio rerio</i> , Determined Under Flow-Through Test Conditions DAS Report No. 160129 ██████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
CA 8.2.1	██████	2016	XDE-777: Acute Toxicity to the Fathead minnow, <i>Pimephales promelas</i> , Determined Under Flow-Through Test Conditions DAS Report No. 160130 ██████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
CA 8.2.1	██████	2016	XDE-777: Acute Toxicity to the Bluegill, <i>Lepomis macrochirus</i> , Determined Under Flow-Through Test Conditions DAS Report No. 161022 ██████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
CA 8.2.2.1 /1	██████	2012	XR-777 TGAI – Early Life-Stage Toxicity Test with Fathead Minnow, <i>Pimephales promelas</i> , Following OECD Guideline #210 DAS Report No.: 110214 ██████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
CA 8.2.2.1/2	██████	2016	XDE-777: Investigation of Larval Toxicity to the Fathead Minnow (<i>Pimephales promelas</i>) Under Static Conditions in a Water-Sediment System DAS Report No. 160128 ██████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
CA 8.2.2.3/1	██████	2014	XDE-777: Investigation of bioconcentration in zebrafish (<i>Danio rerio</i>) under flow-through conditions DAS Report No.: 130983 ██████	Yes	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
			GLP/GEP (Y/N): Yes Published (Y/N): No		
CA 8.2.2.3/2	██████	2015	14C-X696476: Bioconcentration and Metabolism Study with Zebrafish, Danio rerio DAS Report No.: 140481 ██████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
CA 8.2.2.3/3	██████	2014	14C-X12019520: Bioconcentration and Metabolism Study with Zebrafish, Danio rerio DAS Report No.: 140480 ██████ GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Corteva Agriscience
CA 8.2.4.1 /1	Fournier A	2012	XR-777 TGA1 - Acute Toxicity to Water Fleas (Daphnia magna) Under Static-Renewal Conditions, Following OECD Guideline #202 and JMAFF 12 NohSan, No. 8147 Daphnia Acute Immobilization Test (2-7-2-1) Data Requirement OECD Guideline 202 JMAFF 12 NohSan, No. 8147 (Revision) DAS Report No.: 110215 Smithers Viscient GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 DAS Report No.: 120381 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 DAS Report No.: 130372 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 8.2.4.1/04	Huffman	2014	X12264475 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 130371 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
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 DAS Report No.: 130373 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 Static-Renewal Test Conditions DAS Report No.: 130374 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 Static-Renewal Test Conditions DAS Report No.: 130375 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 DAS Report No.: 130376 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 8.2.4.1/09	Stadler T	2014	X12386481 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 130379 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 Static-Renewal Test Conditions DAS Report No.: 130378 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
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 DAS Report No.: 130380 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 DAS Report No.: 130377 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 DAS Report No.: 130383 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 8.2.4.1/14	Lamichhane K	2014	X12255349 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Exposed Under Static-Renewal Test Conditions DAS Report No.: 140484 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 Static-Renewal Test Conditions DAS Report No.: 140485 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 DAS Report No.: 130382 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
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 DAS Report No.: 140486 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 DAS Report No.: 140483 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 8.2.4.2/2	VanHooser, A.	2015a	XDE-777: Acute toxicity to the Freshwater Midge, Chironomus riparius, Determined Under Static-Renewal Test Conditions DAS Report No.: 141002 ABC Laboratories, Inc. GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 DAS Report No.: 141003 ABC Laboratories, Inc. GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 DAS Report No.: 141004 ABC Laboratories, Inc. GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 ABC Laboratories DAS Report No. 140999 GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
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 DAS Report No.: 110216 Smithers Viscient GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 8.2.6.1 /1	Rebstock M	2013	XDE-777: Growth Inhibition Test with the Unicellular Green Alga, <i>Pseudokirchneriella subcapitata</i> DAS Report No.: 120383 ABC Laboratories, Inc GLP/GEP (Y/N): Yes Published (Y/N): No	No	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> DAS Report No.: 120380 ABC Laboratories, Inc GLP/GEP (Y/N): Yes Published (Y/N): No	No	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> DAS Report No.: 130385 ABC Laboratories, Inc GLP/GEP (Y/N): Yes Published (Y/N): No	No	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> DAS Report No.: 130384 ABC Laboratories, Inc GLP/GEP (Y/N): Yes Published (Y/N): No	No	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> DAS Report No.: 141001 ABC Laboratories, Inc GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
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 DAS Report No.: 110168/110169 Institut für Biologische Analytik und Consulting IBACON GmbH GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 8.3.1.1.1/2 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 DAS Report No.: 140217/140221 Institut für Biologische Analytik und Consulting IBACON GmbH GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 DAS Report No.: 120379 Institut für Biologische Analytik und Consulting IBACON GmbH GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 DAS Report No.: 140215 Institut für Biologische Analytik und Consulting IBACON GmbH GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 DAS Report No.: 140216 Institut für Biologische Analytik und Consulting IBACON GmbH GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
CA 8.3.2.1 /1	Moll M	2013	Effects of XDE-777 on the Parasitoid <i>Aphidius rhopalosiphii</i> in the Laboratory (Tier I) - Dose Response Test - (Revision) DAS Report No.: 110170 Institut für Biologische Analytik und Consulting IBACON GmbH GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
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) DAS Report No.: 110171 Institut für Biologische Analytik und Consulting IBACON GmbH GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 8.4.1 /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 DAS Report No.: 110172 Institut für Biologische Analytik und Consulting IBACON GmbH GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 DAS Report No.: 120378 Institut für Biologische Analytik und Consulting IBACON GmbH GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 DAS Report No.: 130204 Institut für Biologische Analytik und Consulting IBACON GmbH GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 DAS Report No.: 130203 Institut für Biologische Analytik und Consulting IBACON GmbH GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 DAS Report No.: 140235 Institut für Biologische Analytik und Consulting IBACON GmbH GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 8.4.1 /6	Witte, B	2015	X12255349 (a metabolite of XDE-777) Effects on Reproduction and Growth of Earthworms <i>Eisenia fetida</i> in Artificial Soil DAS Report No.: 141006 Institut für Biologische Analytik und Consulting IBACON GmbH GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
CA 8.4.2.1 /1	Ganßmann M	2012	Effects of XDE-777 TGAI on Reproduction of the Collembola Folsomia candida in Artificial Soil with 5% Peat DAS Report No.: 120385 Institut für Biologische Analytik und Consulting IBACON GmbH GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
CA 8.4.2.1 /2	Ganßmann M	2012	Effects of XDE-777 TGAI on Reproduction of the Predatory Mite Hypoaspis aculeifer in Artificial Soil with 5% Peat DAS Report No.: 120386 Institut für Biologische Analytik und Consulting IBACON GmbH GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
CA 8.4.2.1 /3	Ganßmann M	2012	Effects of X642188 (metabolite of XDE-777) on Reproduction of the Collembola Folsomia candida in Artificial Soil with 5% Peat DAS Report No.: 120387 Institut für Biologische Analytik und Consulting IBACON GmbH GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
CA 8.4.2.1 /4	Ganßmann M	2012	Effects of X642188 (metabolite of XDE-777) on Reproduction of the Predatory Mite Hypoaspis aculeifer in Artificial Soil with 5% Peat DAS Report No.: 120388 Institut für Biologische Analytik und Consulting IBACON GmbH GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 /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 DAS Report No.: 130208 Institut für Biologische Analytik und Consulting IBACON GmbH GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
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 DAS Report No.: 130210 Institut für Biologische Analytik und Consulting IBACON GmbH GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 DAS Report No.: 130207 Institut für Biologische Analytik und Consulting IBACON GmbH GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 DAS Report No.: 130209 Institut für Biologische Analytik und Consulting IBACON GmbH GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 DAS Report No.: 140229 Institut für Biologische Analytik und Consulting IBACON GmbH GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 /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 DAS Report No.: 140232 Institut für Biologische Analytik und Consulting IBACON GmbH GLP/GEP (Y/N): Yes Published (Y/N): No	No	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 DAS Report No.: 141007 Institut für Biologische Analytik und Consulting IBACON GmbH GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
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 DAS Report No.: 141008 Institut für Biologische Analytik und Consulting IBACON GmbH GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
CA 8.5 /1	Hammesfahr U	2012	Effects of XDE-777 on the Activity of the Soil Microflora in the Laboratory DAS Report No.: 110173 Institut für Biologische Analytik und Consulting IBACON GmbH GLP/GEP (Y/N): Yes Published (Y/N): Yes	No	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 DAS Report No.: 120377 Institut für Biologische Analytik und Consulting IBACON GmbH GLP/GEP (Y/N): Yes Published (Y/N): Yes	No	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 DAS Report No.: 130206 Institut für Biologische Analytik und Consulting IBACON GmbH GLP/GEP (Y/N): Yes Published (Y/N): Yes	No	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 8.5 /4	Hammesfahr U	2013	X12264475 (a metabolite of XDE-777): Effects on the Activity of the Soil Microflora in the Laboratory DAS Report No.: 130205 Institut für Biologische Analytik und Consulting IBACON GmbH GLP/GEP (Y/N): Yes Published (Y/N): Yes	No	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 DAS Report No.: 140238 Institut für Biologische Analytik und Consulting IBACON GmbH GLP/GEP (Y/N): Yes Published (Y/N): Yes	No	Corteva Agriscience
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 (Nitro- gen Transformation) DAS Report No.: 141009 Institut für Biologische Analytik und Consulting IBACON GmbH GLP/GEP (Y/N): Yes Published (Y/N): Yes	No	Corteva Agriscience
CA 8.8 /1	Griffith A	2012	XR-777 TGAI - Activated Sludge Respiration Inhibition Test Following OECD Guideline 209 DAS Report No.: 110217 Smithers Viscient GLP/GEP (Y/N): Yes Published (Y/N): No	No	Corteva Agriscience
CP 10.1.1.1/1	██████	2012	GF-2925: An acute oral toxicity study with the Northern Bobwhite using a sequential testing procedure DAS Report No.: 120389 ██████ GLP/GEP (Y/N): Y Published (Y/N): N	Yes	Corteva Agriscience
CP 10.2.1/1	██████	2013	GF-2925: Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions (Revision) DAS Report No.: 120374 ██████ GLP/GEP (Y/N): Y Published (Y/N): N	Yes	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 10.2.1/2	Stadler T Lamichhane K Goudie, O	2013, amended 2014, revised 2017	GF-2925: Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions (Revision) DAS Report No.: 120375 ABC Laboratories GLP/GEP (Y/N): Y Published (Y/N): N	No	Corteva Agriscience
CP 10.2.1/3	Holou M	2013	GF-2925: Growth inhibition test with the unicellular green alga, <i>Pseudokirchneriella subcapitata</i> DAS Report No.: 120376 ABC Laboratories, Inc. GLP/GEP (Y/N): Y Published (Y/N): N	No	Corteva Agriscience
CP 10.2.3/01	██████	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 DAS Report No.: 130367 ██████ GLP/GEP (Y/N): Y Published (Y/N): N	Yes	DAS
CP 10.2.3/02	██████	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 DAS Report No.: 130368 ██████ GLP/GEP (Y/N): Y Published (Y/N): N	Yes	Corteva Agriscience
CP 10.2.3/03	██████	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 DAS Report No.: 121049 ██████ GLP/GEP (Y/N): Y Published (Y/N): N	Yes	Corteva Agriscience
CP 10.2.3/04	██████	2014	XDE-777: Community level study in outdoor aquatic mesocosms DAS Report No.: 130984 ██████ GLP/GEP (Y/N): Y Published (Y/N): N	Yes	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 10.2.3/05	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 DAS Report No.: 141106 Dow AgroSciences LLC GLP/GEP (Y/N): N Published (Y/N): N	No	Corteva Agriscience
CP 10.2.3/06	Mueller, J.	2015	XDE-777 metabolites: Analysis in aqueous and sediment samples of the outdoor mesocosm study DAS Report No.: 140860 Fraunhofer Institute GLP/GEP (Y/N): Y Published (Y/N): N	No	Corteva Agriscience
CP 10.2.3/07	██████	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 DAS Report No.: 130367, 1st study report ammendment ██████ GLP/GEP (Y/N): Y Published (Y/N): N	Yes	Corteva Agriscience
CP 10.2.3/08	██████	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 DAS Report No.: 130368, 1st study report ammendment ██████ GLP/GEP (Y/N): Y Published (Y/N): N	Yes	Corteva Agriscience
CP 10.2.3/09	██████	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 DAS Report No.: 121049, 1st study report ammendment ██████ GLP/GEP (Y/N): Y Published (Y/N): N	Yes	Corteva Agriscience
CP 10.2.3/10	Kramer V, Lopez-Mancisidor 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 No study number Dow AgroSciences	No	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
			GLP/GEP (Y/N): N Published (Y/N): N		
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 DAS Report No.: 120370, 120371 IBACON GmbH GLP/GEP (Y/N): Y Published (Y/N): N	No	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 IBACON GmbH GLP/GEP (Y/N): Y Published (Y/N): N	No	Corteva Agriscience
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 DAS Report No.: 110174 IBACON GmbH GLP/GEP (Y/N): Y Published (Y/N): N	No	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 DAS Report No.: 110175 IBACON GmbH GLP/GEP (Y/N): Y Published (Y/N): N	No	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 DAS Report No.: 120373 IBACON GmbH GLP/GEP (Y/N): Y Published (Y/N): N	No	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 DAS Report No.: 120390 IBACON GmbH GLP/GEP (Y/N): Y Published (Y/N): N	No	Corteva Agriscience
CP	Ganßmann M	2012c	Effects of GF-2925 on Reproduction of the Predatory Mite <i>Hypoaspis aculeifer</i> in Artificial Soil with 5% Peat	No	Corteva

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
10.4.2.1/2			DAS Report No.: 120391 IBACON GmbH GLP/GEP (Y/N): Y Published (Y/N): N		Agriscience
CP 10.5/1	Hammesfahr U	2012	Effects of GF-2925 on the Activity of the Soil Microflora in the Laboratory DAS Report No.: 120372 IBACON GmbH GLP/GEP (Y/N): Y Published (Y/N): N	No	Corteva Agriscience
CP 10.6.2/1	Friedemann A Teresiak H	2012a	Evaluation of the Phytotoxicity of GF-2925 (XDE-777 130 g as/L, SC), GLP Vegetative Vigour Test DAS Report No.: 110093 agro-check GLP/GEP (Y/N): Y Published (Y/N): N	No	Corteva Agriscience
CP 10.6.2/2	Friedemann A Teresiak H	2012b	Evaluation of the Phytotoxicity of GF-2925 (XDE-777 130 g as/L SC), GLP Seedling Emergence and Seedling Growth Test DAS Report No.: 110094 agro-check GLP/GEP (Y/N): Y Published (Y/N): N	No	Corteva Agriscience

List of data submitted by the applicant and not 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
-	-	-	-	-	-

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

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
-	-	-	-	-	-

Appendix 2 Detailed evaluation of the new studies

zRMS comment:

All studies were evaluated by zRMS-PL previously in Core Dossier for authorisation of ppp Qeen (GF-3307) or Questar 2022 (GF-3308) in their Core Dossiers.
The zRMS's evaluation has been copied from theses reports only for completeness. All studies were peer reviewed during commenting period process and re-opening of the studies' evaluation for extended uses of GF-3307 is not required as no new data are submitted by the Applicant.

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-3307: 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>LD₅₀ > 2000 mg/kg bw</p>
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Reference:	KCP 10.1.1.1/1
Report	█████; 2014; GF-3307: An Acute Oral Toxicity Study with the Northern Bobwhite Using a Sequential Testing Procedure; █████; Lab Study No. 379-366; DAS Study No. 140447; 24 July 2014; Unpublished
Guideline(s):	OECD 223 (adopted July 2010)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable Yes
Duplication (if vertebrate study)	No

COMPLIANCE

Guideline(s):	OECD 223 (adopted July 2010)
US EPA Guideline(s):	Not applicable
Deviations:	None
Dates of work:	23 May 2014 to 06 June 2014
GLP status:	Yes
Number of pages in final report:	55

MATERIALS AND METHODS

Test Item(s)

ISO Common name:	Not applicable
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Test item (chemical/other name):	GF-3307
Purity:	4.8% w/w XDE-777 and 9.4% w/w prothioconazole
Description (physical state):	Liquid
Lot/batch no.:	F1281-135-1 (TSN307579)
CAS no.:	Not applicable

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:	Approximately 46 weeks
Weight range of birds at study initiation:	187-230 grams
Test concentrations:	0 and 2000 mg/kg
No. of feed withholding days before dosing:	Birds were fasted for approximately 17 hours prior to dosing
Method of test item administration:	Oral gavage
Diet:	Wildlife International Game Bird Ration
Number of birds per dose group:	5
Number of birds per control group:	5
Housing:	Birds were individually housed in batteries of pens manufactured by GQF Manufacturing Co. (Model No. 0330)
Environmental conditions:	Temperature: $23.8 \pm 0.4^{\circ}\text{C}$ (SD) with a maximum of 24.3°C , a minimum of 22.8°C and a coefficient of variability of 1.6% Photoperiod: 8 hrs of light per day/16 hours dark during acclimation and throughout the test Humidity: $54 \pm 8\%$ (SD) with a maximum of 67%, a minimum of 44% and a coefficient of variability of 16% Ventilation rate: 15 room air volumes per hour

Methodology

Five northern bobwhite were assigned to the 2000 mg/kg treatment group and the control group. The birds were fasted for approximately 17 hours prior to dosing. At experimental start, a single dose of the test substance in reverse osmosis and deionized water was administered by oral gavage directly into the crop or proventriculus of each bird. Each bird was individually weighed and dosed on the basis of milligrams of test substance per kilogram of body weight. The control birds received a corresponding volume of reverse osmosis deionized water. From test initiation until termination, all birds were observed at least twice a day. 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 Day 1, Day 1 to Day 2 and Day 2 to Day 3. Average feed consumption was then determined from Day 3 to Day 7 and from Day 7 to Day 14.

RESULTS AND DISCUSSION

Table 3: Effect of GF-3307 on mortality of bobwhite quail

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 ₅₀	>2000 mg/kg			
95% C.I.	Not determined			
NOEL	Not determined			

Table 4: Effect of GF-3307 on body weight and feed consumption of bobwhite quail

Treatment (mg/kg bw)	Observation								
	Mean body weight (g)				Feed consumption (g/bird/day)				
	Day 0	Day 3	Day 7	Day 14	Days 0-1	Days 1-2	Days 2-3	Days 3-7	Days 7-14
Negative control	211	209	210	210	15	13	12	14	14
2000	195	191	197	196	5	9	11	15	13

Mortalities and Clinical Observations

No regurgitation was noted among the control birds or among any of the treatment birds. There were no mortalities in the control group. Additionally, there were no mortalities among the five birds dosed at 2000 mg/kg dosage level. One control bird was noted with lesion on the right foot and as limping on Day 2 and 3 of the test. The bird was normal in appearance from Day 4 of the test to test termination. All other control birds were normal in appearance and behavior throughout the test. All birds in the 2000 mg/kg treatment group were normal in appearance and behavior for the duration of the test.

Body Weight and Feed Consumption

When compared to the control group, there was a slight treatment related loss of mean body weight from Day 0 to Day 3 for birds in the 2000 mg/kg treatment group. While the mean body weight change for birds in the 2000 mg/kg treatment group from Day 0 to Day 3 appears comparable to the control group, the control mean was impacted by a 13 g weight loss by the single bird noted with an injury. The mean body weight changes for all other body weight intervals were comparable to the control group.

When compared to the control group, there was a marked, treatment related reduction in mean feed consumption values at the 2000 mg/kg treatment level for the first day of the test (Day 0 to Day 1) and a less pronounced reduction in feed consumption from Day 1 to Day 2. The mean feed consumption values from Day 2 to Day 3, Day 3 to Day 7 and Day 7 to Day 14 for the 2000 mg/kg treatment group were comparable to the control mean values.

Necropsy

A gross necropsy was performed on all birds at test termination. There were no remarkable findings for any control birds or for any birds in the 2000 mg/kg dosage level.

Validity of the test

The conditions for the validity of the test were met and the test is considered valid. The following criteria were used to judge the validity of the test.

- 1) Birds were randomly assigned to control and treatment pens.
- 2) The mortality in the control group did not exceed 10% at the end of the test.
- 3) The test substance was administered orally by gavage.

CONCLUSION

The acute oral LD₅₀ value for northern bobwhite exposed to GF-3307 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-3307	14 day	LD ₅₀	>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
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-3307: 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 minor deviations.</p> <p>It was noted that the study was conducted in 2017 in line with OECD 203 of 1992 where for the tested fish species rainbow trout the recommended test temperature range was 13-17°C (constant within a range of 2°C) while in the current OECD 203 of 2019 recommends the test temperature range of 10-14°C (constant within a range of 1°C). For that reason, the temperature range of 14.3 to 15.4°C in the study is acceptable.</p> <p>It was also noted that the highest pH of 8.7 slightly exceeded the recommended maximum of 8.5.</p> <p>However, in zRMS opinion these deviations are considered to have no impact on the outcome of the study since all the validity criteria were met.</p> <p>It was noted that GF-3307 contains two active substances and in line with the requirements of the Central Zone the test concentrations of both substances should be verified in the respective chemical analyses or, as a minimum, the least stable active substance should be analysed. In the study only the concentrations of one active substance fenpicoxamid were measured and no analyses of the second active substance prothioconazole were performed. No explanation or justification of the substance selected for the measurements was provided in the study report. However, based on the information from the area of environmental fate and behavior of both active substances it can be concluded that fenpicoxamid is the least stable substance with the geomean water DT₅₀ of 0.7 days determined from the EU agreed water/sediment studies as well as DT₅₀ of 0.92 days at pH 7 and DT₅₀ of 0.024 days at pH 9 from the hydrolysis studies. Prothioconazole was determined to be stable in the EU agreed hydrolysis studies with DT₅₀ of > 1 year at pH 7 and 9, and the water DT₅₀ of 1 day from the water/sediment studies indicates that prothioconazole is more stable than fenpicoxamid. Furthermore, based on the calculations of contribution of toxicity of each active substance (provided in this report in Table 9.5-5) fenpicoxamid was determined to be the driver of the toxicity (contribution of > 90%) to fish. For that reason, the zRMS is of the opinion that the concentrations of GF-3307 calculated based on the geometric mean concentrations of fenpicoxamid in the present study are acceptable to derive the endpoint.</p> <p>Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>96-hour LC₅₀ = 33.8 µg product/L (based on geometric mean measured concentrations)</p>
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Reference:	KCP 10.2.1/1
Report:	██████; 2017; GF-3307: Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions; ██████; Lab Study No. 81071; DAS Study No. 140479 ; 12 December 2017; Unpublished
Guideline(s):	OECD Guideline 203 (1992)
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable Yes

Duplication (if vertebrate study)	No
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COMPLIANCE

Guideline(s):	OECD Guideline 203
US EPA Guideline(s):	None
Deviations:	None
Dates of work:	21 July 2014 to 25 July 2014
GLP status:	Yes
Number of pages in final report:	62

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-3307
Purity:	4.8% w/w XDE-777 and 9.4% w/w prothioconazole
Description (physical state):	Brown liquid
Lot/batch no.:	F1281-135-1 (TSN307579)

Test System

Organism (<i>Species</i>):	Rainbow Trout (<i>Oncorhynchus mykiss</i>)
Study type:	Acute
Study design:	Static-renewal
Test duration:	96 hours
Test concentrations:	Nominal: 0 (control), 0.065, 0.13, 0.25, 0.50, and 1.0 mg GF-3307/L (0 (control), 65, 130, 250, 500, and 1,000 µg GF-3307/L) Geometric mean calculated: <MQL (control), 14.6, 19.8, 28.0, 40.8, and 60.0 µg GF-3307/L
Parameters measured:	Mortality
Observation intervals:	24 hours
Age, weight and length of fish at test initiation:	Age: >14 days Mean total length: 48 ± 1.9 mm (45 to 51 mm) Mean blotted wet weight: 1.087 ± 0.137 g (0.848 to 1.315 g) Loading rate: Instantaneous biomass loading: 0.423 g/L
Analytical confirmation of test concentrations:	On days: 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: Instantaneous biomass loading: 0.423 g/L Temperature: 14.3 to 15.4°C Photoperiod: 16-hr light:8-hr dark Dissolved oxygen concentration: (new): 9.1 to 9.9 mg/L (94 to 102% sat) (old): 6.6 to 9.7 mg/L (68 to 100% sat)

pH: 7.3 to 8.7
Alkalinity: 150 mg CaCO₃/L
Total hardness: 140 mg CaCO₃/L
Conductivity: 330 µS/cm
Light Intensity: 992 lux
Salinity: None
XDE-777 technical (TSN302306)

Reference substance:

Methodology

A definitive test was performed at nominal concentrations 0 (control), 0.065, 0.13, 0.25, 0.50, and 1.0 mg GF-3307/L (0 (control), 65, 130, 250, 500, and 1,000 µg GF-3307/L). Seven fish were impartially assigned to treatments by adding one fish per chamber, proceeding from control then the test substance treatments, and repeating steps as necessary until seven fish were present in each test chamber. Observations for mortality and sublethal responses were made at approximately 24, 48, 72, and 96 hours. 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 the water bath to continuously record temperature. Fluorescent lighting was maintained on a 16-hour daylight photoperiod with 30-minute simulated dawn and dusk periods. The measured light intensity at the start of the definitive test was 992 lux as measured with a LI-COR Model LI-189 light meter equipped with a photometric sensor.

RESULTS AND DISCUSSION

The geometric mean calculated concentrations, based on analysis of XDE-777, in the test substance treatment solutions during the 96-hour exposure were 14.6, 19.8, 28.0, 40.8, and 60.0 µg GF-3307/L, which represented recoveries of 6 to 22% of the 65, 130, 250, 500, and 1,000 µg GF-3307/L nominal concentrations. No residues of test substance were detected in the control solutions above the MQL of 0.00833 mg GF-3307/L (8.33 µg GF-3307/L). Recoveries from QC fortifications ranged from 91 to 102% of the nominal concentrations.

The biological response results were reported based upon the geometric mean calculated GF-3307 concentrations, based on analysis of XDE-777. After 96 hours of exposure, mortality was 0% in the control, and 0, 0, 0, 100, and 100% in the 14.6, 19.8, 28.0, 40.8, and 60.0 µg GF-3307/L (geometric mean calculated concentrations) test substance treatments, respectively. Sublethal effects were not observed in any treatment at any time point.

Based on the geometric mean calculated concentrations, the 24-, 48-, 72-, and 96-hour LC₅₀ values were estimated to be 33.8 µg GF-3307/L, with best estimates of 95% confidence limits of 28.0 and 40.8 µg GF-3307/L. The 96-hour NOEC was 28.0 µg GF-3307/L, based on geometric mean calculated concentrations and the absence of mortality and sublethal effects at this, and all lower test substance concentrations.

Table 2. Calculated Concentrations of GF-3307 (Based on Analysis of XDE-777) During the Static-Renewal Acute Toxicity Test with Rainbow Trout, *Oncorhynchus mykiss*

Nominal Concentration, mg T.P./L / μ g T.P./L	Calculated Concentration as mg T.P./L ^a (Percent Nominal)				Geometric Mean Calculated Concentration mg T.P./L / μ g T.P./L
	0 Hour	48 Hours (old)	48 Hours (new)	96 Hours	
0 (control)	<MQL ^b	<MQL ^b	<MQL ^b	<MQL ^b	<MQL ^b
0.065 / 65	0.0557 (86) ^c	<MQL ^b	0.0471 (72)	<MQL ^b	0.0146 / 14.6 (22)
0.13 / 130	0.0950 (73) ^c	<MQL ^b	0.0933 (72)	<MQL ^b	0.0198 / 19.8 (15)
0.25 / 250	0.198 (79) ^c	<MQL ^b	0.179 (72)	<MQL ^b	0.0280 / 28.0 (11)
0.50 / 500	0.400 (80) ^c	<MQL ^b	---	---	0.0408 / 40.8 (8)
1.0 / 1,000	0.863 (86) ^c	<MQL ^b	---	---	0.0600 / 60.0 (6)
QC Fortification Spikes (% Recovery)					
Low Spike (0.00603)	0.00617 (102)	NA	NA	NA	NA
Low Spike (0.0560)	NA	0.0525 (94)	0.0508 (91)	0.0536 (96) ^c	NA
High Spike (0.140)	0.135 (96)	NA	NA	NA	NA
High Spike (1.40)	NA	1.33 (95)	1.36 (97)	1.38 (99)	NA

^a Calculated Concentration (mg/L) = Measured Conc. From Curve (ng/mL) x Analysis Volume (mL) / Sample Volume (mL) / 4.8% (XDE-777) / 1000

^b MQL = 0.00833 mg/L (8.33 μ g/L). When <MQL, ½ MQL (0.00417 mg/L) used for calculation.

^c Re-diluted in duplicate, mean of re-dilutions reported.

--- = Not measured due to 100% mortality in the treatment.

Note: 1,000 μ g/l = 1 mg/L.

Table 2: Effect of GF-3307 on mortality of Rainbow Trout

Treatment (mg GF-3307/L / μ g GF-3307/L)		No. of fish	Cumulative mortality (%)			
Nominal concentration	Geometric mean calculated concentration (based on analysis of XDE-777)		24-hr	48-hr	72-hr	96-hr
Negative control	<MQL	7	0	0	0	0
0.065 / 65	0.0146 / 14.6	7	0	0	0	0
0.13 / 130	0.0198 / 19.8	7	0	0	0	0
0.25 / 250	0.0280 / 28.0	7	0	0	0	0
0.50 / 500	0.0408 / 40.8	7	100	100	100	100*
1.0 / 1,000	0.0600 / 60.0	7	100	100	100	100*
LC ₅₀		33.8 μ g GF-3307/L				
95% C.I.		28.0 and 40.8 μ g GF-3307/L				
NOEC		28.0 μ g GF-3307/L				

* Statistically significant immobility as compared to the control (one-tailed Fisher's test with Hochberg's familywise adjustment for significance, $p < 0.05$)

Table 3:Sub-lethal effects of GF-3307 in Rainbow Trout

Treatment (mg GF-3307/L)		Observation period			
Nominal concentration	Geometric mean calculated concentration (based on analysis of XDE-777)	Observation 1 (% affected)			
		24-hr	48-hr	72-hr	96-hr
Negative control	<MQL	0	0	0	0
0.065	0.0146	0	0	0	0
0.13	0.0198	0	0	0	0
0.25	0.0280	0	0	0	0
0.50	0.0408	0	0	0	0
1.0	0.0600	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., $\geq 90\%$) as stated in the study protocol and the OECD 203 testing guidelines.

Based on the geometric mean calculated concentrations, the 24-, 48-, 72-, and 96-hour LC₅₀ values were estimated to be 33.8 µg GF-3307/L, with best estimates of 95% confidence limits of 28.0 and 40.8 µg GF-3307/L. The 96-hour NOEC was 28.0 µg GF-3307/L, based on geometric mean calculated concentrations and the absence of mortality 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
Rainbow trout	<i>Oncorhynchus mykiss</i>	GF-3307	96-hr	LC ₅₀	33.8	µg GF-3307/L, gm

A 2.2.1.2

Study 2 – GF-3307: 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>All the validity criteria were met.</p> <p>It was noted that GF-3307 contains two active substances and in line with the requirements of the Central Zone the test concentrations of both substances should be verified in the respective chemical analyses or, as a minimum, the least stable active substance should be analysed. In the study only the concentrations of one active substance fenpicoxamid were measured and no analyses of the second active substance prothioconazole were performed. No explanation or justification of the substance selected for the measurements was provided in the study report. However, based on the information from the area of environmental fate and behavior of both active substances it can be concluded that fenpicoxamid is the least stable substance. Additionally, based on the calculations of contribution of toxicity of each active substance (provided in this report in Table 9.5-5) fenpicoxamid was determined to be the driver of the toxicity (contribution of $> 90\%$) to invertebrates. For that reason, the zRMS is of the opinion that the concentrations of GF-3307 calculated based on the geometric mean concentrations of fenpicoxamid in the present study are acceptable to derive the endpoint.</p> <p>Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>EC₅₀ = 8.29 µg product/L (based on geometric mean measured concentration)</p>
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Reference:	KCP 10.2.1/8
Report:	█████; 2014; GF-3307: Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions; █████; Lab Study No. 81070; DAS Study No. 140489; 09 January 2018; Revised ; Unpublished
Guideline(s):	OECD 202
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable Yes
Duplication (if vertebrate study)	No

COMPLIANCE

Guideline(s): OECD 202
 US EPA Guideline(s): Not applicable
 Deviations: None
 Dates of work: 20 June 2014 to 22 June 2014
 GLP status: Yes
 Number of pages in final report: 64

MATERIALS AND METHODS

Test Item(s)

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 (*Species*): Cladoceran (*Daphnia magna*)
 Study type: Acute
 Study design: Static-renewal
 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 (four replicates, five neonates per replicate)
 Number of daphnia per control group: 20 (four replicates, five neonates per replicate)

Environmental conditions:	Loading rate: Not provided Temperature: 20.0 to 20.5°C Photoperiod: 16 hour light:8 hour dark Alkalinity: 150 mg CaCO ₃ /L Hardness: 146 mg CaCO ₃ /L Conductivity: 326 µS/cm Light Intensity: 593 lux 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 ^b	<MQL ^b	<MQL ^b	<MQL ^b	<MQL ^b
0.0097 / 9.7	0.00596 (61)	<MQL ^{b, c}	0.00621 (64)	<MQL ^{b, c}	0.00159 / 1.59 (16)
0.021 / 21	0.0157 (75)	<MQL ^{b, c}	0.0150 (71)	<MQL ^{b, c}	0.00253 / 2.53 (12)
0.047 / 47	0.0329 (70)	<MQL ^{b, c}	0.0321 (68)	<MQL ^{b, c}	0.00368 / 3.68 (8)
0.10 / 100	0.0638 (64)	<MQL ^{b, c}	0.0620 (62)	<MQL ^{b, c}	0.00511 / 5.11 (5)
0.23 / 230	0.150 (65)	<MQL ^{b, c}	0.143 (62)	<MQL ^{b, c}	0.00781 / 7.81 (3)
0.50 / 500	0.328 (66)	<MQL ^{b, c}	0.335 (67)	<MQL ^{b, c}	0.0118 / 11.8 (2)
QC Fortification Spikes (% Recovery)					
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)

^a Initial mean measured concentrations are the average of the 0- and 24-hour (new) measured concentrations.

^b MQL = 0.000833 mg GF-3307/L (0.833 µg GF-3307/L)

^c 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 2: 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
11.8	6	30	20	100
NOEC	Not calculated		5.11 µg GF-3307/L	
EC ₅₀	> 11.8 µg GF-3307/L		8.29 µg GF-3307/L (95% CI: 7.58 and 9.07 µg GF-3307/L)	

Table 3: 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 ≥ 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 EC₅₀ value was estimated to be >11.8 µg GF-3307/L, the highest concentration (95% confidence limits could not be calculated). The 48-hour EC₅₀ value was estimated to be 8.29 µg GF-3307/L (95% confidence limits of 7.58 and 9.07 µg GF-3307/L) based on geometric mean calculated concentrations. The 48-hour NOEC was 5.11 µg 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	µg/L gm

A 2.2.1.3 Study 3 - GF-3307: Growth Inhibition Test with the Unicellular Green Alga

Comments of zRMS:	<p>The study was conducted in line with OECD 201 with no deviations.</p> <p>All the validity criteria were met.</p> <p>The test concentrations of both active substances were verified in the chemical analyses but they were not maintained within 80-120% of nominal nor the initial measured concentrations.</p>
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	<p>Therefore, in line with the recommendations in Appendix J of EFSA Supporting publication 2019:EN-1673, the endpoints should be based on the geometric mean measured concentrations of the sum of both active substances.</p> <p>The Applicant provided the following justification of obtained endpoints from the study.</p> <p>In the final report (██████, 2014), the concentrations of fenpicoxamid (XDE-777) and prothioconazole were measured at 0, 24 and 72 hours. Both molecules degraded during the static study, though fenpicoxamid degraded more rapidly and was <MQL in four of the six treatment solutions at termination. None-the-less, the analytical measurements demonstrated that the formulation was dosed correctly and because it was a static study, three sets of endpoints were calculated based on initial measured concentrations of: (1) fenpicoxamid, (2) prothioconazole, and (3) the mean of fenpicoxamid and prothioconazole.</p> <p>In 2020, a final report addendum was issued with the GF-3307 endpoints calculated based on the time-weighted geomean of the fastest degrading molecule, i.e., fenpicoxamid (ErC50 = 8.0 mg GF-3307/L twgm). Time-weighted geomean was selected versus geomean due to the uneven sampling intervals. Note that an endpoint based on the geometric mean measured concentrations of the sum of both active substances would be higher and therefore less conservative than the endpoint based on fenpicoxamid alone. As such, the time-weighted</p> <p>ErC₅₀ = 8.0 mg GF-3307/L twgm</p>
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Reference:	KCP 10.2.1/3
Report:	Hicks, S.; 2014; GF-3307: Growth Inhibition Test with the Unicellular Green Alga, <i>Pseudokirchneriella subcapitata</i> ; ABC Laboratories, Inc.; Lab Study No. ABC Study No. 81069; DAS Study No. 140491; 24 December 2014, final report addendum 08 January 2020; Unpublished,
Guideline(s):	OECD Guideline 201
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable Yes
Duplication (if vertebrate study)	NA

COMPLIANCE

Guideline(s):	OECD Guideline 201
US EPA Guideline(s):	
Deviations:	None
Dates of work:	16 September 25 May 2014 to 19 September 28 May 2014
GLP status:	Yes
Number of pages in final report:	88

MATERIALS AND METHODS

Test Item(s)

ISO Common name:	Not applicable
Test item (chemical/other name):	GF-3307, a formulation containing the active ingredients XDE-777 and prothioconazole
Purity:	4.8% w/w XDE-777 and 9.4% w/w prothioconazole
Description (physical state):	Brown liquid
Lot/batch no.:	Test Substance No.: TSN307579; Lot No.: F1281-135-1
CAS no.:	Not applicable

Test System

Organism (<i>Species</i>):	Unicellular Green Alga (<i>Pseudokirchneriella subcapitata</i>)
Study type:	Laboratory study assessing algal growth
Study design:	Static
Test concentrations:	<p>Nominal: 0 (control), 0.090, 0.27, 0.80, 2.4, 7.3, 22, and 66 mg GF-3307/L</p> <p>Initial Dosing Solution Calculated Concentrations as Measured XDE-777 Active: <MQL (control), 0.069, 0.29, 0.70, 2.5, 6.6, 24, and 51 mg GF-3307/L</p> <p>Initial Dosing Solution Calculated Concentrations as Measured Prothioconazole Active: <MQL (control), 0.081, 0.31, 0.78, 2.4, 7.0, 25, and 65 mg GF-3307/L</p> <p>Mean Initial Dosing Solution Calculated Concentrations as Measured XDE-777 and Prothioconazole Active: <MQL (control), 0.075, 0.30, 0.74, 2.5, 6.8, 25, and 58 mg GF-3307/L</p> <p>Time-weighted Geometric Mean Calculated Concentrations as Measured XDE-777 Active: <MQL (control), 0.0077, 0.050, 0.14, 0.51, 1.3, 8.0, and 41 mg GF-3307/L</p>
Duration:	72 hrs
Parameters measured:	<p>Test solution pH (range): 7.2 to 7.6</p> <p>Test solution temperature (range): 22.2 to 23.5 °C</p>
Environmental conditions:	<p>Temperature (range): 24 ± 2°C (SD)</p> <p>Photoperiod: continuous light</p> <p>Light intensity (range): 8,533 to 8,679 lux</p>
Observation intervals:	0, 24, 48, 72 hours
Age of inoculum:	4 days
Acclimation period/conditions:	None
Initial cell density:	5.0 × 10 ³ cells/mL
Growth medium:	<p>Name: freshwater algal nutrient medium (FWAM)</p> <p>pH at test initiation: 7.5 to 7.6</p> <p>pH at test termination: 7.2 to 7.5</p> <p>Constant stirring: Orbital shaker</p>
Method of test item added to the test medium:	Test item added to appropriate volume flask, then diluted by bringing the flask to volume with test medium
No. of control replicates:	6
No. of test concentration replicates:	3

Analytical verification:	Method: measuring concentrations of XDE-777 and prothioconazole (active ingredients of GF-3307) xxx using HPLC-MS/MS Samples taken : 0, 24, and 72 hrs Minimum Quantifiable Limit (MQL): 0.000400 mg XDE-777/L = 0.0083 mg GF-3307/L MQL: 0.000400 mg prothioconazole/L = 0.0043 mg GF-3307/L Recoveries from QC fortifications: 90 to 117 (range)
Reference substance:	None

Methodology

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 (replicate G) of the control and each test substance treatment was prepared for analytical confirmation samples at 24 hours. An additional replicate (replicate D) of the 0.090 mg GF-3307/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. At test initiation, all replicates of the control and each A, B, and C replicate of each test substance treatment, as well as all replicate G samples, were inoculated with 1.0 mL of an algal concentrate containing approximately 4.5×10^6 cells/mL, resulting in a final density of approximately 5.0×10^3 cells/mL for each flask. The replicates were inoculated with algae within 30 minutes after test solution preparation. At 24, 48, and 72 hours (± 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. Replicate D of the 0.090 mg GF-3307/L test substance treatment was not inoculated with algae.

Test solutions were analyzed for the concentration of XDE-777 and prothioconazole (active ingredients of GF-3307) using liquid chromatography with tandem mass spectrometry (LC-MS/MS) system. The concentrations of XDE-777 and prothioconazole were measured in test solution samples collected at initiation (0 hour), 24 hours, and termination (72 hours) of the definitive test. The 0-hour samples were collected from parent solutions. The 24-hour samples were collected from replicate G. The 72-hour (termination) samples were collected after combining replicate solutions by treatment. GF-3307 fortified samples were also prepared for analysis at each sample period.

RESULTS AND DISCUSSION

Calculated concentrations of GF-3307, based on analysis of XDE-777, in the test substance treatment solutions at test initiation were 0.069, 0.29, 0.70, 2.5, 6.6, 24, and 51 mg GF-3307/L. This represented recoveries of 77 to 109% of the nominal concentrations. The calculated concentrations of GF-3307, based on analysis of XDE-777, in the test substance treatment solutions at 24 hours were <MQL, 0.035, 0.13, 0.56, 1.4, 7.5, and 41 mg GF-3307/L, representing recoveries of <13 to 62% of the nominal concentrations. The calculated concentrations of GF-3307, based on analysis of XDE-777, in the test substance treatment solutions at 72 hours were <MQL, <MQL, <MQL, <MQL, <MQL, 3.1, and 35 mg GF-3307/L, or <14 to 53% of the nominal concentrations. The geometric mean calculated concentrations of GF-3307, based on analysis of XDE-777, in the 0, 24, and 72 hour test substance treatment solutions were 0.011, 0.035, 0.072, 0.18, 0.34, 8.2, and 42 mg GF-3307/L, representing recoveries of 5 to 64% of the nominal concentrations. The geometric mean calculated concentrations of GF-3307, based on analysis of XDE-777, in the 0 and 72 hour test substance treatment solutions were 0.017, 0.035, 0.054, 0.10, 0.17, 8.6, and 42 mg GF-3307/L, representing recoveries of 2 to 64% of the nominal concentrations.

Calculated concentrations of GF-3307, based on analysis of prothioconazole, in the test substance treatment solutions at test initiation were 0.081, 0.31, 0.78, 2.4, 7.0, 25, and 65 mg GF-3307/L. This represented recoveries of 90 to 115% of the nominal concentrations. The calculated concentrations of GF-3307, based

on analysis of prothioconazole, in the test substance treatment solutions at 24 hours were 0.041, 0.23, 0.81, 2.6, 7.5, 25, and 70 mg GF-3307/L, representing recoveries of 46 to 114% of the nominal concentrations. The calculated concentrations of GF-3307, based on analysis of prothioconazole, in the test substance treatment solutions at 72 hours were 0.035, 0.15, 0.45, 1.8, 5.1, 18, and 54 mg GF-3307/L, or 39 to 82% of the nominal concentrations. The geometric mean calculated concentrations of GF-3307, based on analysis of prothioconazole, in the 0, 24, and 72 hour test substance treatment solutions were 0.049, 0.22, 0.66, 2.2, 6.4, 22, and 63 mg GF-3307/L, representing recoveries of 54 to 100% of the nominal concentrations. The geometric mean calculated concentrations of GF-3307, based on analysis of prothioconazole, in the 0 and 72 hour test substance treatment solutions were 0.053, 0.22, 0.59, 2.1, 6.0, 21, and 59 mg GF-3307/L, representing recoveries of 59 to 95% of the nominal concentrations.

The time-weighted geometric mean calculated concentrations of GF-3307, based on XDE-777 analysis, in the 0-, 24-, and 72-hour test substance treatment solutions were 0.0077, 0.050, 0.14, 0.51, 1.3, 8.0, and 41 mg GF-3307/L, representing recoveries of 9 to 61% of the nominal concentrations.

The biological response results were reported based upon the initial GF-3307 concentration as measured XDE-777 active, initial GF-3307 concentration as measured prothioconazole active, mean initial GF-3307 concentration of the initial measured XDE-777 and prothioconazole actives, and time-weighted geometric mean GF-3307 concentrations as measured XDE-777 active.

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Table 1. Calculated Concentrations of GF-3307 as Measured XDE-777 Active in Test Solutions During the 72-Hour Growth Inhibition Test with the Unicellular Green Alga, *Pseudokirchneriella subcapitata*

Nominal Concentration (mg GF-3307/L)	GF-3307 Concentration as Measured XDE-777 Active (mg GF-3307/L) (Percent Nominal)			
	0 Hour	24 Hour	72 Hour	Time-weighted Geometric Mean ^a
Control	<MQL ^b	<MQL ^b	<MQL ^b	<MQL ^b
0.090	0.069 (77)	<MQL ^b	<MQL ^b	0.0077 (9)
0.270	0.29 (107)	0.035 (13)	<MQL ^b	0.050 (18)
0.80	0.70 (88)	0.13 (16)	<MQL ^b	0.14 (17)
2.4	2.5 (104)	0.56 (23)	<MQL ^b	0.51 (21)
7.3	6.6 (90)	1.4 (19)	<MQL ^b	1.3 (18)
22	24 (109)	7.5 (34)	3.1 (14)	8.0 (37)
66	51 (77)	41 (62)	35 (53)	41 (61)
QC Fortification Spikes (% Recovery)				
Low Spike (0.050)	0.049 (98)	0.045 (90)	0.052 (104)	---
High Spike (70.8)	73 (103)	74 (105)	72 (102)	---

^a Calculated from the 0-, 24-, and 72-hour results. For calculations involving <MQL results, ½ MQL (i.e., 0.00415 mg GF-3307/L) was used in the calculation.

^b MQL = 0.000400 mg XDE-777/L = 0.0083 mg GF-3307/L

Table 2: Effects of GF-3307 on algal growth based on measured XDE-777 active

Hour	EC Type	EC Value [mg test item/L]	95% Confidence Limits [mg test item/L]	NOEC [mg test item/L]
72	ErC ₁₀	20	19 – 20	0.29
	ErC ₂₀	21	20 – 22	0.29
	ErC ₅₀	24	23 – 25	0.29
	EyC ₁₀	0.54	0.34 – 0.74	0.069
	EyC ₂₀	1.1	0.78 – 1.4	0.069
	EyC ₅₀	3.5	3.0 – 4.0	0.069

Table 3: Effects of GF-3307 on algal growth based on measured prothioconazole active

Hour	EC Type	EC Value [mg test item/L]	95% Confidence Limits [mg test item/L]	NOEC [mg test item/L]
72	ErC ₁₀	30	0 – 91	0.31
	ErC ₂₀	32	0 – 93	0.31
	ErC ₅₀	37	0 - 97	0.31
	EyC ₁₀	0.47	0.30 – 0.64	0.081

Hour	EC Type	EC Value [mg test item/L]	95% Confidence Limits [mg test item/L]	NOEC [mg test item/L]
	E _y C ₂₀	0.99	0.73 – 1.2	0.081
	E _y C ₅₀	3.5	3.0 – 4.0	0.081

Table 4: Effects of GF-3307 on algal growth based on measured XDE-777 and prothioconazole active

Hour	EC Type	EC Value [mg test item/L]	95% Confidence Limits [mg test item/L]	NOEC [mg test item/L]
72	E _r C ₁₀	19	0 – 74	0.30
	E _r C ₂₀	21	0 – 60	0.30
	E _r C ₅₀	25	23 – 27	0.30
	E _y C ₁₀	0.51	0.32 – 0.70	0.075
	E _y C ₂₀	1.0	0.76 – 1.3	0.075
	E _y C ₅₀	3.6	3.1 – 4.1	0.075

Table 5: Effects of GF-3307 on algal growth as time-weighted geomean based on measured XDE-777 active

Hour	EC Type	EC Value [mg test item/L]	95% Confidence Limits [mg test item/L]	NOEC [mg test item/L]
72	E _r C ₁₀	1.2	0.97-1.5	
	E _r C ₂₀	2.6	2.0-3.1	
	E _r C ₅₀	8.0	6.4-9.6	0.050
	E _y C ₁₀	0.078	0.049-0.12	
	E _y C ₂₀	0.23	0.18-0.28	
	E _y C ₅₀	0.71	0.55-0.88	0.0077

Table 6: Cell Density Values for *Pseudokirchneriella subcapitata* During a 72-Hour Exposure to GF-3307

Nominal concentration (mg GF-3307)	Mean cell density (cells/mL x 10 ⁴)		
	24 hours	48 hours	72 hours
Control	2.52	8.18	55.2
0.090	2.59	7.93	55.3
0.270	2.41	8.52	47.9
0.80	2.15	8.37	41.5
2.4	1.44	7.78	34.6
7.3	1.71	4.33	18.6
22	1.41	2.52	6.44
66	0.630	0.667	0.333

Table 7: Growth Rate Values from Time Zero for *Pseudokirchneriella subcapitata* During a 72-Hour Exposure to GF-3307

Nominal concentration (mg GF-3307)	Mean growth rate (cells/mL/hour)			% Inhibition
	0-24 hours	0-48 hours	0-72 hours (%CV)	0-72 hours
Control	0.0673	0.0582	0.0653 (1)	--
0.090	0.0685	0.0576	0.0653 (1)	0
0.270	0.0653	0.0591	0.0633 (1)	3
0.80	0.0607*	0.0587	0.0614* (1)	6
2.4	0.0441*	0.0572	0.0588* (2)	10
7.3	0.0510*	0.0449*	0.0501* (4)	23
22	0.0430*	0.0336*	0.0355* (3)	46
66	0.00849*	0.00581*	-0.00619* (-78)	110

*Significant reduction in growth rate as compared to the control (Dunnett's test, p = 0.05).

Table 8: Yield Values for *Pseudokirchneriella subcapitata* During a 72-Hour Exposure to GF-3307

Nominal concentration (mg GF-3307)	Yield (cells/mL x 10 ⁴)			% Inhibition
	24 hours	48 hours	72 hours (%CV)	72 hours
Control	2.02	7.68	54.7 (5)	--
0.090	2.09	7.43	54.8 (9)	0
0.270	1.91	8.02	47.4* (5)	14
0.80	1.65*	7.87	41.0* (4)	25
2.4	0.943*	7.28	34.1* (8)	38
7.3	1.21*	3.83*	18.1* (14)	67
22	0.907*	2.02*	5.94* (9)	89
66	0.130	0.167*	-0.167* (-66)	100

*Significant reduction in yield as compared to the control (Dunnett's test, p = 0.05).

Table 9: Section-by-Section Specific Growth Rates for Controls During a 72-Hour Exposure of *Pseudokirchneriella subcapitata* to GF-3307

Control/Replicate	Section-by-Section Specific Growth Rate (cells/mL/hour) ^a			% Coefficient of Variance	
	0-24 hours	24-48 hours	48-72 hours	Replicate ^b	Overall ^c
A	0.0660	0.0483	0.0810	25	24
B	0.0621	0.0551	0.0777	18	
C	0.0715	0.0435	0.0790	29	
D	0.0698	0.0480	0.0800	25	
E	0.0680	0.0497	0.0769	21	
F	0.0660	0.0506	0.0827	24	

^a Values rounded to three significant figures. The section-by-section specific growth rate in each treatment was calculated for each period, i.e., 0 to 24, 24 to 48, and 48 to 72 hours

^b Replicate % Coefficient of Variance

^c Overall % Coefficient of Variance

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 the number initially inoculated to verify logarithmic phase growth. The mean coefficient of variation for daily growth rates in the control replicates during the course of the test did not exceed 35%. The coefficient of variation of average specific growth rates during the whole test period in control replicates did not exceed 7%. 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
Unicellular Green Alga	<i>Pseudokirchneriella subcapitata</i>	GF-3307	72 hr	E _r C ₅₀	25	mg/L, im
Unicellular Green Alga	<i>Pseudokirchneriella subcapitata</i>	GF-3307	72 hr	E _y C ₅₀	3.6	mg/L, im
Unicellular Green Alga	<i>Pseudokirchneriella subcapitata</i>	GF-3307	72 hr	E _r C ₅₀	8.0	mg/L, twgm
Unicellular Green Alga	<i>Pseudokirchneriella subcapitata</i>	GF-3307	72 hr	E _y C ₅₀	0.71	mg/L, twgm

A 2.2.1.4

Study 4 – X642188 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, *Daphnia magna*, Determined Under Flow-Through Test Conditions

Comments of zRMS:	<p>The study was already evaluated in the course of zonal authorization of the product GF-3308 and was considered acceptable. The zRMS's evaluation was copied here for transparency of the current assessment of the product GF-3307.</p> <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>EC₅₀ = 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, Daphnia magna, 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:	
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	NA

COMPLIANCE

	OECD Guideline 202
Guideline(s):	
US EPA Guideline(s):	None
Deviations:	None
Dates of work:	15 May 2018 to 17 May 2018
GLP status:	Yes
Number of pages in final report:	80

MATERIALS AND METHODS

Test Item(s)

	X642188
Test item (Common name):	
Purity:	99%
Description (physical state):	White solid with a mild odor
Lot/batch no.:	Lot No. SYN-FS08353-048 [TSN303567]

Test System

Organism (<i>Species</i>):	Water flea (<i>Daphnia magna</i>)
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 EC₅₀ 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 EC₅₀ 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) ^a			Overall Mean
	0-Hour	24-Hour	48-Hour	
Control (0)	<MQL ^b	<MQL ^b	<MQL ^b	<MQL ^b
Vehicle Control (0)	<MQL ^b	<MQL ^b	<MQL ^b	<MQL ^b
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)

^a See [Appendix D](#) for individual replicate analytical results.

^b MQL = 0.010 µg X642188/L.

Table 2: 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 ₅₀	>1.3 µg X642188/L		0.79 µg X642188/L (95% confidence limits 0.66 and 0.95 µg X642188/L)	

Table 3: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 ≥ 6.9 mg/L in control, vehicle control, and test substance treatments, which satisfies the acceptability criterion of ≥ 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 EC₅₀ value was estimated to be >1.3 µg X642188/L, the highest concentration tested. The estimated 48-hour EC₅₀ 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 ₅₀	0.79	µg X642188/L

A 2.2.1.5

Study 5 – 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 already evaluated in the course of zonal authorization of the product GF-3308 and was considered acceptable. The zRMS's evaluation was copied here for transparency of the current assessment of the product GF-3307.</p> <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: LC50 > 10 mg pm/L (based on nominal concentration)</p>
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Reference:	KCP 10.2.1/10
Report:	█████; 2018; X12019520 (a metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions; █████, a wholly owned subsidiary of █████; Lab Study No. 87146; DAS Study No. 180560 ; 07 August 2018; Unpublished

Guideline(s):	OECD Guideline 203
Deviations:	
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

COMPLIANCE

Guideline(s): OECD Guideline 203
US EPA Guideline(s): None
Deviations: None
Dates of work: 11 April 2018 to 15 April 2018
GLP status: Yes
Number of pages in final report: 58

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 ₃ /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 ^a	<MQL ^a	<MQL ^a	<MQL ^a	<MQL ^a
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) ^b	5.1 (104) ^b	5.7 (116)	NA
High Spike (14)	15 (104)	15 (104) ^b	14 (100) ^b	15 (109)	NA

^a MQL = 0.80 mg X12019520/L.

^b Samples were re-diluted in duplicate, and the average of the two are reported.

NA= Not Applicable

Table 2: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 ₅₀		>10 mg X12019520/L				
95% C.I.		Not calculated				
NOEC		10 mg X12019520/L				

Table 3: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 LC₅₀ 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

zRMS comments:

The new a.s. data have not been evaluated at zonal level by zRMS.

A 2.2.1.6 Study 11 Fenpicoxamid: Acute Immobilisation Toxicity Test with the Mayfly (*Cloeon dipterum*) under Semi-Static Conditions

Reference:	KCP-10.2.1/11
Report:	Consoir, G.; Fenpicoxamid: Acute Immobilisation Toxicity Test with the Mayfly (<i>Cloeon dipterum</i>) under Semi-Static Conditions; GG-BioTech-Design GmbH, Homberg (Ohm), Germany; Corteva Report No. 2025-0642; Lab Report No. G25-10; 2025; unpublished
Guideline(s):	OECD Guideline 235 (2011)
Deviations:	
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study):	NA

Study Summary

SUMMARY

In a 48-hour static renewal acute immobilisation test, freshwater dipteran *Cloeon dipterum* were exposed to fenpicoxamid. The in-life phase of the definitive test was conducted at nominal concentrations of 0 (control), 0 (solvent control), 32.0, 70.4, 155, 341 and 750 µg fenpicoxamid/L. Assessments of immobilisation and sublethal effects were conducted throughout the test.

Analytical verification of the test concentrations yielded recoveries of 79.4% to 97.7% of nominal at 0 h; of 67.3% to 86.6% of nominal at 2 h; and of 27.1% to 61.9% of nominal at 8 h of each renewal period. The calculated time-weighted mean measured test concentrations were 20.7, 46.8, 102, 232 and 483 µg fenpicoxamid/L.

Based on nominal test concentrations, the 48-hour EC₅₀ value for immobility was 620 µg fenpicoxamid/L.

Based on time-weighted mean measured test concentrations, the 48-hour EC₅₀ value for immobility was 405 µg fenpicoxamid/L.

All the study validity criteria were met.

MATERIALS AND METHODS

Test Item(s)

Test item (common name):	Fenpicoxamid
Purity:	90.0%; all test concentrations have been adjusted for the purity of the test item
Description (physical state):	White powder
Lot identifier/number:	82473677

Test System

Organism (Species):	Mayfly (<i>Cloeon dipterum</i>)
Study type:	48-hour acute immobilisation test
Study design:	Static renewal Renewal timing: 8-, 16-, 24-, 32- and 40-h
Replication:	4 replicates per test item treatment and control groups; 5 individuals/replicate
Test concentrations (nominal):	0 (control), 0 (solvent control), 32.0, 70.4, 155, 341 and 750 µg fenpicoxamid/L
Test concentrations (time-weighted mean measured):	0 (control), 0 (solvent control), 20.7, 46.8, 102, 232, 483 µg fenpicoxamid/L
Parameters measured:	Immobility; behaviour; sublethal effects
Observation intervals:	8-, 16-, 24-, 32-, 40- and 48-hour
Age of test organisms at test initiation:	Nymphs, smallest size available
No. of holding days before dosing:	2 days
Feeding:	None
Loading rate:	5 nymphs in 500-ml solution
Environmental conditions:	Temperature: 16.1 to 17.5°C Photoperiod: 16-hour Light intensity: 714 to 844 lux Dissolved oxygen: 8.09 to 9.20 mg/L; Hardness: 5.8 °dH (103 mg CaCO ₃ /L) pH: 7.79 to 8.31

Analytical verification:	Method: measuring concentrations of test item using HPLC-MS/MS Sample timings: 0 h, 2 h (intermediate), 8 h (aged and fresh), 10 h (intermediate), 16 h (aged and fresh), 18 h (intermediate), 24 h (aged and fresh), 26 h (intermediate), 32 h (aged and fresh), 34 h (intermediate), 40 h (aged and fresh) and 42 h (intermediate) and 48 hours LOQ: 1 µg/L LOD: 0.3 µg/L QC recoveries range: 95 to 105%
Reference toxicant:	none

Solvent substance (concentration): DMF (50 µL/L)

Methodology

Nymphs of the same size (smallest size available) were selected from a natural pond. Afterwards they were held in filtered natural pond water for 2 days prior to the test start to acclimatise. During this establishing phase, the animals were held at a light intensity of 500–1000 lux (16 hours photoperiod daily) and temperature conditions of $16 \pm 2^\circ\text{C}$.

A dose response test was performed at nominal concentrations of 0 (control), 0 (solvent control), 32.0, 70.4, 155, 341 and 750 µg fenpicoxamid/L. The test was conducted with a static renewal design where the test medium was renewed every eight hours; i.e. at 8, 16, 24, 32 and 40 hours. Five nymphs of *Cloeon dipterum* were impartially added to each of four replicates per concentration level at the start of the test. Biological observations, number of immobilized nymphs, and observations of the physical characteristics of the exposure and control solutions (e.g., precipitate, film on solution's surface) were recorded at exposure initiation, at each 8-hour renewal interval, and at exposure termination (48 hours).

The design for location of the test vessels was randomized to minimise the influence of spatial differences. The day of application of the test item was designated as day 0 (\triangleq start of the test). The exposure period was 48 h.

Analytical verification of fenpicoxamid was carried out in each test concentration in freshly prepared samples at test start, 2 h (intermediate), 8 h (aged and fresh), 10 h (intermediate), 16 h (aged and fresh), 18 h (intermediate), 24 h (aged and fresh), 26 h (intermediate), 32 h (aged and fresh), 34 h (intermediate), 40 h (aged and fresh), 42 h (intermediate) and at the end of the test (48 hours).

The quantification of the test item fenpicoxamid in the test samples was performed using High Performance Liquid Chromatography with MS/MS detection (HPLC-MS/MS).

Statistical analysis was performed using the software Toxrat Professional (Version 3.3.0). The 24- and 48-hour EC_{50} values were determined with the probit equation.

RESULTS AND DISCUSSION

Measurements of temperature, pH, light intensity and dissolved oxygen were within the range established for the test. All control and test substance treatment solutions were observed to be clear and colourless, with no visible surface slicks or particulates throughout the exposure.

Recoveries of fenpicoxamid in fresh solutions ranged from 79.4% to 97.7% of nominal at 0 h; of 67.3% to 86.6% of nominal at 2 h; and of 27.1% to 61.9% of nominal at 8 h of each renewal period. The calculated time weighted mean measured concentrations of test item were 20.7, 46.8, 102, 232 and 483 µg fenpicoxamid/L. The endpoint results are reported based on both the nominal and the time weighted mean measured concentrations.

Cumulative immobility at 48 h was 0.0%, 0.0%, 0.0%, 0.0%, 0.0%, 5.0% and 70.0% respectively in the control, solvent control and test item treatment groups of nominal 32.0, 70.4, 155, 341 and 750 µg fenpicoxamid/L (time weighted mean measured <LOD, <LOD, 20.7, 46.8, 102, 232 and 483 µg fenpicoxamid/L).

Sublethal effects were observed in the test item treatment group of nominal 750 µg fenpicoxamid/L (time weighted mean measured 483 µg fenpicoxamid/L). Observations included reduced activity.

Study Validity

To demonstrate the validity of the study, the following conditions were fulfilled:

OECD Criteria	Required	Observed
Immobilisation or other signs of disease or stress in control treatment(s)	≤15%	0%
Dissolved oxygen concentration at the end of the test in all test vessels	≥3 mg/L	≥8.97 mg/L

Table 1: Analytical results

Treatment µg fenpicoxamid/L	% of nominal			
	0-h new	2-h intermediate	8-h aged	9-h new
0 (C)	<LOD	<LOD	<LOD	<LOD
0 (SC)	<LOD	<LOD	<LOD	<LOD
32.0	70.4	71.8	42.5	70.4
70.4	88.4	71.7	40.3	90.3
155	92.8	76.4	42.8	89.7
344	96.2	78.9	44.6	87.7
750	97.4	73.6	39.6	84.5

C: negative control; SC: solvent control

Treatment µg fenpicoxamid/L	% of nominal				
	40-h intermediate	46-h aged	46-h new	48-h intermediate	24-h aged
0 (C)	<LOD	<LOD	<LOD	<LOD	<LOD
0 (SC)	<LOD	<LOD	<LOD	<LOD	<LOD
32.0	87.8	28.6	87.3	72.8	36.4
70.4	87.3	20.8	90.2	72.2	39.4
155	69.7	30.6	80.7	71.0	35.0
344	73.6	27.1	90.0	84.5	37.5
750	74.7	32.3	84.0	70.7	36.1

C: negative control; SC: solvent control

Treatment µg fenpicoxamid/L	% of nominal				
	44-h new	26-h intermediate	32-h aged	32-h new	34-h intermediate
0 (C)	<LOD	<LOD	<LOD	<LOD	<LOD
0 (SC)	<LOD	<LOD	<LOD	<LOD	<LOD
32.0	86.6	76.0	55.0	88.4	80.0
70.4	92.0	80.1	61.9	97.0	86.6
155	89.0	78.1	57.4	88.4	80.0
344	92.4	86.4	60.4	92.4	80.9
750	91.2	76.0	54.2	80.2	77.2

C: negative control; SC: solvent control

Treatment µg fenpicoxamid/L	% of nominal				
	40-h aged	40-h new	42-h intermediate	48-h aged	Time-Weighted Mean
0 (C)	<LOD	<LOD	<LOD	<LOD	NA
0 (SC)	<LOD	<LOD	<LOD	<LOD	NA
32.0	56.0	90.6	81.0	57.2	64.5
70.4	60.9	90.9	78.8	56.0	66.5
155	56.2	87.4	84.5	57.5	65.7
344	54.3	92.8	80.2	58.4	67.0
750	48.0	92.0	76.2	50.8	64.5

C: negative control; SC: solvent control

Table 2: ——— Immobility

Treatment (nominal)	Treatment (time-weighted-mean measured)	Cumulative immobility			
		8-h		16-h	
µg fenpicoxamid/L	µg fenpicoxamid/L	No. immobile	%	No. immobile	%
0 (C)	0 (C)	0	0.0	0	0.0
0 (SC)	0 (SC)	0	0.0	0	0.0
32.0	20.7	0	0.0	0	0.0
70.4	46.8	0	0.0	0	0.0
155	102	0	0.0	0	0.0
341	222	0	0.0	0	0.0
750	482	0	0.0	0	0.0

C: negative control; SC: solvent control

Treatment (nominal)	Treatment (time-weighted-mean measured)	Cumulative immobility			
		24-h		32-h	
µg fenpicoxamid/L	µg fenpicoxamid/L	No. immobile	%	No. immobile	%
0 (C)	0 (C)	0	0.0	0	0.0
0 (SC)	0 (SC)	0	0.0	0	0.0
32.0	20.7	0	0.0	0	0.0
70.4	46.8	0	0.0	0	0.0
155	102	0	0.0	0	0.0
341	222	0	0.0	0	0.0
750	482	4	20.0	0	0.0

C: negative control; SC: solvent control

Treatment (nominal)	Treatment (time-weighted-mean measured)	Cumulative immobility			
		40-h		48-h	
µg fenpicoxamid/L	µg fenpicoxamid/L	No. immobile	%	No. immobile	%
0 (C)	0 (C)	0	0.0	0	0.0
0 (SC)	0 (SC)	0	0.0	0	0.0
32.0	20.7	0	0.0	0	0.0
70.4	46.8	0	0.0	0	0.0
155	102	0	0.0	0	0.0
341	222	0	0.0	0	0.0
750	483	12	60.0	41	70.0

C: negative control; SC: solvent control

Table 3: ——— Sublethal effects

Treatment (nominal)	Treatment (time-weighted-mean measured)	Sublethal effects	
		8-h	16-h
µg fenpicoxamid/L	µg fenpicoxamid/L	Effect (%)	Effect (%)
0 (C)	0 (C)	AN	AN
0 (SC)	0 (SC)	AN	AN
32.0	20.7	AN	AN
70.4	46.8	AN	AN
155	102	AN	AN
341	222	AN	AN
750	482	AN	L

C: negative control; SC: solvent control

AN: all appeared normal; L: reduced activity

Treatment (nominal)	Treatment (time-weighted mean-measured)	Sublethal effects	
		24 h	48 h
$\mu\text{g fenpicoxamid/L}$	$\mu\text{g fenpicoxamid/L}$	Effect (n)	Effect (n)
0 (C)	0 (C)	AN	AN
0.4 (SC)	0.4 (SC)	AN	AN
42.6	20.7	AN	AN
70.4	46.4	AN	AN
155	102	AN	AN
341	326	AN	AN
750	482	L	L

C: negative control; SC: solvent control;
AN: all appeared normal; L: reduced activity

Treatment (nominal)	Treatment (time-weighted mean-measured)	Sublethal effects	
		40 h	48 h
$\mu\text{g fenpicoxamid/L}$	$\mu\text{g fenpicoxamid/L}$	Effect (n)	Effect (n)
0 (C)	0 (C)	AN	AN
0.4 (SC)	0.4 (SC)	AN	AN
42.6	20.7	AN	AN
70.4	46.4	AN	AN
155	102	AN	AN
341	326	AN	AN
750	482	L	L

C: negative control; SC: solvent control;
N: all appeared normal; L: reduced activity

Table 4: Effects of Fenpicoxamid in a caddisfly (*Cloeon dipterum*) acute immobilisation test—based on nominal concentrations

Endpoint type		Endpoint value ($\mu\text{g fenpicoxamid/L}$)	95% Confidence Limits ($\mu\text{g fenpicoxamid/L}$)
24 h immobility	EC_{50}	5750	n.d.
48 h immobility	EC_{50}	620	510—770

Table 5: Effects of Fenpicoxamid in a caddisfly (*Cloeon dipterum*) acute immobilisation test—based on time-weighted mean measured concentrations

Endpoint type		Endpoint value ($\mu\text{g fenpicoxamid/L}$)	95% Confidence Limits ($\mu\text{g fenpicoxamid/L}$)
24 h immobility	EC_{50}	5482	n.d.
48 h immobility	EC_{50}	405	337—495

CONCLUSION

Based on nominal test concentrations, the 48-hour EC_{50} values for immobility was 620 $\mu\text{g fenpicoxamid/L}$. Based on time-weighted mean measured test concentrations, the 48-hour EC_{50} values for immobility was 405 $\mu\text{g fenpicoxamid/L}$.

Common name	Species	Test item	Exposure system	Time scale	Endpoint	Toxicity value	Units of test item
Mayfly	<i>Cladocera dip-</i> <i>serum</i>	Fenproex- amid	Static Re- newal	48-hour	EC ₅₀	405	µg fenproex- amid/L water

zRMS comments:

The new a.s. data have not been evaluated at zonal level by zRMS.

A 2.2.1.7 Study 12: X642188 (a metabolite of XDE-777): Toxicity Test with the Cladoceran, *Daphnia magna*, Conducted Under Pulse-Dosed, Static-Renewal Conditions

Reference:	KCP 10.2.1/12
Report:	Goudie, O., 2020; X642188 (a metabolite of XDE-777): Toxicity Test with the Cladoceran, <i>Daphnia magna</i> , Conducted Under Pulse-Dosed, Static-Renewal Conditions, Eurofins EAG Agrosience LLC, Columbia, Missouri, USA; Corteva Report No. 491071, Lab Report No. 88456, 2020, unpublished
Guidelines:	OECD 211 (2012)
Deviations:	Compared to OECD TG 211 (2012): Exposure and assessment were modified: <ul style="list-style-type: none"> Test medium renewals at days 0, 3 or at days 0, 8 Only mortality was assessed
GLP	Yes
Acceptability:	Yes
Duplication (if vertebrate study):	NA

Study Summary

SUMMARY

The 21-day pulse-dosed, static-renewal toxicity of X642188 to *Daphnia magna* was studied based on the methodology of OECD Guideline 211 (2012).

A definitive test was conducted with a single negative control, and two discrete test groups consisting of a vehicle control and five X642188 target nominal concentrations of 10, 18, 23, 28, and 65 µg X642188/L.

During the definitive test, a pulse-dose regimen consisted of two separate dosing events at two different time intervals was utilized. Each dosing event consisted of freshly prepared control and pertinent test group solutions. The first dosing event was at test initiation, where fresh test solutions were prepared for the vehicle control and all test substance treatment replicates in both test groups. The second dosing event in each case (day 3 or day 8) consisted of freshly prepared solutions of the vehicle control and all test substance treatment replicates in the pertinent test group. On renewal days that were non-dosing events for a particular test group, each test chamber was renewed with dilution water only. Renewal occurred every two to three days up to test termination. On each dosing event or renewal day, the parent daphnids were transferred to clean test chambers containing fresh dilution water or test solutions, as applicable.

The negative control, vehicle control, and all test substance solutions were clear and colorless with no visible particulates, surface film, un-dissolved test substance, or precipitate throughout the test. Water quality parameters of temperature, dissolved oxygen concentration and pH remained within acceptable limits throughout the definitive test. The total hardness ranged from 140 to 148 mg CaCO₃/L in the dilution water control, 28, and 65 µg X642188/L test treatment solutions. The total alkalinity ranged from 148 to 160 mg CaCO₃/L in the dilution water control, 28, and 65 µg X642188/L test treatment solutions. The conductivity ranged from 322 to 351 µS/cm in the dilution water control, 28, and 65 µg X642188/L test treatment solutions.

After 21 days of exposure, survival of *D. magna* exposed to a second pulse dose (Test Group 1) of X642188 on day 3, was 100, 100, 90, 60, 50, and 0% in the 0 (vehicle control), 10, 18, 23, 28, and 65 µg X642188/L nominal treatments, respectively. After 21 days of exposure, survival of *D. magna* exposed to a second pulse dose (Test Group 2) of X642188 on day 8, was 100, 90, 90, 70, 80, and 10% in the 0 (vehicle control), 10, 18, 23, 28, and 65 µg X642188/L nominal treatments, respectively. The negative control survival after 21 days of exposure was 100%. No morphological or behavioral changes were observed.

MATERIALS AND METHODS

Test Item(s)

Test item (common name):	X642188
Purity:	90.7%
Description (physical state):	White solid
Lot/batch no.:	HY-2020151001-02 (TSN310968)

Test System

Organism (Species):	Water flea (<i>Daphnia magna</i>)
Study type:	21-day Pulse dose
Study design:	Static renewal, pulse dosed, 1 individual per replicate, 10 replicates per test concentration
Test concentrations:	Nominal: 0 (negative control), 0 (vehicle control) 10, 18, 23, 28, and 65 µg X642188/L
Parameters measured:	Survival
Observation intervals:	Daily
Age of test organisms at test initiation:	<24 hours old
No. of holding days before dosing:	None
Number of Daphnids per dose group:	20
Number of Daphnids per control group:	Negative control: 10 Vehicle control: 20 (10 per Test Group)
Feeding regime:	Daily
Environmental conditions:	Loading rate: Not given Temperature: Fresh: 19.7 to 20.4°C Spent: 19.2 to 20.1°C Photoperiod: 16-hour Light intensity: 553 to 561 lux

	Dissolved oxygen concentration: Fresh: 8.1 to 8.6 mg/L (93 to 99% sat) Spent: 7.9 to 9.0 mg/L (91 to 104% sat)
	pH: Fresh: 8.1 to 8.4 Spent: 8.2 to 8.5
	Total hardness: Fresh: 144 to 148 mg CaCO ₃ /L Spent: 140 to 148 mg CaCO ₃ /L
	Alkalinity: Fresh: 158 to 160 mg CaCO ₃ /L Spent: 148 to 158 mg CaCO ₃ /L
Analytical verification:	Method: measuring concentrations of X642188 using LC-MS/MS Sampling intervals: LOQ: 0.030 µg X642188/L LOD: 0.0090 µg X642188/L QC recoveries range: 89 to 118%
Reference toxicant:	Potassium chloride conducted as a separate GLP study
Solvent substance (concentration):	DMF (100 µL/L)

Methodology

The definitive test was performed with a single negative control, and two discrete test groups consisting of a vehicle control and five X642188 target nominal concentrations of 10, 18, 23, 28, and 65 µg X642188/L. The definitive test was conducted for 21 days, commencing when daphnids were added to the test chambers. The negative control was replicated ten times (A through J, 1 daphnid each) for a total of 10 daphnids. The vehicle control and all test substance treatments were replicated ten times (A through J, 1 daphnid each) for each test group, resulting in a total of 20 daphnids for the vehicle control and all test substance treatments. The two discrete test groups were maintained separately from each other, but were contained within the same water bath to ensure environmental conditions were as consistent as possible for all test chambers. The negative control test chambers were split and associated with an individual test group using a random number generator. Daphnids were fed 1.0 to 1.2 mL of a prepared feeding solution daily, excepting the day of test termination. The prepared feeding solution contained a mixture of algae (*Pseudokirchneriella subcapitata*, formerly *Selenastrum capricornutum*) and YCT invertebrate supplement.

During the definitive test, a pulse dose regimen consisted of two separate dosing events at two different time intervals was utilized. Each dosing event consisted of freshly prepared control and pertinent test group solutions. The first dosing event was at test initiation, where fresh test solutions were prepared for the vehicle control and all test substance treatment replicates in both test groups. The second dosing event in each case consisted of freshly prepared solutions of the vehicle control and all test substance treatment replicates in the pertinent test group. The test substance treatment groups for each dosing event were as follows: Dosing event one—test groups 1 and 2; Dosing event two—Day 3 (test group 1) and Day 8 (test group 2). On renewal days that were not dosing events for a particular test group, each test chamber was renewed with dilution water only. Renewal occurred every two to three days up to test termination. On each dosing event or renewal day, the parent daphnids were transferred to clean test chambers containing fresh dilution water or test solutions, as applicable. Observations of survival (i.e., mortality), were conducted for all treatments at least daily throughout the test. Observations were conducted using a random number generator on all non-dosing event days. On days with dosing events, pertinent biological test chambers were observed concurrent with each analytical sampling time point (e.g., at dosing [0 hour], 2 hours, and 4 hours post-dosing), and in order of negative control, vehicle control, and then ascending order of

treatment. No sub-lethal effect observations were recorded; daphnids were recorded as either alive or dead. Any daphnids that did not exhibit movement by macroscopic visual observation were then examined microscopically to determine if mortality had occurred. If mortality had not occurred, the daphnid was noted as alive. If mortality was verified, the daphnid was recorded as dead and discarded. Total hardness, total alkalinity, and conductivity were measured in the freshly prepared parent solutions in the negative control and the 65 µg X642188/L treatment at initiation. Total hardness, total alkalinity, and conductivity were measured in a composite of all available spent replicates (i.e., all those containing live organisms) in the negative control and test treatments as follows: Negative control—day 3, 6, 11, and 21; 28 µg X642188/L treatment—day 6 (test group 1), day 11 (test group 2), and day 21 (test groups 1 and 2); 65 µg X642188/L treatment—day 3 (test group 1). Temperature, dissolved oxygen concentration, and pH were measured in the dilution water (negative control), fresh parent solutions, and composites of spent replicate test solutions as listed above. No aeration was provided to any negative control, vehicle control, or test substance treatment during the test. Test solutions were analyzed for X642188 using a liquid chromatography system with tandem mass spectrometry (LC-MS/MS).

The data for survival was summarized and percent mortality reported by treatment group for each day of the exposure. No additional statistical analyses were performed on the data.

RESULTS AND DISCUSSION

Measurements of temperature, dissolved oxygen, pH and light intensity were within the range established for the test. The negative control, vehicle control, and all test substance solutions were clear and colorless with no visible particulates, surface film, un-dissolved test substance, or precipitate throughout the test.

No residues of X642188 were detected in the control solutions above the LOD.

Recoveries of X642188 ranged from 102% to 120% in fresh solutions at 0 hours on day 0, day 3 (Test Group 1) and day 8 (Test group 2) and from 6 to 12% after 2 hour and from 1 to 2% after 4 hours. After 3 days, the concentrations ranged from <LOD to 0% (slightly above LOQ).

The biological results are reported based on the nominal test concentrations.

No morphological or behavioral changes were observed. The negative and vehicle control survival after 21 days of exposure was 100%.

After 21 days of exposure, survival of *D. magna* exposed to a second pulse dose (Test Group 1) of X642188 on day 3, was 100, 100, 90, 60, 50, and 0% in the 0 (vehicle control), 10, 18, 23, 28, and 65 µg X642188/L nominal treatments, respectively. After 21 days of exposure, survival of *D. magna* exposed to a second pulse dose (Test Group 2) of X642188 on day 8, was 100, 90, 90, 70, 80, and 10% in the 0 (vehicle control), 10, 18, 23, 28, and 65 µg X642188/L nominal treatments, respectively.

Study Validity

To demonstrate the validity of the study, the following conditions were fulfilled:

OECD Criteria	Required	Observed
Parental mortality at the end of the test in the control	≤20%	0%

USEPA Criteria	Required	Observed
Organisms in any control showing signs of disease, stress and/or death	≤10%	0%

Table 1: Analytical results

Treatment µg X642188/L	Control	Vehicle control	10	10	50	50	50	Range
Sampling day	% nominal							
0	0h post dosing ^a	<LOD	<LOD	113	113	110	107	107 to 113
0	2h post dosing ^a	<LOD	<LOD	7	8	8	8	7 to 8
0	4h post dosing ^{a,b}	<LOD	<LOD	2	1	1	2	1 to 2
2	spent ^{a,b}	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0 (<LOQ)
3	0h post dosing ^a	<LOD	<LOD	102	111	108	114	102 to 113
3	2h post dosing ^a	<LOD	<LOD	7	8	7	12	7 to 12
3	4h post dosing ^a	<LOD	<LOD	1	1	1	2	1 to 2
6	spent ^a	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
6	0h post dosing ^a	<LOD	<LOD	112	116	108	111	108 to 120
6	2h post dosing ^a	<LOD	<LOD	8	6	6	8	6 to 8
6	4h post dosing ^a	<LOD	<LOD	1	1	1	1	1 to 2
14	spent ^a	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0 (<LOQ)

LOD = 0.0090 µg X642188/L

LOQ = 0.030 µg X642188/L

^aNot measured due to 100% mortality in Test Group 1

^aTest group 1

^bTest group 2

Table 2: Effect of X642188 on daphnid survival (% survival) – Test Group 1 (test medium renewal on day 3)

Nominal Treatment (µg/L)	Study Day (number surviving (% survival))								
	Day 1			Day 2		Day 3			
	0-hr	2-hr	4-hr			0-hr	2-hr	4-hr	6-hr
VC	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	5 (100) ^a	5 (100) ^a	5 (100) ^a
0 (VC)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
10	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
18	10 (100)	10 (100)	10 (100)	10 (100)	9 (90)	9 (90)	9 (90)	9 (90)	9 (90)
28	10 (100)	10 (100)	9 (90)	8 (80)	8 (80)	8 (80)	8 (80)	8 (80)	8 (80)
28	10 (100)	10 (100)	9 (90)	8 (80)	8 (80)	8 (80)	8 (80)	8 (80)	8 (80)
85	10 (100)	9 (90)	7 (70)	5 (50)	2 (20)	2 (20)	2 (20)	1 (10)	0 (0)

C: control; VC: vehicle control; ^a Five of the ten control replicates positioned in testing area with each Test Group. Only replicates associated with each particular Test Group were observed at that Test Group's dosing event hourly time points on study days 3 and 8.

Table 2: Effect of X642188 on daphnid survival (% survival) – Test Group 1 (continued) (test medium renewal on day 3)

Nominal Treatment (µg/L)	Study Day (number surviving (% survival))							
	Day 4	Day 5	Day 6	Day 7	Day 8			
					0-hr	2-hr	4-hr	6-hr
VC	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	5 (100) ^a	5 (100) ^a	5 (100) ^a
0 (VC)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
10	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	N/A	N/A	N/A
18	9 (90)	9 (90)	9 (90)	9 (90)	9 (90)	N/A	N/A	N/A
28	7 (70)	7 (70)	7 (70)	6 (60)	6 (60)	N/A	N/A	N/A
28	6 (60)	5 (50)	5 (50)	5 (50)	5 (50)	N/A	N/A	N/A
85	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	N/A	N/A	N/A

C: control; VC: vehicle control; ^a Five of the ten control replicates positioned in testing area with each Test Group. Only replicates associated with each particular Test Group were observed at that Test Group's dosing event hourly time points on study days 3 and 8.

Table 2: *Effect of X642188 on daphnid survival (% survival) — Test Group 1 (continued) (test medium renewal on day 3)*

Nominal Treatment (µg/L)	Study Day (number surviving (% survival))							
	9	10	11	12	13	14	15	16
3 (C)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
0 (VC)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
10	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
18	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
25	6 (60)	6 (60)	6 (60)	6 (60)	6 (60)	6 (60)	6 (60)	6 (60)
28	5 (50)	5 (50)	5 (50)	5 (50)	5 (50)	5 (50)	5 (50)	5 (50)
65	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

C: control; VC: vehicle control; * Five of the ten control replicates positioned in testing area with each Test Group. Only replicates associated with each particular Test Group were observed at that Test Group's dosing event hourly time points on study days 3 and 8.

Table 2: *Effect of X642188 on daphnid survival (% survival) — Test Group 1 (continued) (test medium renewal on day 3)*

Nominal Treatment (µg/L)	Study Day (number surviving (% survival))				
	17	18	19	20	21
3 (C)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
0 (VC)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
10	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
18	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
25	6 (60)	6 (60)	6 (60)	6 (60)	6 (60)
28	5 (50)	5 (50)	5 (50)	5 (50)	5 (50)
65	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

C: control; VC: vehicle control; * Five of the ten control replicates positioned in testing area with each Test Group. Only replicates associated with each particular Test Group were observed at that Test Group's dosing event hourly time points on study days 3 and 8.

Table 3: Effect of X642188 on daphnid survival (% survival) – Test Group 2 (test medium renewal on day 8)

Nominal Treatment (µg/L)	Study Day (number surviving (% survival))								
	Day 3			Day 4	Day 5	Day 8			
	0-1h	2-4h	4-6h			0-1h	2-4h	4-6h	6-12h
0 (VC)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	NA	NA	NA
10	10 (100)	10 (100)	10 (100)	10 (100)	9 (90)	9 (90)	NA	NA	NA
18	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	NA	NA	NA
22	10 (100)	10 (100)	9 (90)	9 (90)	7 (70)	7 (70)	NA	NA	NA
28	10 (100)	10 (100)	10 (100)	10 (100)	8 (80)	8 (80)	NA	NA	NA
65	10 (100)	8 (80)	6 (60)	5 (50)	3 (30)	1 (10)	NA	NA	NA

VC: vehicle control. * Five of the ten control replicates positioned in testing area with each Test Group. Only replicates associated with each particular Test Group were observed at that Test Group's dosing event hourly time points on study days 3 and 8.

Table 3: Effect of X642188 on daphnid survival (% survival) – Test Group 2 (continued) (test medium renewal on day 8)

Nominal Treatment (µg/L)	Study Day (number surviving (% survival))							
	Day 4	Day 5	Day 6	Day 7	Day 8			
					0-1h	0-3h	3-12h	1-12h
0 (VC)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
10	9 (90)	9 (90)	9 (90)	9 (90)	9 (90)	9 (90)	9 (90)	9 (90)
18	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
22	7 (70)	7 (70)	7 (70)	7 (70)	7 (70)	7 (70)	7 (70)	7 (70)
28	8 (80)	8 (80)	8 (80)	8 (80)	8 (80)	8 (80)	8 (80)	8 (80)
65	1 (10)	1 (10)	1 (10)	1 (10)	1 (10)	1 (10)	1 (10)	1 (10)

VC: vehicle control. * Five of the ten control replicates positioned in testing area with each Test Group. Only replicates associated with each particular Test Group were observed at that Test Group's dosing event hourly time points on study days 3 and 8.

Table 3: Effect of X642188 on daphnid survival (% survival) – Test Group 2 (continued) (test medium renewal on day 8)

Nominal Treatment (µg/L)	Study Day [number surviving (% survival)]							
	0	16	32	48	64	80	96	112
0 (VC)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
16	9 (90)	9 (90)	9 (90)	9 (90)	9 (90)	9 (90)	9 (90)	9 (90)
32	10 (100)	10 (100)	9 (90)	9 (90)	9 (90)	9 (90)	9 (90)	9 (90)
48	7 (70)	7 (70)	7 (70)	7 (70)	7 (70)	7 (70)	7 (70)	7 (70)
64	8 (80)	8 (80)	8 (80)	8 (80)	8 (80)	8 (80)	8 (80)	8 (80)
80	1 (10)	1 (10)	1 (10)	1 (10)	1 (10)	1 (10)	1 (10)	1 (10)

VC: vehicle control Five of the ten control replicates positioned in testing area with each Test Group. Only replicates associated with each particular Test Group were observed at that Test Group's dosing event hourly time points on study days 3 and 8.

Table 3: Effect of X642188 on daphnid survival (% survival) – Test Group 2 (continued) (test medium renewal on day 8)

Nominal Treatment (µg/L)	Study Day [number surviving (% survival)]				
	16	32	48	64	80
0 (VC)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
16	9 (90)	9 (90)	9 (90)	9 (90)	9 (90)
32	9 (90)	9 (90)	9 (90)	9 (90)	9 (90)
48	7 (70)	7 (70)	7 (70)	7 (70)	7 (70)
64	8 (80)	8 (80)	8 (80)	8 (80)	8 (80)
80	1 (10)	1 (10)	1 (10)	1 (10)	1 (10)

Five of the ten control replicates positioned in testing area with each Test Group. Only replicates associated with each particular Test Group were observed at that Test Group's dosing event hourly time points on study days 3 and 8.

CONCLUSION

At the nominal concentration of 18 µg X642188/L, the 21-day survival was 90% in both treatments, i.e. with test medium renewal after 3 days or after 8 days.

A 2.2.1.8 Study 6 - X12446477 (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 already evaluated in the course of zonal authorization of the product GF-3308 and was considered acceptable. The zRMS's evaluation was copied here for transparency of the current assessment of the product GF-3307.</p> <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>LC50 > 10 mg pm/L (based on nominal concentration)</p>
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Reference:	KCP 10.2.1/6
Report:	█████; 2018; X12446477 (a metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions; █████, a wholly owned subsidiary of █████; Lab Study No. 87147; DAS Study No. 180561 ; 18 July 2018; Unpublished
Guideline(s):	OECD Guideline 203
Deviations:	
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

COMPLIANCE

Guideline(s):	OECD Guideline 203
US EPA Guideline(s):	None
Deviations:	None
Dates of work:	26 March 2018 to 30 March 2018
GLP status:	Yes
Number of pages in final report:	58

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 (<i>Species</i>):	Rainbow trout (<i>Oncorhynchus mykiss</i>)
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 ± 0.1858 g (0.6130 to 1.2036 g) Mean total length: 46 ± 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 ₃ /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 ^a	<MQL ^a	<MQL ^a	<MQL ^a	<MQL ^a
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

^a MQL = 0.050 mg X12446477/L.

NA= Not Applicable

Table 2: 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 ₅₀		>10 mg X12446477/L				
95% C.I.		Not calculated				
NOEC		10 mg X12446477/L				

Table 3:Sub-lethal effects of X12446477in 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 LC₅₀ 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 ₅₀	>10	mg X12446477/L, nom

A 2.2.1.9

Study 7– GF-3308: Acute Toxicity to the Cladoceran, *Daphnia magna*, Determined Under Static-Renewal Test Conditions

Comments of zRMS:	The study evaluated by zRMS-PL during zonal evaluation of GF-3308, available on Circa platform. The study was not used in the current dossier EC50 = 0.048 mg product/L (based on time weighted geometric mean concentration), corresponding to 0.0023 mg a.s./L
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Reference:	KCP 10.2.1/4
Report:	Goudie, O.; 2016; GF-3308: Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions; ABC Laboratories, Inc. a wholly owned subsidiary of EAG, Inc., Columbia, Missouri, USA; Lab Study No. 83495; DAS Study No. 160102 ; 01 December 2016; Unpublished
Guideline(s):	OECD 202
Deviations:	
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

COMPLIANCE

Guideline(s):	OECD 202
US EPA Guideline(s):	None
Deviations:	None

Dates of work: 07 June 2016 to 09 June 2016
GLP status: Yes
Number of pages in final report: 66

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 (*Species*): Water flea (*Daphnia magna*)
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
Reference substance: XDE-777 (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 characterize 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 hr^{-1} ($R^2=0.94$; $p<0.0001$; 95% CI -0.730 to -0.587 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 1). 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_{50} , 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 $1/2\text{LoQ}$ 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.

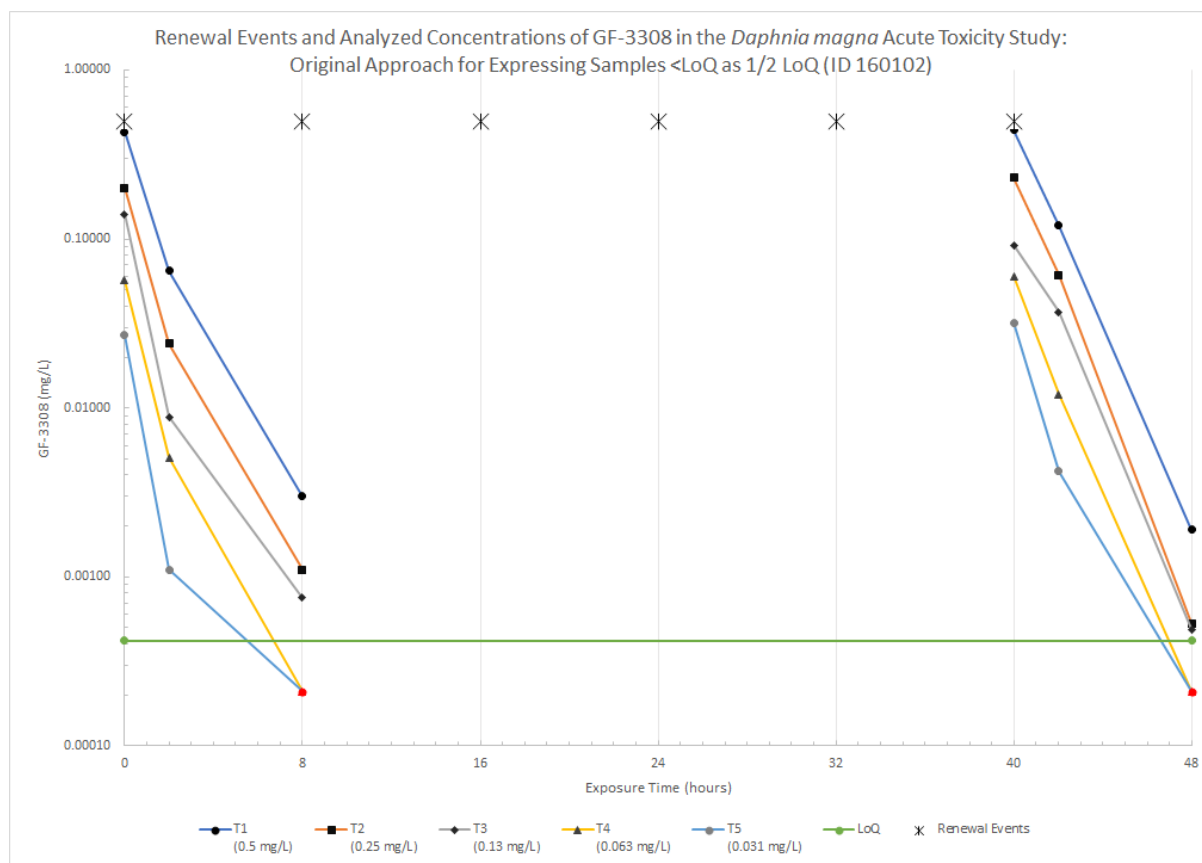
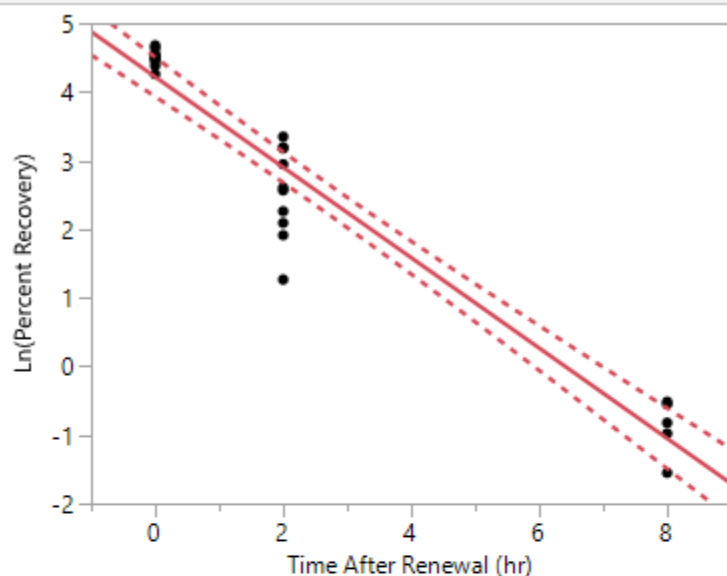


Figure 1a Graphic depiction of analytically verified concentrations of GF-3308 (containing XDE-777 active ingredient) and the 6 dosing renewal events in the *Daphnia* acute toxicity study. Symbols in black/gray represent analytically verified samples >LOQ. Symbols in red represent the 4 samples that 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 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 EC₅₀ value was estimated to be >0.073 mg GF-3308/L, the highest concentration tested. The 48-hour EC₅₀ 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 EC₅₀ and NOEC were 0.0023 and 0.00086 mg XDE-777/L, respectively, as expressed as the active ingredient purity of 4.8%.

Revised Table 1. Revised Calculated Concentrations of GF-3308 (based on analysis of XDE-777) During the 48-hour 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

Nominal Concentration (mg GF-3308/L)	Calculated Concentration as mg GF-3308/L (Percent Nominal) ^a						Time Weighted Geometric Mean Concentration: 0-8 hours ^c	Time Weighted Geometric Mean Concentration: 40-48 hours ^c	Average Time Weighted Geometric Mean Concentration ^d
	0-Hour	2-Hour (spent)	8-Hour (spent)	40-Hour (fresh)	42-Hour (spent)	48-Hour			
Control	<MQL ^b	<MQL ^{b,j}	<MQL ^{b,j}	<MQL ^b	<MQL ^b	<MQL ^{b,h}	<MQL ^b	<MQL ^b	<MQL ^b
0.031	0.027 (87) ^e	0.0011 (4) ^j	0.00011 (0.35) ^j	0.032 (103)	0.0042 (14)	0.00011 (0.35)	0.0023	0.0043	0.0033
0.063	0.057 (90) ^e	0.0051 (8) ^j	0.00022 (0.35) ^j	0.060 (95)	0.012 (19)	0.00022 (0.35)	0.0065	0.0097	0.0081
0.13	0.14 (108)	0.0088 (7) ^j	0.00075 (0.6) ^j	0.092 (71) ^f	0.037 (28)	0.00049 (0.4) ^g	0.014 (11)	0.021 (16)	0.018 (14)
0.25	0.20 (80) ^e	0.024 (10) ^j	0.0011 (0.4) ^j	0.23 (92)	0.061 (24)	0.00053 (0.2) ^g	0.026 (11)	0.041 (17)	0.034 (14)
0.50	0.43 (86) ^e	0.065 (13) ^j	0.00299 (0.6) ^j	0.44 (88)	0.12 (24)	0.0019 (0.4) ^g	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) ^f	0.033 (118) ^f	0.031 (111)	0.032 (115) ^f	0.027 (97) ^e	NA	NA	NA
High Spike (0.572)	0.63 (110) ^e	0.68 (119) ^f	0.60 (105) ^f	0.62 (108)	0.60 ^e (105)	<MQL ^{b,h}	NA	NA	NA

^a The Calculated Concentration, mg GF-3308/L = ((Measured conc. from curve × Analysis Vol / Sample Vol) / 1000) / purity of XDE-777, 4.8%

^b MQL = 0.0200 ng XDE-777/L = 0.00042 mg GF-3308/L

^c For <MQL values, ½ MQL (0.00021 mg GF-3308/L used for calculations.

^d Average of 0-8 and 40-48 hour time weighted geometric mean concentrations.

^e Re-diluted in duplicate and re-analyzed, mean of duplicate re-analysis reported.

^f Re-diluted in duplicate and re-analyzed, mean of original and duplicate re-analysis reported.

^g Original concentration from curve had no peak. Samples re-analyzed twice. The third analysis is reported.

^h Sample re-diluted in duplicate and re-analyzed, results matched the original.

ⁱ Sample re-diluted and re-analyzed, results of the re-analyses reported.

^j Sample re-analyzed, results of the re-analyses reported.

NA = Not applicable.

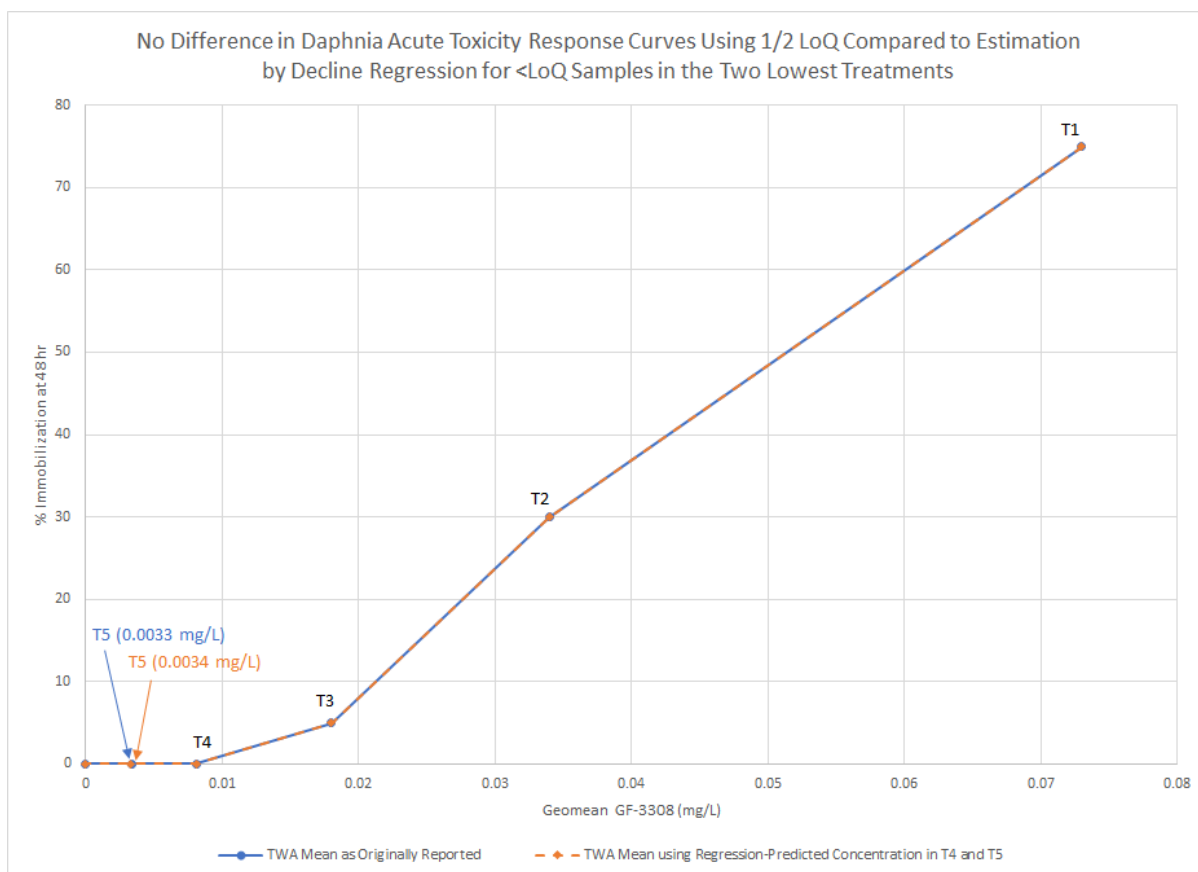


Figure 2 Comparison of dose response of *Daphnia magna* to GF-3308 using the originally calculated geometric mean concentrations (using 1/2 LoQ for 4 samples at the two lowest treatment levels) and geometric means concentrations calculated using the decline regression estimated values (0.35% recovery at 8 hours).

Table 1: Calculated Concentrations of GF-3308 (based on analysis of XDE-777) During the 48-hour Acute Toxicity Test with *D. magna*

Nominal Concentration (mg GF-3308/L)	Calculated Concentration as mg GF-3308/L (Percent Nominal) ^a						Time Weighted Geometric Mean Concentration: 0-8 hours ^c	Time Weighted Geometric Mean Concentration: 40-48 hours ^c	Average Time Weighted Geometric Mean Concentration ^d
	0-Hour	2-Hour (spent)	8-Hour (spent)	40-Hour (fresh)	42-Hour (spent)	48-Hour			
Control	<MQL ^b	<MQL ^{b,j}	<MQL ^{b,j}	<MQL ^b	<MQL ^b	<MQL ^{b,g}	<MQL ^b	<MQL ^b	<MQL ^b
0.031	0.027 (87) ^e	0.0011 (4) ⁱ	<MQL ^{b,i}	0.032 (103)	0.0042 (14)	<MQL ^{b,g}	0.0024 (8)	0.0044 (14)	0.0034 (11)
0.063	0.057 (90) ^e	0.0051 (8) ⁱ	<MQL ^{b,i}	0.060 (95)	0.012 (19)	<MQL ^{b,g}	0.0065 (10)	0.0096 (15)	0.0081 (13)
0.13	0.14 (108)	0.0088 (7) ⁱ	0.00075 (0.6) ⁱ	0.092 (71) ^f	0.037 (28)	0.00049 (0.4) ^g	0.014 (11)	0.021 (16)	0.018 (14)
0.25	0.20 (80) ^e	0.024 (10) ⁱ	0.0011 (0.4) ⁱ	0.23 (92)	0.061 (24)	0.00053 (0.2) ^g	0.026 (11)	0.041 (17)	0.034 (14)
0.50	0.43 (86) ^e	0.065 (13) ⁱ	0.00299 (0.6) ⁱ	0.44 (88)	0.12 (24)	0.0019 (0.4) ^g	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) ^f	0.033 (118) ^f	0.031 (111)	0.032 (115) ^f	0.027 (97) ^e	NA	NA	NA
High Spike (0.572)	0.63 (110) ^e	0.68 (119) ^f	0.60 (105) ^f	0.62 (108)	0.60 ^e	<MQL ^{b,h}	NA	NA	NA

^a The Calculated Concentration, mg GF-3308/L = (Measured conc. from curve × Analysis Vol / Sample Vol) / 1000 / purity of XDE-777, 4.8%

^b MQL = 0.0200 ng XDE-777/L = 0.00042 mg GF-3308/L

^c For <MQL values, ½ MQL (0.00021 mg GF-3308/L used for calculations.

^d Average of 0-8 and 40-48 hour time weighted geometric mean concentrations.

^e Re-diluted in duplicate and re-analyzed, mean of duplicate re-analysis reported.

^f Re-diluted in duplicate and re-analyzed, mean of original and duplicate re-analysis reported.

^g Original concentration from curve had no peak. Samples re-analyzed twice. The third analysis is reported.

^h Sample re-diluted in duplicate and re-analyzed, results matched the original.

ⁱ Sample re-diluted and re-analyzed, results of the re-analyses reported.

^j Sample re-analyzed, results of the re-analyses reported.

NA = Not applicable.

Table 2: 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 ₅₀	>0.073 mg GF-3308/L		0.048 mg GF-3308/L	

Table 3: 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 ≥ 8.4 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*. The 24-hour EC₅₀ value was estimated to be >0.073 mg GF-3308/L, the highest concentration tested. The 48-hour EC₅₀ 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 EC₅₀ 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 ₅₀	0.048	mg/L twgm
Water flea	<i>Daphnia magna</i>	GF-3308	48-hr	EC ₅₀	0.0023	mg a.i./L

A 2.2.1.10

Study 2 - GF-3307: 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 a minor deviation.</p> <p>It was noted that the study was conducted in 2018 in line with OECD 203 of 1992 where for the tested fish species rainbow trout the recommended test temperature range was 13-17°C (constant within a range of 2°C) while in the current OECD 203 of 2019 recommends the test temperature range of 10-14°C (constant within a range of 1°C). For that reason, the temperature range of 14.3 to 15.0°C in the study is acceptable.</p> <p>It was also noted that the highest pH of 8.6 slightly exceeded the recommended maximum of 8.5.</p> <p>However, in zRMS opinion these deviations are considered to have no impact on the outcome of the study since all the validity criteria were met.</p> <p>It was noted that GF-3307 contains two active substances and in line with the requirements of the Central Zone the test concentrations of both substances should be verified in the respective chemical analyses or, as a minimum, the least stable active substance should be analysed. In the study the concentrations of both active substances fenpicoxamid and prothioconazole were measured. The geometric mean measured concentrations of prothioconazole were maintained within 80-120% of nominal but the mean concentrations of fenpicoxamid were between 29 and 34% of nominal. For that reason, the concentrations of GF-3307 were calculated based on the geometric mean measured concentration of the least stable substance fenpicoxamid. However, according to the recommendations in Appendix J EFSA Supporting publication 2019:EN-1673 in such a case the geomean measured concentrations of both active substances should be re-calculated to the sum of both active substances. Nevertheless, based on the information from the area of environmental fate and behavior of both active substances it can be concluded that fenpicoxamid is the least stable substance. Furthermore, based on the calculations of contribution of toxicity of each active substance (provided in this report in Table 9.5-5) fenpicoxamid was determined to be the driver of the toxicity (contribution of $> 90\%$) to fish. For that reason, the zRMS is of the opinion that the concentrations of GF-3307 calculated based on the geometric mean concentrations of fenpicoxamid in the present study are acceptable to derive the endpoint.</p> <p>Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>96-hour LC₅₀ = 72 µg product/L (based on geometric mean measured concentrations)</p>
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Reference:	KCP 10.2.1/2
Report:	█████; 2018; GF-3307: Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Flow-Through Test Conditions; █████, a wholly owned subsidiary of █████; Lab Study No. 87719; DAS Study No. 180975 ; 23 October 2018; Unpublished
Guideline(s):	OECD Guideline 203
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable ^{Yes}
Duplication (if vertebrate study)	Yes, the previous GF-3307 acute trout study was done under static-renewal conditions and concentrations were not maintained above MQL between renewal periods, therefore the study was repeated under flow-through conditions in order to satisfy EFSA, 2015.

COMPLIANCE

Guideline(s):	OECD Guideline 203
US EPA Guideline(s):	None
Deviations:	Test solution pH deviated slightly from the range recommended by the OECD 203 guideline (6.0-8.5) because the pH of the control and all measured test treatments at 0 and 72 hours was 8.6.
Dates of work:	10 August 2018 to 14 August 2018
GLP status:	Yes
Number of pages in final report:	80

MATERIALS AND METHODS

Test Item(s)

	GF-3307
Test item (Common name):	
Purity:	4.9 wt% (51 g/L) Fenpicoxamid (XDE-777), 9.8 wt% (102 g/L) Prothioconazole
Description (physical state):	brown liquid with a fragrant odor
Lot/batch no.:	ENBK-166226-026-2 (TSN314331)

Test System

Organism (<i>Species</i>):	Rainbow trout (<i>Oncorhynchus mykiss</i>)
Study type:	Acute
Study design:	Flow through
Test duration:	96 hours
Test concentrations:	Nominal: 0 (control), 28, 56, 110, 230, and 450 µg GF-3307/L Mean calculated (based on XDE-777 analysis): <LOD, 9.4, 17, 33, 67, and 150 µg GF-3307/L
Parameters measured:	Mortality
Observation intervals:	Daily
Age, weight and length of fish at test initiation:	Age: juvenile Mean blotted wet weight: 1.7631 ± 0.3076 g (1.0467 to 2.0716 g) Mean total length: 57 ± 4.0 mm (46 to 60 mm) Loading rate: dynamic biomass loading: 0.08 g/L/day

Analytical confirmation of test concentrations:	On days: Day -1
No. of holding days before dosing:	On hours: 0, 48, and 96 hours
Number of fish per dose group:	14
Number of fish per control group:	7
Feeding regime:	7
Environmental conditions:	Fish were fed salmon starter daily.
	Loading rate: dynamic biomass loading: 0.08 g/L/day
	Temperature: 14.3 to 15.0°C
	Photoperiod: 16-hr light:8-hr dark
	Dissolved oxygen concentration: 8.4 to 9.2 mg/L (85 to 95% sat.)
	pH: 8.4 to 8.6
	Alkalinity: 160 mg CaCO ₃ /L
	Conductivity: 348 µS/cm
	Light Intensity: 775 lux
	Total hardness: 152 mg CaCO ₃ /L
	Salinity: not applicable
Reference substance:	XDE-777 (TSN302306)
	Prothioconazole (TSN312881)

Methodology

The flow-through definitive test was performed from 10 to 14 August 2018 at nominal concentrations of 0 (control), 28, 56, 110, 230, and 450 µg GF-3307/L. Seven fish were assigned to each treatment by adding one fish per chamber, proceeding from control, then low to high test substance treatments, and repeating steps as necessary. A single replicate test chamber was used for each treatment. Observations for mortality and sublethal responses were made at approximately 24, 48, 72, and 96 hours. The undiluted test substance was used as the diluter stock solution starting on 08 August 2018. Each time the diluter system cycled, the syringe dispenser introduced 0.0017 mL of the diluter stock solution to the chemical mixing box of the diluter system where the solution was diluted with approximately 3.95 L of dilution water; following adjustment for test substance density of 1.0383 g/mL, this generated the highest nominal test substance treatment. The four lower test substance treatment solutions were generated by mixing appropriate volumes of the highest test substance treatment solution with dilution water at each cycle of the proportional diluter system. Temperature, dissolved oxygen, and pH were measured in each test chamber daily using a WTW Multi 3500i multi meter. In addition, a continuous record of the temperature from a centrally located test chamber was also maintained. Total hardness and alkalinity of the dilution water were measured at test initiation using titrimetric methods adapted from Standard Methods. The light intensity at definitive test initiation was 775 lux.

RESULTS AND DISCUSSION

The concentration of GF-3307 was calculated, based on XDE-777 and prothioconazole active ingredient analysis, in test solution samples collected from the test chambers one day prior to initiation (day -1), initiation (0 hour), 48 hours, and 96 hours (termination).

Calculated concentrations of GF-3307, based on analysis of prothioconazole, in the test substance treatment solutions one day prior to initiation (day -1) were 21, 48, 97, 220, and 380 µg GF-3307/L, which represented recoveries of 76 to 94% of the nominal concentrations. Calculated concentrations of GF-3307 in the test substance treatment solutions at initiation (0 hour) were 30, 58, 130, 260, and 500 µg GF-3307/L, which represented recoveries of 103 to 114% of nominal. Calculated concentrations in the test substance treatment solutions at 48 hours were 14, 44, 110, 210, and 390 µg GF-3307/L, which represented recoveries of 49 to 100% of nominal. Calculated concentrations in the test solutions at 96 hours (termination) were 30, 61, 120, and 240 µg GF-3307/L, which represented recoveries of 104 to 113% of nominal. No samples were collected from highest test substance treatment at 96 hours due to

100% mortality prior to that time. The mean calculated GF-3307 concentrations, based on prothioconazole analysis, were 25, 54, 120, 240, and 440 µg GF-3307/L, which represented recoveries of 88 to 109% of nominal.

The concentration of GF-3307, based on the analysis of XDE-777 active ingredient, was calculated in test solution samples. Calculated concentrations of GF-3307, based on analysis of XDE-777, in the test substance treatment solutions one day prior to study initiation (day -1) were 6.8, 16, 27, 57, and 150 µg GF-3307/L, which represented recoveries of 24 to 33% of the nominal concentrations. Calculated concentrations of GF-3307 in the test substance treatment solutions at initiation (0 hour) were 6.4, 15, 28, 56, and 150 µg GF-3307/L, which represented recoveries of 23 to 34% of nominal. Calculated concentrations in the test substance treatment solutions at 48 hours were 7.8, 17, 38, 81, and 140 µg GF-3307/L, which represented recoveries of 28 to 35% of nominal. Calculated concentrations in the test substance treatment solutions at 96 hours (termination) were 14, 20, 34, and 65 µg GF-3307/L, which represented recoveries of 28 to 50% of nominal. No sample was collected from the highest test substance treatment at 96 hours due to 100% mortality prior to that time. The mean calculated concentrations in the test substance treatment solutions during the 96-hour exposure were 9.4, 17, 33, 67, and 150 µg GF-3307/L, which represented recoveries of 29 to 34% of the nominal concentrations. The biological response results were reported based upon the mean calculated GF-3307 concentrations. After 96 hours of exposure, survival was 100, 100, 100, 100, 57, and 0% in the 0 (control), 9.4, 17, 33, 67, and 150 µg GF-3307/L mean calculated concentration, respectively. No sublethal effects were observed.

Table 8. Calculated Concentrations of GF-3307, Based on Analysis of XDE-777, During the Flow-through Acute Toxicity Test with Rainbow Trout, *Oncorhynchus mykiss*

Nominal Concentration (µg GF-3307/L)	Calculated Concentration ^a as µg GF-3307/L (Percent Nominal)						96-Hour Mean Calculated Concentration ^d	
	Day -1	0 Hour	48 Hours		96 Hours			
0 (control)	<LOD ^b	<LOD ^b	<LOD ^b		<LOD ^b		<LOD ^b	
28	6.8 (24)	6.4 (23)	7.8 (28)		14 (50)		9.4 (34)	
56	16 (28) ^c	15 (27)	17 (31)		20 (35)		17 (31)	
110	27 (25)	28 (26)	38 (35)		34 (31)		33 (30)	
230	57 (25)	56 (24)	81 (35)		65 (28)		67 (29)	
450	150 (33) ^c	150 (34)	140 (32)		---		150 (33)	
450 (Mixing Box)	430 (96)	NA	NA		NA		NA	
QC Fortification Spikes (% Recovery)								
Low Spike (5.00)	NA	A	4.7 (94)	A	4.9 (97)	A	5.0 (100)	NA
		B	4.9 (98)	B	4.8 (95)	B	4.8 (97)	
Low Spike (10.0)	9.7 (97)	NA		NA		NA		NA
High Spike (600)	580 (97)	A	590 (98)	A	580 (96)	A	590 (98)	NA
		B	610 (102)	B	570 (95)	B	550 (91)	

^a Calculated Concentration (µg GF-3307/L) = Measured concentration of XDE-777 from the curve (ng a.i./mL) × analysis volume (mL) / sample volume (mL) / purity of XDE-777 (4.9%)

^b LOD = 1.50 µg GF-3307/L.

^c Original results outside the standard curve. Less dilute aliquots analyzed, and the results reported.

^d Day -1 not included in calculations.

NA = Not applicable.

--- = not collected due to 100% mortality.

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Table 9. Calculated Concentrations of GF-3307, Based on Analysis of Prothioconazole, During the Flow-through Acute Toxicity Test with Rainbow Trout, *Oncorhynchus mykiss*

Nominal Concentration (µg GF-3307/L)	Calculated Concentration ^a as µg GF-3307/L (Percent Nominal)					96-Hour Mean Calculated Concentration ^e
	Day -1	0 Hour	48 Hours	96 Hours		
0 (control)	<LOD ^b	<LOD ^b	<LOD ^b	<LOD ^b		<LOD ^b
28	21 (76)	30 (106)	14 (49) ^d	30 (109)		25 (88)
56	48 (86)	58 (103)	44 (78) ^c	61 (110)		54 (97)
110	97 (88)	130 (114)	110 (100)	120 (113)		120 (109)
230	220 (94)	260 (111)	210 (93)	240 (104) ^d		240 (103)
450	380 (84)	500 (111)	390 (86) ^d	---		440 (99)
450 (Mixing Box)	390 (87)	NA	NA	NA		NA
QC Fortification Spikes (% Recovery)						
Low Spike (10.0)	7.3 (73)	A 11 (109)	A 5.1 (51) ^c	A 10 (105)		NA
		B 11 (112)	B 4.3 (43) ^c	B 12 (116)		
High Spike (600)	580 (96)	A 770 (129) ^c	A 580 (97)	A 650 (108) ^d		NA
		B 760 (127) ^c	B 590 (99)	B 680 (114) ^d		

^a Calculated Concentration (µg GF-3307/L) = Measured concentration of prothioconazole from the curve (ng a.i./mL) × analysis volume (mL) / sample volume (mL) / purity of prothioconazole (9.8%)

^b LOD = 0.150 µg GF-3307/L.

^c Re-diluted in duplicate and re-analyzed, average of original and duplicate re-analyses reported.

^d Re-diluted in duplicate and re-analyzed, average of duplicate re-analyses reported.

^e Day -1 not included in calculations.

NA = Not applicable.

--- = not collected due to 100% mortality.

Table 3: Effect of GF-3307, based on analysis of XDE-777, on mortality of rainbow trout

Treatment (µg GF-3307/L)		No. of fish	Cumulative mortality (%)				Cumulative % Survival
Nominal concentra- tion	Mean measured concentra- tion		24 Hours	48 Hours	72 Hours	96 Hours	
0 (Negative control)	<LOD	7	0 (0)	0 (0)	0 (0)	0 (0)	100
28	9.4	7	0 (0)	0 (0)	0 (0)	0 (0)	100
56	17	7	0 (0)	0 (0)	0 (0)	0 (0)	100
110	33	7	0 (0)	0 (0)	0 (0)	0 (0)	100
230	67	7	0 (0)	0 (0)	3 (43)	3 (43)	57
450	150	7	7 (100)	7 (100)	7 (100)	7 (100)	0 *
96-hr LC ₅₀		72 µg GF-3307/L					
96-hr 95% C.I.		55 to 96 µg GF-3307/L					
96-hr NOEC		67 µg GF-3307/L					

* Statistically significant decrease in survival as compared to the control (Fisher's One-Tailed Test with Hochberg's familywise adjustment)

Table 4: Sub-lethal effects of GF-3307, based on analysis of XDE-777, in rainbow trout

Treatment (µg GF-3307/L)		Observation period			
Nominal concentration	Mean measured concentration	Observation 1 (% affected)			
		24-hr	48-hr	72-hr	96-hr
0 (Negative control)	<LOD	0 (0)	0 (0)	0 (0)	0 (0)
28	9.4	0 (0)	0 (0)	0 (0)	0 (0)
56	17	0 (0)	0 (0)	0 (0)	0 (0)
110	33	0 (0)	0 (0)	0 (0)	0 (0)
230	67	0 (0)	0 (0)	0 (0)	0 (0)
450	150	0 (0)	0 (0)	0 (0)	0 (0)

CONCLUSION

There was no mortality among control animals during the study. Therefore, control animals satisfied test acceptability criteria for survival (i.e., $\geq 90\%$ or at most one mortality) as stated in the study protocol and the OECD 203 testing guideline.

Based on mean calculated GF-3307 concentrations and mortality, the estimated 24- and 48-hour LC_{50} value was 100 μg GF-3307/L, with best estimates for confidence limits of 67 and 150 μg GF-3307/L (based on the highest concentration whose smoothed proportion was zero and the lowest concentration whose smoothed proportion was one). Based on mean calculated GF-3307 concentrations and mortality, the estimated 72- and 96-hour LC_{50} value was 72 μg GF-3307/L, with 95% confidence limits of 55 and 96 μg GF-3307/L. The slope of the 24-, 48-, 72-, and 96-hour concentration-response lines could not be calculated due to lack of more than one partial response. The 96-hour NOEC was 67 μg GF-3307/L, based on mean calculated GF-3307 concentrations and a lack of statistically significant mortality at this and all lower test substance concentrations.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Rainbow trout	<i>Oncorhynchus mykiss</i>	GF-3307	96-hr	LC_{50}	72	μg GF-3307/L, mm

A 2.2.1.11 Study 7– 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>All the validity criteria were met.</p> <p>The analytical measurements showed that during the study the concentrations of both active substances were not maintained within 80-120% of nominal concentrations. Therefore, the results were based on the time weighted geometric mean GF-3307 concentrations, calculated from measured fenpicoxamid (the least stable substance) concentrations. However, according to the recommendations in Appendix J EFSA Supporting publication 2019:EN-1673 in such a case the time weighted geomean measured concentration should be re-calculated to the sum of both active substances. Nevertheless, based on the calculations of contribution of toxicity of each active substance (provided in this report in Table 9.5-5) fenpicoxamid was determined to be the driver of the toxicity (contribution of $> 90\%$) to invertebrates. For that reason, the zRMS is of the opinion that the concentrations of GF-3307 calculated based on the time weighted geometric mean concentrations of fenpicoxamid in the present study are acceptable to derive the endpoint.</p> <p>Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>$EC_{50} = 15 \mu\text{g}$ product/L (based on time weighted geometric mean measured concentration)</p>
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Reference:	KCP 10.2.1/7
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 Agroscience, LLC, Easton, Maryland, USA; Lab Study No. 379A-305; DAS Study No. 191366 ; 20 February 2020; Unpublished
Guideline(s):	OECD 202 (2004)
Deviations:	None
GLP:	Yes

Acceptability:	Acceptable Yes
Duplication (if vertebrate study)	No

COMPLIANCE

Guideline(s): OECD 202 (2004)
US EPA Guideline(s): None
Deviations: None
Dates of work: 28 October 2019 to 31 October 2019
GLP status: Yes
Number of pages in final report: 98

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 (*Species*): Cladoceran (*Daphnia magna*)
Study type: Acute
Study design: Static-renewal
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

Measured concentrations of fenpicoxamid and prothioconazole in the samples collected from freshly prepared test solutions at 0 and 40 hours ranged from approximately 82.2 to 98.9%, and 87.8 to 109%, of nominal, respectively. Measured concentrations of fenpicoxamid and prothioconazole in the samples collected at 2 and 42 hours ranged from approximately 45.8 to 61.1%, and 81.6 to 97.8% of nominal, respectively. Measured concentrations of fenpicoxamid and prothioconazole in the samples collected from the spent test solutions at 8 and 48 hours ranged from <LOQ to approximately 16.2%, and 65.6 to 86.9% of nominal, respectively. Additionally, the measured concentrations of fenpicoxamid and prothioconazole in the samples collected from the freshly prepared nominal 500 µg GF-3307 test solutions at 16, 24, and 32 hours ranged from approximately 86.0 to 91.5%, and 91.0 to 93.6% of nominal, respectively.

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 EC₅₀ value was estimated to be 32 µg GF-3307/L (95% CI: 24 and 53 µg GF-3307/L). The 48-hour EC₅₀ value was estimated to be 15 µg GF-3307/L (95% CI: 1.7 and 18 µg GF-3307/L). The sublethal 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 ^{1,2} Fenpicoxamid (µg a.i./L)	Fenpicoxamid Percent of Nominal ²	Fenpicoxamid Time-Weighted Geometric Mean Measured Concentration ³ (µg a.i./L)	Fenpicoxamid Time-Weighted Geometric Mean Measured Percent of Nominal ³
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 ⁴	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 ⁵	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		

¹ 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.

² Results were generated using Analyst Version 1.6.3 and Excel 2010 in full precision mode. Manual calculations may differ slightly.

³ Results were generated using Excel 2010 in full precision mode. Manual calculations may differ slightly.

⁴ Sample result is below LOQ but quantifiable because the peak area count is within the calibration curve range.

⁵ 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 ^{1,2} Prothioconazole (µg a.i./L)	Prothioconazole Percent of Nominal ²	Prothioconazole Time-Weighted Geometric Mean Measured Concentration ³ (µg a.i./L)	Prothioconazole Time-Weighted Geometric Mean Measured Percent of Nominal ¹
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 ⁴	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		

¹ 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.

² Results were generated using Analyst Version 1.6.3 and Excel 2010 in full precision mode. Manual calculations may differ slightly.

³ Results were generated using Excel 2010 in full precision mode. Manual calculations may differ slightly.

⁴ 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 (µg GF-3307/L)	Nominal Fenpicoxamid Concentration (µg a.i./L)	Nominal Prothioconazole Concentration (µg a.i./L)	Time-Weighted Geometric Mean Measured Concentration ^{1,2}			GF-3307 Time- Weighted Mean Percent of Nominal ²
			Fenpicoxamid (µg a.i./L)	Prothioconazole (µg a.i./L)	Measured GF-3307 Based on Fenpicoxamid (µg GF-3307/L) ^{3,4}	
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

¹ The limit of quantitation (LOQ) of GF-3307 for these analyses was set at 15.0 µg GF-3307/L (0.705 and 1.46 µg 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%.

² Results were generated using Analyst Version 1.6.3 and Excel 2010 in full precision mode. Manual calculations may differ slightly.

³ 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.

⁴ Reported to two significant figures.

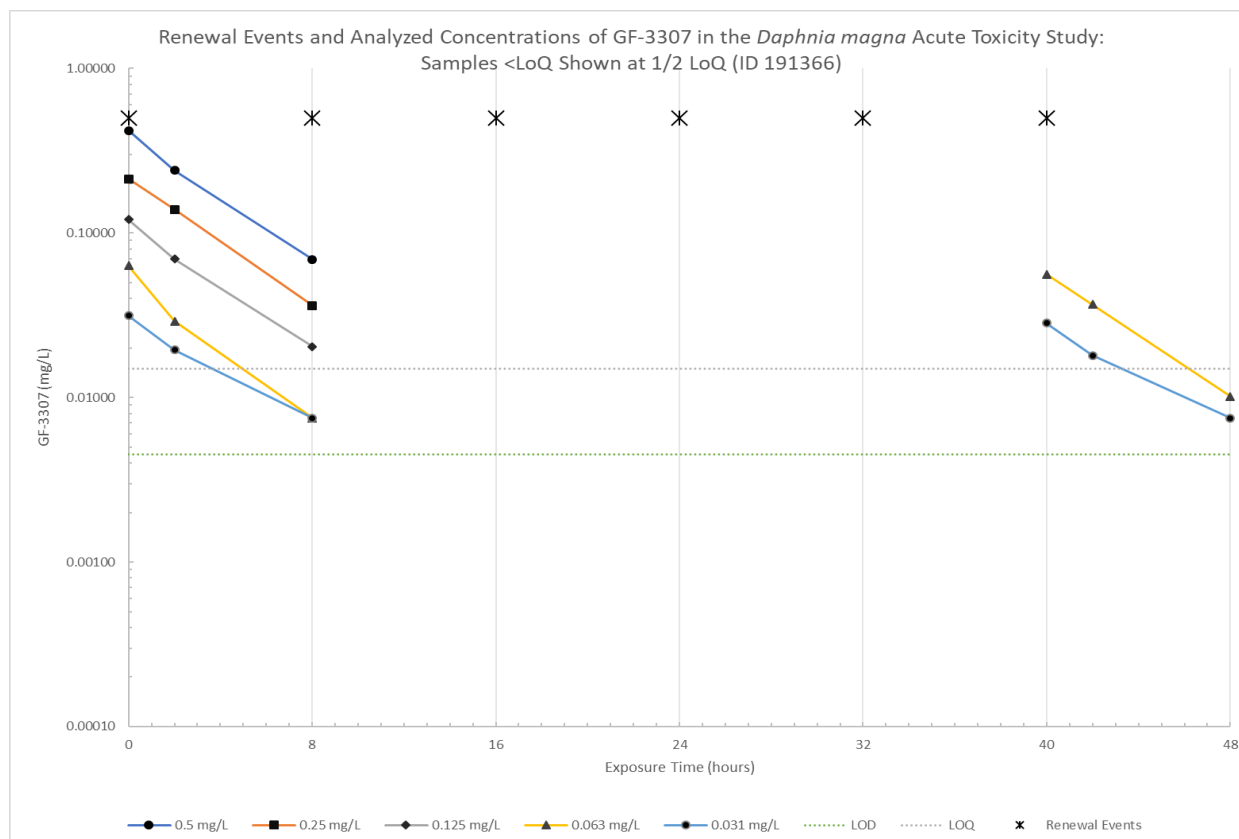


Figure 1. Decline of fenpicoxamid in the GF-3307 acute daphnid toxicity test with 8 hour renewals (Goudie, 2020, DAS 191366). Test solutions were measured at 0, 2, 8 hours and 40, 42, and 48 hours. Low QC spike (LOQ = 0.015 mg GF-3307/L; limit of detection, LOD = 0.0045 mg GF-3307/L)

Table 4: Effect of GF-3307 on immobilisation

Average Time Weighted Geometric Mean Calculated Treatment (µg 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 ₅₀	32 µg GF-3307/L		15 µg GF-3307/L	

Table 5: Sub-lethal effects of GF-3307

Average Time Weighted Geometric Mean Calculated Treatment (µg 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 ≥ 8.8 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 time-weighted geometric mean concentrations, the 48-hour EC₅₀ value was 15 µg GF-3307/L, with a 95% confidence interval of 1.7 to 18 µg GF-3307/L. The slope of the concentration-response curve was calculated to be 5.49. The NOEC and no-immobility concentration could not be determined. The lowest 100% immobility concentration was 53 µg GF-3307/L.

Common name	Species	Test Substance	Time-scale	Endpoint	Toxicity value	Units of GF-3307
Cladoceran	<i>Daphnia magna</i>	GF-3307	48-hr	EC ₅₀	15	µg/L, twgm

A 2.2.1.12 Study 10 7 – GF-2925: A Static-Renewal Acute Toxicity Test with the Cladoceran (*Daphnia magna*)

Comments of zRMS:	<p>The study was already evaluated in the course of zonal authorization of the product GF-3308 and was considered acceptable. The zRMS's evaluation was copied here for transparency of the current assessment of the product GF-3307.</p> <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</p>
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	<p>are selected (with more frequent intervals the mortality in controls exceeds the validity criterion of 10%). Problems with analytical verification of fenpicoxamid 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 EC50 could not be determined, but it may be concluded that it is <0.00165 mg test item/L (based on time weighted geometric mean concentration), corresponding to <0.000203 mg a.s./L</p>
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Reference:	KCP 10.2.1/10
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 202 (2004)
Deviations:	
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	NA

COMPLIANCE

Guideline(s):	OECD 202 (2004)
US EPA Guideline(s):	None
Deviations:	Test duration was 32 hours instead of 48 hours due to 100% immobility in the treatment concentrations. A definitive EC ₅₀ was not achieved at the concentrations tested.
Dates of work:	20 January 2021 to 22 January 2021
GLP status:	Yes
Number of pages in final report:	84

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.:	F47919O001 (TSN403981)

Test System

Organism (<i>Species</i>):	Cladoceran (<i>Daphnia magna</i>)
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 GF-2925 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 colorless 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 EC₅₀ 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 1: Calculated Concentrations of GF-2925 in Test Solution Samples

Nominal Test Substance Concentration (µg GF-2925/L)	Calculated GF-2925 Concentration as µg/L (Percent of Nominal) ^{1,2,3}											
	Sample ID ³	0-Hour	Sample ID ³	2-Hour (old)	Sample ID ³	8-Hour (old)	Sample ID ³	8-Hour (new)	Sample ID ³	10-Hour (old)	Sample ID ³	16-Hour (old)
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 ⁴	3	14.7 (121)	8	13.3 (109)	15 ⁶	4.31 (35.3)	---	---	---	---	---	---
122 ⁵	5	120 (98.6)	---	---	---	---	12	131 (107)	---	---	---	---
8000 ⁵	4	8460 (106)	---	---	---	---	11	11380 (142)	---	---	---	---

¹ 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.

² Results were generated using Microsoft Excel, based on the fenpicoxamid active ingredient at 12.3%. Manual calculations may differ slightly.

³ Sample ID: 379A-343-n, where n = sample number.

⁴ Not sampled after 8-Hour old solutions due to 100% immobility in treatment.

⁵ Stock solutions sampled at fresh preparation only.

Table 1: Calculated Concentrations of GF-2925 in Test Solution Samples (cont.)

Nominal Test Substance Concentration (µg GF-2925/L)	Calculated GF-2925 Concentration as µg/L (Percent of Nominal) ^{1,2,3}											
	Sample ID ³	16-Hour (new)	Sample ID ³	18-Hour (old)	Sample ID ³	24-Hour (old)	Sample ID ³	24-Hour (new)	Sample ID ³	26-Hour (old)	Sample ID ³	32-Hour (old)
Negative Control	18	0 (N/A)	24	0 (N/A)	30	0 (N/A)	26	0 (N/A)	32	0 (N/A)	38	0 (N/A)
2.44	19 ⁶	2.20 (90.3)	25 ⁶	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 ⁴	---	---	---	---	---	---	---	---	---	---	---	---
122 ⁵	21	146 (119)	---	---	---	---	30	141 (116)	---	---	---	---
8000 ⁵	20	12850 (161)	---	---	---	---	29	8700 (109)	---	---	---	---

¹ 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.
² Results were generated using Microsoft Excel, based on the fenpicoxamid active ingredient at 12.3%. Manual calculations may differ slightly.
³ Sample ID: 379A-343-n, where n = sample number.
⁴ Not sampled after 8-Hour old solutions due to 100% immobility in treatment.
⁵ Stock solutions sampled at fresh preparation only.
⁶ Backup samples were analyzed to confirm original results. Average of the original and backup analyses is reported.

Table 2: Overall Test Treatment Exposure Concentrations of GF-2925

Nominal GF-2925 Concentration (µg/L)	Time-Weighted Geometric Mean Concentration in µg GF-2925/L ¹				Overall Time Weighted Geometric Mean Concentration in µg GF-2925/L (% Nominal)
	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 ²	9.50	---	---	---	9.50 (77.8)

¹ Results were generated using Microsoft Excel, based on calculated individual GF-2925 concentrations. Manual calculations may differ slightly.

² Not sampled after the 8-hour time point due to 100% immobility in treatment.

Table 3:Effect of GF-2925 on immobilisation

Average Time Weighted Geometric Mean Calculated Treatment ($\mu\text{g GF-2925/L}$)	Average Time Weighted Geometric Mean Calculated Treatment ($\mu\text{g a.s./L}$)	8-hr		32-hr	
		No. immobile	% Immobil-ity	No. immobile	% Immobil-ity
Negative control	Negative control	0	0	0	0
1.65	0.203	11	55	20	100
9.50	1.17	20	100	--	--
NOEC		--		--	
EC ₅₀		--		< 1.65 $\mu\text{g GF-2925/L}$ < 0.203 $\mu\text{g a.s./L}$	

Table 4:Sub-lethal effects of GF-2925

Average Time Weighted Geometric Mean Calculated Treatment ($\mu\text{g GF-2925/L}$)	Average Time Weighted Geometric Mean Calculated Treatment ($\mu\text{g a.s./L}$)	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 ≥ 8.7 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 time-weighted geometric mean concentrations, the 32-hour EC₅₀ value was <1.65 $\mu\text{g GF-2925/L}$ (<0.203 a.s./L). The NOEC was not determined. The lowest 100% immobility concentration was 1.65 $\mu\text{g GF-2925/L}$ (0.203 a.s./L).

Common name	Species	Test Sub-stance	Time-scale	End-point	Tox-icity value	Units
Cladoceran	<i>Daphnia magna</i>	GF-2925	32-hr	EC ₅₀	< 1.65	$\mu\text{g GF-2925/L, twgm}$
Cladoceran	<i>Daphnia magna</i>	GF-2925	32-hr	EC ₅₀	< 0.203	$\mu\text{g fencicoxamid/L, twgm}$

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 already evaluated in the course of zonal authorization of the product GF-3308 and was considered acceptable. The zRMS's evaluation was copied here for transparency of the current assessment of the product GF-3307.</p> <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>
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	<p>Reliability of the EC10 value was evaluated in line with recommendations of EFSA Supporting publication 2019:EN-1673:</p> <ul style="list-style-type: none"> - 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, - although there is no EC20,low available for evaluation, the median EC10 is lower than EC50,low which indicates a medium level of protection, - the dose-response curve is shallow with steepness of 0.22 (im) and 0.21 (twm) (i.e. <0.33). <p>Based on above indications the calculated EC10 are considered to be sufficiently reliable. 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) = 1.9 mg pm/kg (based on initial measured concentration) EC10 (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) EC10 (based on survival as most sensitive parameter) = 0.58 mg X642188/kg (based on time weighted mean measured concentration)</p>
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Reference:	KCP 10.2.2/1
Report:	Beasley, J.; 2018; X642188 (a metabolite of XDE-777): Chronic Toxicity in Whole Sediment to Freshwater Midge, Chironomus riparius, Using Spiked Sediment; Analytical Bio-Chemistry Laboratories, Inc., a wholly owned subsidiary of EAG, Inc., Columbia, Missouri, USA; Lab Study No. 87149; DAS Study No. 180563 ; 30 August 2018; Unpublished
Guideline(s):	OECD Guideline 218
Deviations:	
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	NA

COMPLIANCE

Guideline(s):	OECD Guideline 218
US EPA Guideline(s):	none
Deviations:	none
Dates of work:	24 April 2018 to 22 May 2018
GLP status:	Yes
Number of pages in final report:	93

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.:	SYN-FS08353-048 (TSN303567)

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
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
No. of Chironomid per control group:	120
Environmental conditions:	Temperature: 20.1 to 21.2 °C Photoperiod: 16 hr light: 8-hr dark Light intensity: 735 lux

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 wide-bore 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.

RESULTS AND DISCUSSION

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. 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. 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 time-weighted mean measured sediment treatments), respectively. 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.

Table 1: 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 ₃ /L Alkalinity: not determined Conductivity: not determined Ammonia concentration: 0.00 mg/L (un-ionized) 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 2: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	0.00089, 0.0011	0.00034, 0.00038	0.00010, 0.00013	0.000013, 0.000014	
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 3: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	0.42	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				

Table 4:Effect of X642188 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 (%)	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
		Biomass (mg)	0.75	0.92	0.84
8.2	2.8	Survival (%)	40	75	58
		Biomass (mg)	1.04	0.76	0.90

Table 5:Effect of X642188 on adult emergence and development rate at day 28

Day-0 Mean Measured Sedi-ment (mg/kg)	Time-Weighted Mean Meas-ured Sedi-ment (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	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
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

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 EC₁₀, EC₁₅, and EC₅₀ 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 EC₁₀, EC₁₅, and EC₅₀ 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 EC₁₀, EC₁₅, and EC₅₀ 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 EC₁₀, EC₁₅, and EC₅₀ 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 EC₁₀, EC₁₅, and EC₅₀ 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 2 – X12335723 (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 already evaluated in the course of zonal authorization of the product GF-3308 and was considered acceptable. The zRMS's evaluation was copied here for transparency of the current assessment of the product GF-3307.</p> <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 X12335723. Taking this into account, the study was evaluated by the zRMS.</p> <p>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 <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>
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	<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 10.2.2/2
Report:	Leak, T.; 2018; X12335723 (a metabolite of XDE-777): Chronic Toxicity in Whole Sediment to Freshwater Midge, <i>Chironomus riparius</i> , Using Spiked Sediment; Analytical Bio-Chemistry Laboratories, Inc., a wholly owned subsidiary of EAG, Inc., Columbia, Missouri, USA; 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

COMPLIANCE

Guideline(s):	OECD Guideline 218
US EPA Guideline(s):	none
Deviations:	none
Dates of work:	30 April 2018 to 28 May 2018
GLP status:	Yes
Number of pages in final report:	91

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

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), 0.69, 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
Feeding:	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
Environmental conditions:	Temperature: 20.2 to 20.7 °C Photoperiod: 16 hr light: 8-hr dark Light intensity: 702 lux

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. Aeration was provided to each test chamber two to three centimeters 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 wide-bore 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.

RESULTS AND DISCUSSION

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. 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. 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 time-weighted mean measured sediment treatments), respectively. 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.

Table 1: 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 ₃ /L Alkalinity: not determined Conductivity: not determined Ammonia concentration: 0.00 mg/L (un-ionized) 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 2: 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 3: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				

Table 4: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 5: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

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
3.6	1.2	Tot Dev. rate	0.0606	0.0635	0.0615	0.0716	0.0643
		% 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 EC₁₀, EC₁₅, and EC₅₀ 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 2.2.3.1.1.1 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>The study was already evaluated in the course of zonal authorization of the product GF-3308 and was considered acceptable. The zRMS's evaluation was copied here for transparency of the current assessment of the product GF-3307.</p> <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 LC₅₀), aquatic invertebrates (48h EC₅₀), and algae (96h EC₅₀). The zRMS agrees with the proposed endpoints and their justification.</p> <p>Fish LC₅₀ = 81.990 mg/L Aquatic invertebrate EC₅₀ = 48.857 mg/L Algae EC₅₀ = 44.437 mg/L</p> <p>Information regarding log K_{ow} 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):	
Deviations:	
GLP:	
Acceptability:	Yes
Duplication (if vertebrate study)	NA

Abstract

This document provides the results of “non-testing methods” for X12433979, a metabolite of XDE-777. The “non-testing methods” included Quantitative Structure Property Relationship (QSPR) models for prediction of octanol-water partition coefficient (reported as log₁₀ K_{ow}) and Quantitative Structure Activity Relationship (QSAR) models for prediction of acute toxicity in fish (LC₅₀), aquatic invertebrates (EC₅₀), and population-level toxicity (EC₅₀) 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 LC₅₀, daphnid 48 hr EC₅₀, and green algae 96 hr EC₅₀ values for X12433979 were 81.990, 48.857, and 44.437 mg/L, respectively. Predicted fish 96 hr LC₅₀, mysid 96 hr EC₅₀, and green algae 96 hr EC₅₀ values for X12314005 were 16.203, 17.264, and 12.182, respectively. Predicted fish 96 hr LC₅₀, mysid 96 hr EC₅₀, and green algae 96 hr EC₅₀ values for XDE-777 were 4.569, 0.600, and 0.267 mg/L, respectively.

Methodology

The log₁₀ K_{ow} and acute toxicity in fish (LC₅₀), aquatic invertebrates (EC₅₀), and population-level toxicity (EC₅₀) to algae was estimated for metabolite X12433979, metabolite X12314005, and XDE-777. 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-suitetm-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 XDE-777 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 1 Concordance of predicted with measured octanol-water partition coefficient (log K_{ow}) for XDE-777.

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)⁷. 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 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 2 Concordance of predicted fish 96 hr LC₅₀, aquatic invertebrate EC₅₀, and algae EC₅₀ for XDE-777, X12314005 and predicted values for X12433979.

DAS ID Number	Pred. Fish 96 hr LC ₅₀ (mg/L)	Actual Trout 96 hr LC ₅₀ (mg/L)	Pred. Aq. Invert. EC ₅₀ (mg/L)	Actual <i>D. magna</i> . EC ₅₀ (mg/L)	Pred. Algae EC ₅₀ (mg/L)	Actual Green Algae EC ₅₀ (mg/L)	Study References
XDE-777	4.569	=0.0022	0.600	=0.00093	0.267	>0.522	Fournier, 2012a Fournier, 2012b Rebstock, 2012
X12314005	16.203	>1.9	17.264	>8.5	12.182	--	Dinehart, 2014a Dinehard, 2014b

⁷ 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).

X12433979	81.990	--	48.857	--	44.437	--	
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^a >1.9 indicates a 50% effect level was not achieved in the study, hence the LC50 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 **bold** are considered to be concordant with predicted values.

^b -- indicates that an actual study result was not available.

A 2.2.3.1.1.2 Study 2 - GF-3308: Population Effects Study in an Indoor Aquatic Microcosm with *Daphnia magna*

A 2.2.3.1.1.3 Study 3 – XDE-777: Population Effects Study in an Indoor Aquatic Microcosm with *Daphnia magna*

Comments of zRMS:	<p>The study was already evaluated in the course of zonal authorization of the product GF-3308 and was considered acceptable. The zRMS's evaluation was copied here for transparency of the current assessment of the product GF-3307.</p> <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 <i>Daphnia</i> 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 <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 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 EC10 values with exception of the NOEC determined for the abundance of neonates (day 2 and 7) and juveniles (day 7) when clear effects >20% were observed at concentration set as NOEC (1.88 µg a.s./L). Nevertheless, EC10 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>“Although refined exposure tests with standard test species that more or less re-semble 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. <i>Daphnia</i>) for this purpose when recovery is also considered. These population-level</p>
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	<p>laboratory experiments with invertebrates are usually per-formed 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 trig-ger an unrealistic strong recovery as no competitors are present (Knillmann et al., 2012b).”</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 on evaluation of GF-3308 Core dossier available on Circa platform) 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.</p> <p>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 EC10-juveniles = 0.770 µg a.s./L (based on clear reduction on day 7) EC10-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:	
GLP:	
Acceptability:	Yes
Duplication (if vertebrate study)	NA

COMPLIANCE

Guideline(s):	None
US EPA Guideline(s):	None
Deviations:	None
Dates of work:	23 March 2016 to 27 April 2016
GLP status:	Yes
Number of pages in final report:	799

MATERIALS AND METHODS

Test Item(s)

Test item (Common name): XDE-777
Purity: 73.2%
Description (physical state): White solid with a slight odor
Lot/batch no.: E3485-83 (TSN301100)

Test System

Organism (<i>Species</i>):	Water flea (<i>Daphnia magna</i>)
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 (6.0×10^7 cells/mL) algal suspension (<i>Pseudokirchneriella subcapitata</i> , formerly <i>Selenastrum capricornutum</i>). 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 ₃ /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. 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 t-test (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.

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. Daphnid abundance (i.e., neonate, juvenile, adults, and all age groups combined) was unaffected in the 0.690 and 1.88 µg 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 µg 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 µg 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 µg 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 µg a.i./L groups and on Day 7 in the 4.75, 13.3, and 34.6 µg 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 µg 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 µg 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 µg a.i./L treatment group. Male daphnia were observed in the adult daphnids population on day -5 (1.88 and 13.3 µg 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 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 ^a	--			<MQL ^a	--	
	B	<MQL ^a	--			<MQL ^a	--	
	C	<MQL ^a	--	<MQL ^a	--	<MQL ^a	--	<MQL ^a
	D	<MQL ^a	--			<MQL ^a	--	
0 (Vehicle Control)	A	<MQL ^a	--			<MQL ^a	--	
	B	<MQL ^a	--			<MQL ^a	--	
	C	<MQL ^a	--	<MQL ^a	--	<MQL ^a	--	<MQL ^a
	D	<MQL ^a	--			<MQL ^a	--	
1.0	A	0.692	69			0.532	53	
	B	0.720	72	0.690 (69)	4	0.533	53	
	C	0.649	65			0.479	48	0.526 (53)
	D	0.700	70			0.561	56	
2.5	A	1.77	71			1.59	64	
	B	1.96	78	1.88 (75)	4	1.62	65	
	C	1.93	77			1.48	59	1.52 (61)
	D	1.86	74			1.40	56	
6.3	A	4.74	75			3.66	58	
	B	4.73	75	4.75 (75)	5	3.70	59	
	C	4.50	71			3.49	55	3.68 (58)
	D	5.04	80			3.88	62	
16	A	13.3	83			10.5	66	
	B	13.3	83	13.3 (83)	1	9.49	59	
	C	13.1	82			10.8	68	10.2 (64)
	D	13.3	83			10.0	63	
40	A	34.2	86			26.1	65	
	B	34.0	85	34.6 (87)	3	25.9	65	
	C	33.8	85			26.3	66	26.1 (65)
	D	36.3	91			26.0	65	

^a Minimum Quantifiable Limit (MQL) = 0.0200 ng a.i./mL.

^b 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 ^a	--		<MQL ^a	--	
	B	<MQL ^a	--		<MQL ^a	--	
	C	<MQL ^a	--	<MQL ^a	<MQL ^a	--	<MQL ^a
	D	<MQL ^a	--		<MQL ^a	--	
0 (Vehicle Control)	A	<MQL ^a	--		<MQL ^a	--	
	B	<MQL ^a	--		<MQL ^a	--	
	C	<MQL ^a	--	<MQL ^a	<MQL ^a	--	<MQL ^a
	D	<MQL ^a	--		<MQL ^a	--	
1.0	A	0.573	57		0.290	29	
	B	0.421	42		0.291	29	
	C	0.384	38	0.457 (46)	0.242	24	0.290 (29)
	D	0.449	45		0.338	34	
2.5	A	0.992	40		0.729	29	
	B	1.14	46		0.723	29	
	C	1.03	41	1.04 (42)	0.718	29	0.716 (29)
	D	0.997	40		0.692	28	
6.3	A	2.67	42		1.68	27	
	B	2.79	44		1.49	24	
	C	2.83	45	2.79 (44)	1.86	30	1.74 (28)
	D	2.86	45		1.91	30	
16	A	7.95	50		4.86	30	
	B	6.42	40		4.17	26	
	C	8.19	51	7.33 (46)	6.03	38	4.88 (31)
	D	6.77	42		4.47	28	
40	A	20.0	50		13.2	33	
	B	14.3	36		9.85	25	
	C	19.4	49	18.3 (46)	13.5	34	12.3 (31)
	D	19.3	48		12.8	32	

^a Minimum Quantifiable Limit (MQL) = 0.0200 ng a.i./mL.

^b 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 ^a	--		<MQL ^a	--	
	B	<MQL ^a	--		<MQL ^a	--	
	C	<MQL ^a	--	<MQL ^a	<MQL ^a	--	<MQL ^a
	D	<MQL ^a	--		<MQL ^a	--	
0 (Vehicle Control)	A	<MQL ^a	--		<MQL ^a	--	
	B	<MQL ^a	--		<MQL ^a	--	
	C	<MQL ^a	--	<MQL ^a	<MQL ^a	--	<MQL ^a
	D	<MQL ^a	--		<MQL ^a	--	
1.0	A	0.107	11		<MQL ^a	--	
	B	0.125	13		<MQL ^a	--	
	C	0.0783	8	0.117 (12)	<MQL ^a	--	<MQL ^a
	D	0.156	16		<MQL ^a	--	
2.5	A	0.318	13		<MQL ^a	--	
	B	0.392	16		<MQL ^a	--	
	C	0.305	12	0.340 (14)	<MQL ^a	--	<MQL ^a
	D	0.346	14		<MQL ^a	--	
6.3	A	0.637	10		0.0376	1	
	B	0.682	11		<MQL ^a	0	
	C	0.773	12	0.750 (12)	<MQL ^a	0	0.0227 (0)
	D	0.907	14		0.0331	1	
16	A	2.31	14		0.220	1	
	B	1.56	10		0.0224	0	
	C	3.30	21	2.31 (14)	0.218	1	0.125 (1)
	D	2.05	13		0.0415	0	
40	A	5.87	15		0.302	1	
	B	3.30	8		0.0404	0	
	C	6.27	16	5.24 (13)	0.185	0	0.164 (0)
	D	5.53	14		0.127	0	

^a Minimum Quantifiable Limit (MQL) = 0.0200 ng a.i./mL.

^b 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		
		Measured	48 hour ^b % of Nominal	Mean (% nominal)
0 (Control)	A	<MQL ^a	--	<MQL ^a
	B	<MQL ^a	--	
	C	<MQL ^a	--	
	D	<MQL ^a	--	
0 (Vehicle Control)	A	<MQL ^a	--	<MQL ^a
	B	<MQL ^a	--	
	C	<MQL ^a	--	
	D	<MQL ^a	--	
1.0	A	<MQL ^a	--	<MQL ^a
	B	<MQL ^a	--	
	C	<MQL ^a	--	
	D	<MQL ^a	--	
2.5	A	<MQL ^a	--	<MQL ^a
	B	<MQL ^a	--	
	C	<MQL ^a	--	
	D	<MQL ^a	--	
6.3	A	<MQL ^a	--	<MQL ^a
	B	<MQL ^a	--	
	C	<MQL ^a	--	
	D	<MQL ^a	--	
16	A	<MQL ^a	--	<MQL ^a
	B	<MQL ^a	--	
	C	<MQL ^a	--	
	D	<MQL ^a	--	
40	A	<MQL ^a	--	<MQL ^a
	B	<MQL ^a	--	
	C	<MQL ^a	--	
	D	<MQL ^a	--	

^a Minimum Quantifiable Limit (MQL) = 0.0200 ng a.i./mL.

^b 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 2: 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 ₁₀	5.54	4.75	1.52	6.06	NE	0.770	1.11	NSS
EC ₂₀	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

^a Data on this day was not statistically analysed because the terms of recovery had been met prior to this day.

Table 3: 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 ₁₀	NE	3.38	NE	NE	NE	NE	NE	NE

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
EC ₂₀	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

^a Data on this day was not statistically 279analysed because the terms of recovery had been met prior to this day.

Table 4: 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 ^a	No. juveniles	No. neonates ^a	Total ^a	No. adults ^a	No. juveniles ^a	No. neonates ^a	Total ^a
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 ₁₀	NA	NE	NA	NA	NA	NA	NA	NA
EC ₂₀	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

^a Data on this day was not statistically 279analysed because the terms of recovery had been met prior to this day.

Table 5: 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 ₁₀	NSS	1.84	NE	NE	NE	1.86	NSS	NE
EC ₂₀	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

^a The terms of recovery had been met prior to this day.

Table 6: Effects of XDE-777 on mean daphnid abundance (Daphnids/L) (continued)

Initial Mean Measured Treatment (µg a.i./L)	Day 0-21				Day 0-28			
	Adults	Juveniles	Neonates	Total	Adults ^a	Juveniles	Neonates ^a	Total ^a
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

Initial Mean Measured Treatment (µg a.i./L)	Day 0-21				Day 0-28			
	Adults	Juveniles	Neonates	Total	Adults ^a	Juveniles	Neonates ^a	Total ^a
Negative control	0.123	0.137	0.0324	0.0940	NA	0.0989	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 ₁₀	NE	NE	NE	NE	NA	NE	NA	NA
EC ₂₀	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

^a The terms of recovery had been met prior to this day.

Table 7: Effects of XDE-777 on mean daphnid abundance (Daphnids/L) (continued)

Initial Mean Measured Treatment (µg a.i./L)	Day 0-35			
	Adults ^a	Juveniles	Neonates ^a	Total ^a
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 ₁₀	NA	NE	NA	NA
EC ₂₀	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

^a The terms of recovery had been met prior to this day.

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	NOEC	1.88	µg a.i./L

A 2.2.3.2 Study 4 - X642188 (a metabolite of XDE-777): Population Effects Study in an Indoor Aquatic Microcosm with *Daphnia magna*

Comments of zRMS:	Study not evaluated by zRMS. The study evaluated at EU level.
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Reference:	CP 10.2.3/4
Report:	Lamichhane, K.; 2015; X642188 (a metabolite of XDE-777): Population Effects Study in an Indoor Aquatic Microcosm with <i>Daphnia magna</i> ; ABC Laboratories, Inc.; Lab Study No. 81786; 131295; 12 June 2015; Unpublished
Acceptability:	Yes
Duplication	NA

(if vertebrate study)	
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COMPLIANCE

Guideline(s):	None
US EPA Guideline(s):	None
Deviations:	None
Dates of work:	06 February 2015 to 04 March 2014
GLP status:	Yes
Number of pages in final report:	89

MATERIALS AND METHODS

Test Item(s)

ISO Common name:	Not applicable
Test item (chemical/other name):	X642188
Purity:	99%
Description (physical state):	White solid
Lot/batch no.:	SYN-FS08353-048
CAS no.:	Not applicable

Test System

Organism (<i>Species</i>):	<i>Daphnia magna</i>
Study type:	Population effects
Study design:	Static
Test concentrations:	Nominal: 0 (control), 0 (vehicle control), 2.5, 5.0, 10, 20, and 40 µg/L Initial Mean measured: <MQL (control), <MQL (vehicle control), 2.0, 4.4, 8.6, 17, and 35µg/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:	At initiation, 1 hour, 2 hour, 4 hour, 8 hour, 24 hour, 48 hour, and day 7
No. of holding days before dosing:	7 days
Number of daphnia per dose group:	800 juvenile and 800 adult
Number of daphnia per control group:	800 juvenile and 800 adult
Environmental conditions:	Loading rate: none Temperature: 19.5 to 20.4 °C Photoperiod: 16-hr light: 8-hr dark Dissolved oxygen concentration: 5.6 to 8.9 mg/L (64 to 102% sat.) pH: 7.8 to 8.5
Reference substance:	None

Methodology

A definitive test was performed from 06 February to 04 March 2014 with target nominal concentrations of 0 (control), 0 (vehicle control), 2.5, 5.0, 10, 20, and 40 µg/L. Daphnids were transferred to each test chamber resulting in a total of 200 juvenile and 200 adult daphnids per replicate of the control, vehicle control, and test substance treatments, or five juvenile and five adult daphnids per liter. The daphnids were added to the test chambers seven days prior to the start of the definitive study (Day -7). Abundance (number of individuals per liter) of each life stage over time was determined by sampling the population

prior test initiation (days -4 and -2), at initiation (day 0), and on days 2, 7, 14, and 21 after test initiation. Adult daphnids morphological sex was determined on days 2, 7, 14, and 21. 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 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 either counted immediately after collection and, following counting, discarded appropriately, or were preserved with 1-2 mL Lugols solution 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 daphnid counting. Observations were made daily for immobile daphnids and occurrence of abnormalities. All statistical comparisons were made to the pooled control group.

RESULTS AND DISCUSSION

The mean initial (0 hour) measured concentrations were 2.0, 4.4, 8.6, 17, and 35 µg/L, 80 to 88% of nominal, for the 2.5, 5.0, 10, 20, and 40 µg/L nominal treatments, respectively. No residues of X642188 were detected in the control or vehicle control solutions above the MQL for the analyte (0.10 µg/L) during the exposure period.

Table 2. Measured Concentrations of X642188 (a metabolite of XDE-777) During the Population Effects Study with *Daphnia magna*

Nominal Concentration (µg/L)	R E P	Measured Concentrations Expressed as µg/L									
		0 hour		1 hour		2 hour		4 hour		8 hour	
		Measured (µg/L)	% of Nominal	Measured (µg/L)	% of Nominal	Measured (µg/L)	% of Nominal	Measured (µg/L)	% of Nominal	Measured (µg/L)	% of Nominal
0 (Control)	A	<MQL ^a	NA	<MQL ^a	NA	<MQL ^a	NA	<MQL ^a	NA	<MQL ^a	NA
	B	<MQL ^a	NA	<MQL ^a	NA	<MQL ^a	NA	<MQL ^a	NA	<MQL ^a	NA
	C	<MQL ^a	NA	<MQL ^a	NA	<MQL ^a	NA	<MQL ^a	NA	<MQL ^a	NA
	D	<MQL ^a	NA	<MQL ^a	NA	<MQL ^a	NA	<MQL ^a	NA	<MQL ^a	NA
0 (Vehicle Control)	A	<MQL ^a	NA	<MQL ^a	NA	<MQL ^a	NA	<MQL ^a	NA	<MQL ^a	NA
	B	<MQL ^a	NA	<MQL ^a	NA	<MQL ^a	NA	<MQL ^a	NA	<MQL ^a	NA
	C	<MQL ^a	NA	<MQL ^a	NA	<MQL ^a	NA	<MQL ^a	NA	<MQL ^a	NA
	D	<MQL ^a	NA	<MQL ^a	NA	<MQL ^a	NA	<MQL ^a	NA	<MQL ^a	NA
2.5	A	2.0 ^c	80%	0.81	32%	0.40	16%	0.11	4%	<MQL ^a	NA
	B	1.8	72%	0.80	32%	0.35	14%	0.11	4%	<MQL ^a	NA
	C	2.0	80%	0.76	30%	0.30	12%	<Lowest Std. ^e	NA	<MQL ^a	NA
	D	2.1	84%	0.91	36%	0.37	15%	<Lowest Std. ^e	NA	<MQL ^a	NA
5.0	A	4.3	86%	1.7	34%	0.67	13%	0.20 ^d	4%	<MQL ^a	NA
	B	4.4	88%	2.3	46%	0.99	20%	0.29 ^d	6%	<Lowest Std. ^e	NA
	C	4.2	84%	2.2	44%	0.76	15%	0.17 ^d	3%	<MQL ^a	NA
	D	4.5	90%	1.4	28%	0.71	14%	0.26 ^d	5%	<Lowest Std. ^e	NA
10	A	9.2	92%	3.3	33%	1.3	13%	0.45 ^d	5%	0.17	2%
	B	8.5 ^c	85%	3.7	37%	1.7	17%	0.44 ^d	4%	0.14	1%
	C	8.6 ^c	86%	3.5	35%	1.5	15%	0.44 ^d	4%	0.20	2%
	D	7.9	79%	3.6	36%	1.8	18%	0.35 ^d	4%	0.21	2%
20	A	17	85%	7.3	37%	4.1	21%	0.95 ^d	5%	0.48	2%
	B	17	85%	7.7	39%	3.7	19%	1.4 ^d	7%	0.74	4%
	C	16	80%	8.7	44%	3.5	18%	1.3 ^d	7%	0.60	3%
	D	18	90%	7.1	36%	2.6	13%	1.5 ^d	8%	0.81	4%
40	A	37	93%	13	33%	6.0	15%	3.0 ^d	8%	1.7	4%
	B	35	88%	12	30%	6.3	16%	2.2 ^d	6%	1.4	4%
	C	34	85%	17	43%	9.8	25%	2.8 ^d	7%	1.4	4%
	D	32	80%	20	50%	9.5	24%	3.0 ^d	8%	3.1	8%

^a MQL = 0.10 µg/L

^b Calculated using ½ MQL for <MQL and < Lowest Std results.

^c Re-analyzed in duplicate, average of re-analyses reported.

^d Re-analyzed using the first dilution of 5.0 mL of sample with 5.0 mL of 0.2:50:50 formic acid:acetonitrile:water to improve chromatography.

^e <Lowest Standard = the peak area counts were less than 90% of the lowest standard.

Table 2. Measured Concentrations of X642188 (a metabolite of XDE-777) During the Population Effects Study with *Daphnia magna* (continued)

Nominal Concentration (µg/L)	R E P	Measured Concentrations Expressed as µg/L					
		24 hour		48 hour		168 hour	
		Measured (µg/L)	% of Nominal	Measured (µg/L)	% of Nominal	Measured (µg/L)	% of Nominal
0 (Control)	A	<MQL ^a	NA	<MQL ^a	NA	<MQL ^a	NA
	B	<MQL ^a	NA	<MQL ^a	NA	<MQL ^a	NA
	C	<MQL ^a	NA	<MQL ^a	NA	<MQL ^a	NA
	D	<MQL ^a	NA	<MQL ^a	NA	<MQL ^a	NA
0 (Vehicle Control)	A	<MQL ^a	NA	<MQL ^a	NA	<MQL ^a	NA
	B	<MQL ^a	NA	<MQL ^a	NA	<MQL ^a	NA
	C	<MQL ^a	NA	<MQL ^a	NA	<MQL ^a	NA
	D	<MQL ^a	NA	<MQL ^a	NA	<MQL ^a	NA
2.5	A	<MQL ^a	NA	<MQL ^a	NA	NA	NA
	B	<MQL ^a	NA	<MQL ^a	NA	NA	NA
	C	<MQL ^a	NA	<MQL ^a	NA	NA	NA
	D	<MQL ^a	NA	<MQL ^a	NA	NA	NA
5.0	A	<MQL ^a	NA	<MQL ^a	NA	NA	NA
	B	<MQL ^a	NA	<MQL ^a	NA	NA	NA
	C	<MQL ^a	NA	<MQL ^a	NA	NA	NA
	D	<MQL ^a	NA	<MQL ^a	NA	NA	NA
10	A	<MQL ^a	NA	<MQL ^a	NA	NA	NA
	B	<MQL ^a	NA	<MQL ^a	NA	NA	NA
	C	<MQL ^a	NA	<MQL ^a	NA	NA	NA
	D	<MQL ^a	NA	<MQL ^a	NA	NA	NA
20	A	0.26	1%	0.17	1%	<MQL ^a	NA
	B	0.41	2%	0.24	1%	<MQL ^a	NA
	C	0.28	1%	0.19	1%	<MQL ^a	NA
	D	0.33	2%	0.24	1%	<MQL ^a	NA
40	A	0.83	2%	0.51	1%	<MQL ^a	NA
	B	0.69	2%	0.37	1%	<MQL ^a	NA
	C	0.73	2%	0.39	1%	<MQL ^a	NA
	D	1.4	4%	0.67	2%	<MQL ^a	NA

^a MQL = 0.10 µg/L

^b Calculated using ½ MQL for <MQL and < Lowest Std results.

^c Re-analyzed in duplicate, average of re-analyses reported.

^d Re-analyzed using the first dilution of 5.0 mL of sample with 5.0 mL of 0.2:50:50 formic acid:acetonitrile:water to improve chromatography.

Table 2:Effect of X642188 on mean daphnid abundance (Daphnids/L)

Initial Mean Measured Treatment (µg/L)	Day 2				Day 7			
	No. adults	No. juveniles	No. neonates	Total	No. adults	No. juveniles	No. neonates	Total
Negative control	11	18	41	69	27	28	110	164
Vehicle control	8	15	35	57	33	39	94	165
Pooled control	9	17	38	63	30	34	102	165
2.0	13	24	40	77	26	59	127	211
4.4	12	22	45	78	38	45	143	225
8.6	11	20	34	65	39	38	115	191
17	9	21	54	84	53	38	113	204
35	10	11	72	93	13	21	24*	57*
NOEC	35 µg/L				17 µg/L			
EC ₅₀	Not Calculated				Not Calculated			

*statistically different from pooled controls (Dunnett's test; $p = 0.05$)

Table 3:Effect of X642188 on mean daphnid abundance (Daphnids/L) (continued)

Initial Mean Measured Treatment (µg/L)	Day 14				Day 21			
	No. adults	No. juveniles	No. neonates	Total	No. adults	No. juveniles	No. neonates	Total
Negative control	100	92	76	267	92	90	31	212
Vehicle control	125	96	95	316	142	101	29	271
Pooled control	112	94	86	291	117	96	30	242
2.0	98	87	84	268	135	112	54	300
4.4	86	89	75	249	139	100	54	293

Initial Mean Measured Treatment (µg/L)	Day 14				Day 21			
	No. adults	No. juveniles	No. neonates	Total	No. adults	No. juveniles	No. neonates	Total
Negative control	100	92	76	267	92	90	31	212
8.6	140	121	135	396	144	107	65	316
17	64	57	98	219	112	98	46	255
35	36*	23*	85	143	99	51	114	264
NOEC	17 µg/L				35 µg/L			
EC ₅₀	Not Calculated				Not Calculated			

*statistically different from pooled controls (Dunnett's test; $p = 0.05$)

No statistically significant differences were noted in the abundance of adults, juveniles, neonates, or total daphnid on Day 21, which confirmed the population recovery. No males were observed in the adult daphnids population on days 2, 7, 14, and 21. All juveniles and neonates from these time points were preserved in an appropriately labeled scintillation vial with 70% ethanol for future sexing, if necessary. No abnormal or immobile daphnids were observed over the course of the exposure.

CONCLUSION

Daphnid abundance was unaffected in the 2.0, 4.4, 8.6, and 17 µg/L test substance concentrations during the 21-day post-exposure. A significant reduction of daphnid abundance was observed at the 35 µg/L test substance concentration; neonates abundance was significantly decreased on day 7, but recovered by day 14 of the post-exposure phase, while juvenile and adult daphnid abundance was significantly decreased on day 14 of the post-exposure phase. All daphnid age groups, including neonate, juvenile, and adult, recovered by day 21 of the post-exposure phase

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Water flea	<i>Daphnia magna</i>	X642188	Days 2 and 21	NOEC	35	µg/L
Water flea	<i>Daphnia magna</i>	X642188	Days 7 and 14	NOEC	17	µg/L
Water flea	<i>Daphnia magna</i>	X642188	21 d	NOECoverall	17	µg/L

A 2.2.3.2.1.1 Study 5 – Efficacy of XDE-777 metabolites to Septoria tritici on wheat

Comments of zRMS:	<p>The study was already evaluated in the course of zonal authorization of the product GF-3308 and was considered acceptable. The zRMS's evaluation was copied here for transparency of the current assessment of the product GF-3307.</p> <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, GF-3308.</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
Guideline(s):	
Deviations:	
GLP:	
Acceptability:	Yes

Duplication (if vertebrate study)	NA
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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, 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. 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 cm² 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 GF-3308 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 2.2.3.2.1.2 Study 6 – *Septoria tritici* Biological screening report for five metabolites 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., GF-3308.</p> <p>The summary below was struck through as no evaluated in area of Section 9.</p>
Reference:	KCP 10.2.3/6

Report:	Yao, C.; 2014; <i>Septoria tritici</i> Biological Screening Report for Five Metabolites of XDE-777; Dow AgroSciences LLC, Zionsville, Indiana, USA; Lab Study No. DAI 1370; 04 November 2014; Unpublished
Guideline(s):	
Deviations:	
GLP:	
Acceptability:	Yes
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 cm² 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 oC) to permit spores to germinate and infect the leaf. The plants were then transferred to a greenhouse set at 20 oC 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 2.2.3.2.1.1 Study 7 – GF-3307: 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-3307 on <i>Daphnia magna</i> population in an indoor aquatic microcosms.</p> <p>The test was performed under static test conditions with two test item applications at day 0 and day 14. The abundance of three different size classes (neonates, juveniles and adults) were investigated over 35 days after the first application of the test item. Counting of daphnia abundance was performed at days - 5, - 1, 0, 2, 7, 14, 16, 21, 28 and 35.</p> <p>To assess the actual concentrations of XDE-777 and X642188 sampling was performed at test start (0h) and after 1, 2, 4, 8, 24, 48 hours, on day 7 and 14, and after the 2nd application within 30 minutes and after 1, 2, 4, 8, 24 and 48 hours, on day 21, 28 and 35.</p> <p>To assess the actual concentrations of Prothioconazole sampling was performed at test start (0h) and after 24 and 48 hours, on day 7 and 14, and after the 2nd test item application within 30 minutes, after 24 and 48 hours, and on day 21, 28 and 35.</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>Based on the evaluations, the two applications of GF-3307 had a temporary effect on the <i>Daphnia magna</i> population at 532 µg/L.</p> <p>Daphnid abundance for juvenile and adult daphnids as well as for the sum of all age groups was affected two days after the 2nd test item application (day 16), resulting in a NOEC of 180 µg/L and a LOEC of 532 µg/L GF-3307, corresponding to 8.46 and 25.0 µg/L XDE-777, based on arithmetic mean measured initial concentrations. Afterwards, no significant differences to controls were found until the end of the test.</p> <p>In reference to Daphnid abundance for neonates it was unaffected in all tested concentrations during the 35-day test following the 1st and 2nd test item application, resulting in a NOEC of 532 µg/L GF-3307 and a LOEC of > 532 µg/L GF-3307, corresponding to 25.0 µg/l and > 25.0 µg/L XDE-777, based on mean measured initial concentrations.</p> <p>The MDD (minimum detectable difference in percent of control) for the sum of all age</p>
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	<p>groups was < 50 % on most sampling days, which allows the determination of small effects according to the current aquatic guidance document (EFSA PPR 2013). For the single development stages, MDDs were higher, but it should be noted that if mean abundances in treated vessels were higher than in the controls, direct effects can be excluded without statistical testing.</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>“Although refined exposure tests with standard test species that more or less re-semble 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. <i>Daphnia</i>) 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).”</p> <p>It should be, however, pointed out that this population study with GF-3307 together with similar study with fenpicoxamid (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 GF-3307 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 mean measured concentrations..</p> <p>Overall, the study is considered acceptable for comparative purposes with following endpoints (all based on initial mean measured concentrations):</p> <p>21-d NOEC = 180 µg prep/L (4.65 µg fenpicoxamid/L)</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:	Brüggemann, M., Böhmer, W., Kosak, L.; 2020; GF-3307: Population Effects Study in an Indoor Aquatic Microcosm with <i>Daphnia magna</i> ; Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Schmallingenberg, Germany; Lab Study No. DOW-051/7-50/G; DAS Study No. 181382 ; 19 February 2020; Unpublished
Guideline(s):	None
Deviations:	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.
GLP:	
Acceptability:	Yes
Duplication (if vertebrate study)	NA

COMPLIANCE

Guideline(s):	None
US EPA Guideline(s):	None
Deviations:	None
Dates of work:	05 February 2019 to 12 March 2019
GLP status:	Yes
Number of pages in final report:	401

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-3307
Purity:	4.7 % XDE-777 (synonym of Fenpicoxamid) 9.3 % Prothioconazole
Description (physical state):	orange liquid, emulsifying concentrate (EC)
Lot/batch no.:	ENBK-169144-026 (TSN314279)

Test System

Organism (<i>Species</i>):	Daphnia (<i>Daphnia magna</i>)
Study type:	Population Effects
Duration of study:	42 days (7 days acclimation + 35 days post 1 st application)
Test conditions:	Static
Test concentrations:	Nominal: 0 (control), 7.2, 23, 65, 200, and 590 µg GF-3307/L Calculated initial concentrations: 7.30, 24.9, 61.8, 180 and 532 µg/L
Parameters measured:	Abundance of Daphnia (three size classes: neonates, juveniles, adults)
Observation intervals:	Days -5, -2 and 0 (prior to first test item application), Days 2, 7 and 14 (prior to the second test item application), Days 16, 21, 28 and 35
Reference substance:	XDE-777 (TSN302306) X642188 (TSN312858) Prothioconazole (TSN312881)
Number of organisms per dose group:	4 juvenile daphnids (aged 4-5 days) and 4 adult daphnids (aged 10-11 days) per liter
Number of organisms per control group:	4 juvenile daphnids (aged 4-5 days) and 4 adult daphnids (aged 10-11 days) per liter
Feeding regime, if applicable:	Feeding with unicellular alga <i>Desmodesmus subspicatus</i> . The food content was determined daily by measuring the optical density of the test medium at 585 nm. The food content was kept at an optical density of 0.015.
Environmental conditions:	Loading rate: not applicable Temperature: 19.5 – 20.8 °C Photoperiod: 16/8 hours Dissolved oxygen concentration: 6.70 - 9.30 mg O ₂ /L pH: 7.36 – 8.65 Total hardness: 294 and 345 mg CaCO ₃ /L

Methodology

At test initiation (day -7), daphnids of two different life stages (4 juvenile daphnids aged 4-5 days and 4 adult daphnids aged 10-11 days per liter) were introduced to test beakers (25 L aquaria) to acclimate for 7 days prior to the first test item application (referred to as test start or day 0). The daphnids were exposed to the test item added to water at a range of five concentrations and a control with four replicates each. The test was performed under static test conditions with two test item applications at day 0 and day 14. The abundance of three different size classes (neonates, juveniles and adults) were investigated over 35 days after the first application of the test item. Counting of daphnia abundance was performed at days - 5, - 1, 0, 2, 7, 14, 16, 21, 28 and 35.

The concentrations of XDE-777, X642188 and Prothioconazole were analysed in the control and all treatments. To assess the actual concentrations of XDE-777 and X642188 sampling was performed at test start (0h) and after 1, 2, 4, 8, 24, 48 hours, on day 7 and 14, and after the 2nd application within 30 minutes and after 1, 2, 4, 8, 24 and 48 hours, on day 21, 28 and 35. To assess the actual concentrations of Prothioconazole sampling was performed at test start (0h) and after 24 and 48 hours, on day 7 and 14, and after the 2nd test item application within 30 minutes, after 24 and 48 hours, and on day 21, 28 and 35. The concentrations of the active ingredients and the metabolite in the aqueous phase of all treatment levels were determined by LC-MS/MS. The method LOQs were 0.025 µg XDE-777/L, 0.0015 µg X642188/L (metabolite of XDE-777), and 0.05 µg Prothioconazole/L based on the validation fortifications. The experimental LOQs were 0.025 µg XDE-777/L, 0.0025 µg X642188/L (metabolite of XDE-777), and 0.0625 µg Prothioconazole/L based on the concurrent fortifications that occurred during sample analysis.

RESULTS AND DISCUSSION

For XDE-777, the initial measured concentrations were 0.39, 1.34, 2.98, 8.55 and 25.2 µg/L (90.8 and 124 % of nominal) after the first application and 0.30, 1.00, 2.83, 8.37 and 24.8 µg/L (87.6 and 92.7 % of nominal) after the second application. A fast decrease of test concentrations was observed. After the first application, XDE-777 concentrations were below the LOQ of 0.025 µg/L after only 24 hours for all treatment levels (mean DT₅₀ = 2.19 hours). After the second application, XDE-777 concentrations were below the LOQ within the first 48 hours after application (DT₅₀ = 5.71 hours). X642188 showed decreasing concentrations that were below the LOQ of 0.0015 µg/L in measurements performed after 24 hours and after 7 days after the first and second application, respectively (DT₅₀ = 1.98 hours and 11.3 hours, respectively). Prothioconazole concentrations were less fast decreasing, but were below the LOQ of 0.05 µg/L for the two lower concentrations after 14 days after the first and second application.

Table 1: Mean measured concentrations of XDE-777 [µg/L] after the 1st application.

Nominal concentration [µg/L]	Mean measured concentration XDE-777							
	Hours after 1st application							
	0		1	2	4	8	24	48
	[µg/L]	[%]	[µg/L]	[µg/L]	[µg/L]	[µg/L]	[µg/L]	[µg/L]
Control	<LOQ	-	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
0.338	0.390	115	0.345	0.235	0.134	0.063	<LOQ	<LOQ
1.08	1.34	124	0.968	0.795	0.298	0.110	<LOQ	<LOQ
3.06	2.98	97.5	2.64	1.28	1.25	0.180	<LOQ	<LOQ
9.40	8.55	90.9	7.03	5.37	2.85	1.04	<LOQ	<LOQ
27.7	25.2	90.8	20.6	13.5	7.21	2.06	<LOQ	<LOQ

LOQ: 0.025 µg/L

Table 2: Mean measured concentrations of XDE-777 [µg/L] after the 2nd application.

Nominal concentration [µg/L]	Mean measured concentration XDE-777							
	Hours after 2nd application							
	0		1	2	4	8	24	48
	[µg/l]	[%]	[µg/L]	[µg/L]	[µg/L]	[µg/L]	[µg/L]	[µg/L]
Control	<LOQ	-	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
0.338	0.296	87.6	0.254	0.232	0.176	0.116	<LOQ	<LOQ
1.08	1.00	92.7	0.942	0.777	0.672	0.369	0.061	<LOQ
3.06	2.83	92.6	2.29	2.16	1.51	1.16	0.066	<LOQ
9.40	8.37	89.0	7.66	6.83	5.24	3.70	0.417	0.049
27.7	24.8	89.6	21.2	19.1	15.6	10.6	1.37	0.094

LOQ: 0.025 µg/L

Table 3: Mean measured concentrations of X642188 [µg/L] after the 1st application.

Nominal concentration of XDE-777 [µg/L]	Mean measured concentration X642188 [µg/L]						
	Hours after 1st application						
	0	1	2	4	8	24	48
	[µg/l]	[µg/L]	[µg/L]	[µg/L]	[µg/L]	[µg/L]	[µg/L]
Control	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
0.338	0.011	0.008	0.005	0.002	<LOQ	<LOQ	<LOQ
1.08	0.036	0.022	0.018	0.005	0.003	<LOQ	<LOQ
3.06	0.080	0.065	0.023	0.025	0.004	<LOQ	<LOQ
9.40	0.236	0.176	0.116	0.065	0.020	0.002	<LOQ
27.7	0.689	0.451	0.262	0.113	0.033	0.003	<LOQ

LOQ: 0.0015 µg/L

Table 4: Mean measured concentrations of X642188 [µg/L] after the 2nd application.

Nominal concentration of XDE-777 [µg/L]	Mean measured concentration X642188 [µg/L]						
	Hours after 2nd application						
	0	1	2	4	8	24	48
	[µg/l]	[µg/L]	[µg/L]	[µg/L]	[µg/L]	[µg/L]	[µg/L]
Control	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
0.338	0.008	0.007	0.007	0.006	0.007	0.002	<LOQ
1.08	0.028	0.026	0.030	0.029	0.033	0.012	0.002
3.06	0.075	0.063	0.072	0.055	0.067	0.007	0.006
9.40	0.215	0.198	0.210	0.164	0.171	0.031	0.011
27.7	0.624	0.556	0.544	0.480	0.389	0.094	0.016

LOQ: 0.0015 µg/L

Table 5: Mean measured concentrations of Prothioconazole [µg/L] after the 1st application.

Nominal concentration [µg/L]	Mean measured concentration Prothioconazole [µg/L]					
	Day after 1st application					
	0		1	2	7	14
	[µg/l]	[%]	[µg/L]	[µg/L]	[µg/L]	[µg/L]
Control	<LOQ	-	<LOQ	<LOQ	<LOQ	<LOQ
0.670	0.221	33.0	<LOQ	<LOQ	<LOQ	<LOQ
2.14	1.64	76.9	0.357	0.104	0.053	<LOQ
6.05	4.96	82.0	1.25	0.867	0.125	0.052
18.6	16.9	90.8	8.07	4.31	0.521	0.232
54.9	52.8	96.2	33.6	25.8	2.54	0.884

LOQ: 0.05 µg/L

Table 6: Mean measured concentrations of Prothioconazole [µg/L] after the 2nd application.

Nominal concentration [µg/L]	Mean measured concentration Prothioconazole [µg/L]						
	Day after 2nd application						
	0		1	2	7	14	21
	[µg/l]	[%]	[µg/L]	[µg/L]	[µg/L]	[µg/L]	[µg/l]
Control	<LOQ	-	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
0.670	0.406	60.6	0.106	0.055	<LOQ	<LOQ	<LOQ
2.14	2.32	109	0.644	0.144	0.070	0.054	>LOQ
6.05	6.15	102	1.56	0.542	0.167	0.126	0.065
18.6	19.5	105	7.04	1.84	0.492	0.328	0.194
54.9	57.6	105	27.7	16.2	1.70	0.877	0.523

LOQ: 0.05 µg/L

Table 7: Calculated initial concentrations of GF-3307 (based on unrounded measured XDE-777 concentrations).

Nominal concentration GF-3307 [µg/L]	Calculated initial concentrations of GF-3307 [µg/L]					
	1 st application		2 nd application		Mean initial	
	[µg/L]	[%]	[µg/L]	[%]	[µg/L]	[%]
Control	<LOQ	-	<LOQ	-	<LOQ	-
7.2	8.29	115	6.30	87.6	7.30	101
23.0	28.52	124	21.32	92.7	24.92	108
65.0	63.38	97.5	60.21	92.6	61.80	95.1
20	181.82	90.9	178.10	89.0	179.96	90.0
590	535.47	90.8	528.44	89.6	531.95	90.2

All concentration-effect relationships were based on the initial concentrations of GF-3307 calculated based on the arithmetic mean measured XDE-777 initial concentrations.

Calculated initial concentrations of GF-3307 were 7.30, 24.9, 61.8, 180 and 532 µg/L (90.0 – 108 % of nominal).

The biology results obtained during the study are provided below:

**Table 8. Assessment of Neonate Daphnid Abundance (Number of Neonates/L).
Concentrations given as arithmetic mean measured initial concentrations of GF-3307.**

Date	Replicate	Concentration [µg/L]					
		Control	7.3	24.9	61.8	180	532
Day -5	1	0.00	0.00	0.00	0.00	0.00	0.00
	2	0.00	0.00	0.00	0.00	0.00	0.00
	3	0.00	0.00	0.00	0.00	0.00	0.00
	4	0.00	0.00	0.00	0.00	0.00	0.00
	Mean (SD)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Day -3	1	0.32	0.00	0.00	0.00	0.00	0.32
	2	0.00	0.16	0.00	0.00	0.00	0.16
	3	0.00	0.48	0.16	0.00	0.32	0.32
	4	0.00	0.16	0.00	0.00	0.32	0.16
	Mean (SD)	0.08 (0.16)	0.20 (0.20)	0.04 (0.08)	0.00 (0.00)	0.16 (0.18)	0.24 (0.09)
Day 0	1	0.48	0.32	0.00	0.00	0.00	0.32
	2	0.32	0.00	0.16	0.16	0.32	0.00
	3	0.32	0.32	0.00	0.00	0.32	0.16
	4	0.16	0.00	0.16	0.16	0.00	0.32
	Mean (SD)	0.32 (0.13)	0.16 (0.18)	0.08 (0.09)	0.08 (0.09)	0.16 (0.18)	0.20 (0.15)
Day 2	1	0.64	0.96	2.56	0.8	0.8	0.8
	2	1.76	1.12	0.48	0.96	0.64	0.64
	3	0.64	1.6	0.64	0.48	1.6	1.28
	4	2.72	0.48	0.16	0.96	0.8	0.48
	Mean (SD)	1.44 (1.00)	1.04 (0.46)	0.96 (1.09)	0.80 (0.23)	0.96 (0.43)	0.80 (0.35)
Day 7	1	0.80	3.52	3.04	4.32	8.80	4.00
	2	1.60	3.20	7.84	12.96	3.20	1.12
	3	4.80	2.56	12.8	6.24	4.80	3.84
	4	3.04	3.52	3.52	5.44	1.76	6.24
	Mean (SD)	2.56 (1.76)	3.20 (0.45)	6.80 (4.55)	7.24 (3.89)	4.64 (3.04)	3.80 (2.10)
Day 14	1	11.20	35.04	6.88	40.64	47.20	53.12
	2	32.48	19.52	3.84	11.52	33.28	58.24
	3	15.36	14.88	23.2	16.96	54.08	59.04
	4	7.84	6.08	20.8	42.56	60.32	50.56
	Mean (SD)	16.72 (10.95)	18.88 (12.13)	13.68 (9.74)	27.92 (15.97)	48.72 (11.60)	55.24 (4.08)
Day 16	1	10.24	18.24	4.32	68.96	25.76	10.4
	2	17.12	35.36	7.040	19.84	29.12	5.44
	3	10.56	42.40	18.40	33.92	30.40	1.28
	4	28.80	18.08	11.68	51.20	19.20	0.48
	Mean (SD)	16.68 (8.68)	28.52 (12.30)	10.36 (6.16)	43.48 (21.28)	26.12 (5.01)	4.40 (4.55)
Day 21	1	0.48	0.80	2.88	32.00	18.72	4.80
	2	0.80	1.12	5.92	12.96	40.80	0.80
	3	15.2	23.52	10.4	42.4	6.88	1.76
	4	11.52	29.76	3.20	50.56	20.16	1.12
	Mean (SD)	7.00 (7.50)	13.80 (15.04)	5.60 (3.48)	34.48 (16.23)	21.64 (14.09)	2.12 (1.83)
Day 28	1	15.36	2.56	33.12	4.00	3.84	47.84
	2	14.56	12.16	31.84	10.24	13.28	40.8
	3	8.00	4.64	8.32	9.12	7.04	52.64
	4	5.44	0.96	9.76	4.16	5.76	24.64
	Mean (SD)	10.84 (4.88)	5.08 (4.95)	20.76 (13.56)	6.88 (3.27)	7.48 (4.08)	41.48 (12.23)
Day 35	1	2.88	6.08	7.20	6.72	3.52	15.84
	2	18.24	17.92	8.64	2.88	4.80	2.72
	3	4.16	5.76	5.92	4.64	2.08	11.04
	4	36.48	4.00	8.64	3.68	2.88	35.52
	Mean (SD)	15.44 (15.66)	8.44 (6.39)	7.60 (1.31)	4.48 (1.66)	3.32 (1.15)	16.28 (13.92)

* Photo was lost during transmission, thus, daphnids fixed in ethanol were counted again.

No statistically significant decrease was noted in the abundance of neonate daphnids during the 35-days exposure period (Dunnett's test, Williams' test or Welch-t-test after Bonferroni Holm; $p = 0.05$, one sided smaller) at any sampling point.

Table 9. Assessment of Juvenile Daphnid Abundance (Number of Juveniles/L).

Concentrations given as arithmetic mean measured initial concentrations of GF-3307.

Date	Replicate	Concentration [µg/L]					
		Control	7.3	24.9	61.8	180	532
Day -5	1	1.12	0.48	0.32	0.48	0.16	0.16
	2	0.32	0.32	0.48	1.12	0.16	0.80
	3	1.12	0.96	0.32	2.24	0.96	0.48
	4	0.80	0.64	0.16	0.00	0.32	0.80
Mean (SD)		0.84 (0.38)	0.60 (0.27)	0.32 (0.13)	0.96 (0.97)	0.40 (0.38)	0.56 (0.31)
Day -3	1	0.32	0.00	0.00	0.32	0.00	0.00
	2	0.16	0.16	0.00	0.00	0.16	0.00
	3	0.00	0.16	0.16	0.00	0.00	0.00
	4	0.00	0.00	0.16	0.16	0.00	0.16
Mean (SD)		0.12 (0.15)	0.08 (0.09)	0.08 (0.09)	0.12 (0.15)	0.04 (0.08)	0.04 (0.08)
Day 0	1	0.00	0.00	0.00	0.00	0.00	0.00
	2	0.00	0.00	0.00	0.00	0.00	0.16
	3	0.00	0.00	0.00	0.00	0.00	0.00
	4	0.32	0.00	0.00	0.00	0.16	0.00
Mean (SD)		0.08 (0.16)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.04 (0.08)	0.04 (0.08)
Day 2	1	0.32	2.24	1.60	0.00	0.00	0.16
	2	0.48	0.96	0.00	1.92	0.00	0.00
	3	2.08	0.00	0.00	1.92	1.76	1.12
	4	6.88	1.92	0.48	0.00	0.00	0.8
Mean (SD)		2.44 (3.06)	1.28 (1.01)	0.52 (0.75)	0.96 (1.11)	0.44 (0.88)	0.52 (0.53)
Day 7	1	0.32	8.00	3.84	5.76	8.64	7.20
	2	3.20	2.56	4.64	7.20	3.68	2.08
	3	8.48	3.04	11.36	3.84	4.64	5.76
	4	2.4	2.08	4.80	4.64	2.08	5.28
Mean (SD)		3.60 (3.47)	3.92 (2.75)	6.16 (3.49)	5.36 (1.46)	4.76 (2.79)	5.08 (2.16)
Day 14	1	9.28	16.48	2.56	8.48	15.04	15.68
	2	17.76	9.44	3.68	2.24	9.28	14.24
	3	14.72	6.24	16.48	4.64	20.32	20.00
	4	5.12	2.56	20.32	16.8	13.60	14.40
Mean (SD)		11.72 (5.63)	8.68 (5.91)	10.76 (8.97)	8.04 (6.38)	14.56 (4.55)	16.08 (2.69)
Day 16	1	0.96	13.76	2.40	6.56	10.88	0.96
	2	4.96	7.04	3.68	7.04	4.48	0.32
	3	4.16	7.20	5.12	12.64	12.64	0.32
	4	8.16	3.68	7.04	10.72	3.84	0.16
Mean (SD)		4.56 (2.96)	7.92 (4.22)	4.56 (1.99)	9.24 (2.93)	7.96 (4.45)	0.44† (0.35)
Day 21	1	1.76	4.00	2.40	9.44	20.16	3.52
	2	1.28	0.96	4.48	14.24	19.36	1.44
	3	11.04	4.64	3.68	13.12	21.76	0.32
	4	2.4	5.12	5.12	34.40	14.56	1.28
Mean (SD)		4.12 (4.64)	3.68 (1.87)	3.92 (1.17)	17.80 (11.26)	18.96 (3.10)	1.64 (1.35)
Day 28	1	3.68	6.88	17.76	14.72	18.4	6.56
	2	5.28	2.24	27.52	13.12	23.84	13.60
	3	2.40	16.48	15.20	21.12	31.68	29.12
	4	0.48	7.36	2.88	16.80	43.04	18.72
Mean (SD)		2.96 (2.03)	8.24 (5.96)	15.84 (10.14)	16.44 (3.46)	29.24 (10.69)	17.00 (9.49)
Day 35	1	0.48	9.60	28.96	34.88	26.40	32.8
	2	46.56	22.24	37.60	6.88	22.24	12.32
	3	4.80	23.84	28.32	10.72	9.92	42.56
	4	24.80	8.48	26.40	40.96	14.24	28.16
Mean (SD)		19.16 (21.12)	16.04 (8.12)	30.32 (4.97)	23.36 (17.07)	18.20 (7.48)	28.96 (12.61)

† statistically significant difference from the control

Table 10. Assessment of Adult Daphnid Abundance (Number of Adults/L).Concentrations given as arithmetic mean measured initial concentrations of GF-3307.

Date	Replicate	Concentration [µg/L]					
		Control	7.3	24.9	61.8	180	532
Day -5	1	0.64	0.00	0.32	0.00	0.00	0.00
	2	0.00	0.00	0.00	0.16	0.00	0.00
	3	0.00	0.48	0.16	0.00	0.32	0.16
	4	0.00	0.16	0.00	0.00	0.80	0.48
	Mean (SD)	0.16 (0.32)	0.16 (0.23)	0.12 (0.15)	0.04 (0.08)	0.28 (0.38)	0.16 (0.23)
Day -3	1	0.64	1.28	0.32	1.60	2.56	0.80
	2	1.92	0.16	0.64	0.16	0.32	0.00
	3	0.16	0.48	1.60	0.16	0.48	0.00
	4	1.12	0.96	0.16	0.48	0.00	1.60
	Mean (SD)	0.96 (0.75)	0.72 (0.50)	0.68 (0.64)	0.60 (0.68)	0.84 (1.16)	0.60 (0.77)
Day 0	1	0.48	0.48	0.64	0.32	0.48	0.16
	2	0.00	1.44	0.00	0.00	0.64	0.48
	3	0.32	1.28	1.60	0.16	0.48	0.48
	4	1.28	0.16	0.00	0.64	0.96	0.32
	Mean (SD)	0.52 (0.54)	0.84 (0.62)	0.56 (0.76)	0.28 (0.27)	0.64 (0.23)	0.36 (0.15)
Day 2	1	0.32	1.28	1.92	0.00	0.00	0.32
	2	0.48	0.32	0.00	0.00	0.48	0.32
	3	0.96	0.00	0.16	0.96	1.60	0.64
	4	3.04	0.16	0.00	0.00	0.16	0.00
	Mean (SD)	1.20 (1.26)	0.44 (0.58)	0.52 (0.94)	0.24 (0.48)	0.56 (0.72)	0.32 (0.26)
Day 7	1	0.32	2.56	0.80	1.92	3.52	1.12
	2	0.96	0.16	0.48	4.00	1.28	0.48
	3	2.08	1.28	4.48	0.32	1.28	4.64
	4	0.80	1.12	0.80	1.12	0.00	0.80
	Mean (SD)	1.04 (0.74)	1.28 (0.99)	1.64 (1.90)	1.84 (1.58)	1.52 (1.46)	1.76 (1.94)
Day 14	1	2.88	10.24	0.96	4.00	4.96	7.68
	2	11.2	4.16	0.96	0.32	13.92	23.84
	3	8.96	4.96	5.60	1.44	7.68	3.84
	4	3.04	2.88	5.76	7.20	4.48	4.32
	Mean (SD)	6.52 (4.21)	5.56 (3.24)	3.32 (2.73)	3.24 (3.06)	7.76 (4.34)	9.92 (9.44)
Day 16	1	0.80	2.72	0.80	5.12	1.60	0.00
	2	1.92	1.12	1.28	3.04	3.84	0.00
	3	0.96	4.16	5.28	7.84	2.40	0.00
	4	4.16	0.96	1.92	2.40	0.00	0.00
	Mean (SD)	1.96 (1.55)	2.24 (1.51)	2.32 (2.03)	4.60 (2.45)	1.96 (1.60)	0.00† (0.00)
Day 21	1	0.16	0.80	1.76	0.80	1.76	4.96
	2	0.00	0.00	2.56	1.44	2.88	2.08
	3	2.72	2.08	0.48	7.52	1.12	1.44
	4	0.96	2.56	2.08	8.00	2.40	0.64
	Mean (SD)	0.96 (1.25)	1.36 (1.17)	1.72 (0.89)	4.44 (3.85)	2.04 (0.77)	2.28 (1.88)
Day 28	1	1.92	2.88	5.28	2.08	4.96	7.52
	2	1.60	5.28	5.76	3.36	2.40	4.80
	3	5.12	3.84	3.68	7.20	4.64	3.84
	4	1.12	1.12	9.60	3.20	4.96	4.32
	Mean (SD)	2.44 (1.82)	3.28 (1.75)	6.08 (2.51)	3.96 (2.23)	4.24 (1.24)	5.12 (1.65)
Day 35	1	0.00	7.36	2.56	12.48	7.20	4.96
	2	2.88	2.40	7.20	3.04	2.24	3.20
	3	0.00	10.08	13.92	3.68	3.20	8.64
	4	6.24	7.36	5.12	20.64	6.88	17.44
	Mean (SD)	2.28 (2.97)	6.80 (3.20)	7.20 (4.87)	9.96 (8.32)	4.88 (2.53)	8.56 (6.34)

† statistically significant difference from the control

Table 11. Assessment of Total Daphnid Abundance (Number of Daphnids/L).
Concentrations given as arithmetic mean measured initial concentrations of GF-3307.

Date	Replicate	Concentration [$\mu\text{g/L}$]					
		Control	7.3	24.9	61.8	180	532
Day -5	1	1.76	0.48	0.64	0.48	0.16	0.16
	2	0.32	0.32	0.48	1.28	0.16	0.80
	3	1.12	1.44	0.48	2.24	1.28	0.64
	4	0.80	0.80	0.16	0.00	1.12	1.28
	Mean (SD)	1.00 (0.60)	0.76 (0.50)	0.44 (0.20)	1.00 (0.98)	0.68 (0.60)	0.72 (0.46)
Day -3	1	1.28	1.28	0.32	1.92	2.56	1.12
	2	2.08	0.48	0.64	0.16	0.48	0.16
	3	0.16	1.12	1.92	0.16	0.80	0.32
	4	1.12	1.12	0.32	0.64	0.32	1.92
	Mean (SD)	1.16 (0.79)	1.00 (0.35)	0.80 (0.76)	0.72 (0.83)	1.04 (1.03)	0.88 (0.81)
Day 0	1	0.96	0.80	0.64	0.32	0.48	0.48
	2	0.32	1.44	0.16	0.16	0.96	0.64
	3	0.64	1.60	1.60	0.16	0.80	0.64
	4	1.76	0.16	0.16	0.80	1.12	0.64
	Mean (SD)	0.92 (0.62)	1.00 (0.66)	0.64 (0.68)	0.36 (0.30)	0.84 (0.27)	0.60 (0.08)
Day 2	1	1.28	4.48	6.08	0.80	0.80	1.28
	2	2.72	2.40	0.48	2.88	1.12	0.96
	3	3.68	1.60	0.80	3.36	4.96	3.04
	4	12.64	2.56	0.64	0.96	0.96	1.28
	Mean (SD)	5.08 (5.14)	2.76 (1.22)	2.00 (2.72)	2.00 (1.31)	1.96 (2.00)	1.64 (0.95)
Day 7	1	1.44	14.08	7.68	12.00	20.96	12.32
	2	5.76	5.92	12.96	24.16	8.16	3.68
	3	15.36	6.88	28.64	10.40	10.72	14.24
	4	6.24	6.72	9.12	11.20	3.84	12.32
	Mean (SD)	7.20 (5.85)	8.40 (3.81)	14.60 (9.62)	14.44 (6.51)	10.92 (7.27)	10.64 (4.73)
Day 14	1	23.36	61.76	10.40	53.12	67.20	76.48
	2	61.44	33.12	8.48	14.08	56.48	96.32
	3	39.04	26.08	45.28	23.04	82.08	82.88
	4	16.00	11.52	46.88	66.56	78.40	69.28
	Mean (SD)	34.96 (20.10)	33.12 (21.11)	27.76 (34.73)	39.20 (24.73)	71.04 (11.59)	81.24 (11.49)
Day 16	1	12.00	34.72	7.52	80.64	38.24	11.36
	2	24.00	43.52	12.00	29.92	37.44	5.76
	3	15.68	53.76	28.80	54.4	45.44	1.60
	4	41.12	22.72	20.64	64.32	23.04	0.64
	Mean (SD)	23.20 (12.96)	38.68 (13.18)	17.24 (9.44)	57.32 (21.23)	36.04 (9.38)	4.84 (4.88)
Day 21	1	2.40	5.60	7.04	42.24	40.64	13.28
	2	2.08	2.08	12.96	28.64	63.04	4.32
	3	28.96	30.24	14.56	63.04	29.76	3.52
	4	14.88	37.44	10.40	92.96	37.12	3.04
	Mean (SD)	12.08 (12.73)	18.84 (17.63)	11.24 (3.28)	56.72 (28.00)	42.64 (14.34)	6.04 (4.86)
Day 28	1	20.96	12.32	56.16	20.80	27.20	61.92
	2	21.44	19.68	65.12	26.72	39.52	59.20
	3	15.52	24.96	27.20	37.44	43.36	85.60
	4	7.04	9.44	22.24	24.16	53.76	47.68
	Mean (SD)	16.24 (6.70)	16.60 (7.05)	42.68 (21.26)	27.28 (7.19)	40.96 (10.97)	63.60 (15.91)
Day 35	1	3.36	23.04	38.72	54.08	37.12	53.60
	2	67.68	42.56	53.44	12.80	29.28	18.24
	3	8.96	39.68	48.16	19.04	15.20	62.24
	4	67.52	19.84	40.16	65.28	24.00	81.12
	Mean (SD)	36.88 (35.55)	31.28 (11.50)	45.12 (6.93)	37.80 (25.80)	26.40 (9.21)	53.80 (26.34)

The abundance of particular Daphnids age groups over the test period is illustrated on the following Figure 1.

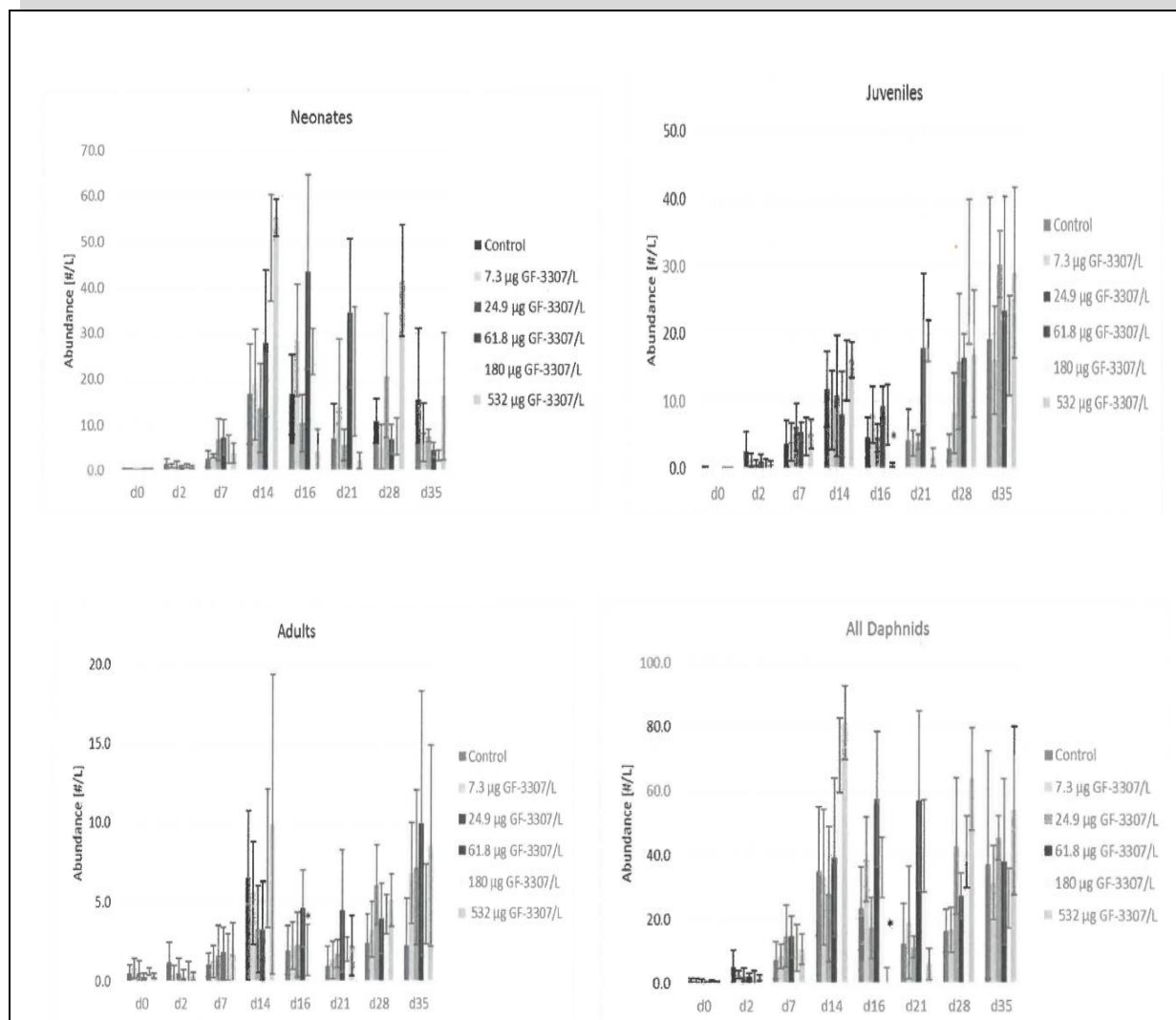


Figure 1. The abundance of particular Daphnids age groups over the test period

Daphnid abundance for juvenile and adult daphnids as well as for the sum of all age groups was affected two days after the 2nd test item application (day 16) (Williams' test, $p = 0.05$; one-sided smaller). For all other sampling points, no significant difference could be observed for all development stages (Dunnett's test, Williams' test, Welch-t-test after Bonferroni Holm or median test with Bonferroni correction; $p = 0.05$, one sided smaller).

Table 8: Effects of GF-3307 on abundance of neonate, juvenile and adult daphnids.

Timepoint	Biological Parameter (Abundance of daphnids)	GF-3307 [µg/L]		XDE-777 [µg/L]		MDDs [%]
		NOEC	LOEC	NOEC	LOEC	
Day 2	All Age Groups	532.0	> 532.0	25.0	> 25.0	-40.2
	Neonates	532.0	> 532.0	25.0	> 25.0	-54.5
	Juveniles	532.0	> 532.0	25.0	> 25.0	-98.6
	Adults	532.0	> 532.0	25.0	> 25.0	-95.7
Day 7	All Age Groups	532.0	> 532.0	25.0	> 25.0	-40.8
	Neonates	532.0	> 532.0	25.0	> 25.0	-54.1
	Juveniles	532.0	> 532.0	25.0	> 25.0	-55.5
	Adults	532.0	> 532.0	25.0	> 25.0	-115
Day 14	All Age Groups	532.0	> 532.0	25.0	> 25.0	-16.9
	Neonates	532.0	> 532.0	25.0	> 25.0	-22.3
	Juveniles	532.0	> 532.0	25.0	> 25.0	-35.3

	Adults	532.0	> 532.0	25.0	> 25.0	-49.8
Day 16	All Age Groups	180.0	532.0	8.46	25.0	-20.8
	Neonates	532.0	> 532.0	25.0	> 25.0	-65.0
	Juveniles	180.0	532.0	8.46	25.0	-30.9
	Adults	180.0	532.0	8.46	25.0	-54.2
Day 21	All Age Groups	532.0	> 532.0	25.0	> 25.0	-93.8
	Neonates	532.0	> 532.0	25.0	> 25.0	-143
	Juveniles	532.0	> 532.0	25.0	> 25.0	-50.2
	Adults	532.0	> 532.0	25.0	> 25.0	-118
Day 28	All Age Groups	532.0	> 532.0	25.0	> 25.0	-14.9
	Neonates	532.0	> 532.0	25.0	> 25.0	-25.8
	Juveniles	532.0	> 532.0	25.0	> 25.0	-49.1
	Adults	532.0	> 532.0	25.0	> 25.0	-34.1
Day 35	All Age Groups	532.0	> 532.0	25.0	> 25.0	-45.6
	Neonates	532.0	> 532.0	25.0	> 25.0	-39.2
	Juveniles	532.0	> 532.0	25.0	> 25.0	-100
	Adults	532.0	> 532.0	25.0	> 25.0	-95.5

CONCLUSION

Daphnid abundance for neonates was unaffected in all tested concentrations during the 35-day test following the 1st and 2nd test item application, resulting in a NOEC of 532 µg/L GF-3307 and a LOEC of > 532 µg/L GF-3307, corresponding to 25.0 µg/l and > 25.0 µg/L XDE-777, based on arithmetic mean measured initial concentrations.

Daphnid abundance for juvenile and adult daphnids as well as for the sum of all age groups was affected two days after the 2nd test item application (day 16), resulting in a NOEC of 180 µg/L and a LOEC of 532 µg/L GF-3307, corresponding to 8.46 and 25.0 µg/L XDE-777, based on arithmetic mean measured initial concentrations. Afterwards, no significant differences to controls were found until the end of the test.

Based on the evaluations, the two applications of GF-3307 had only a temporary effect on the *Daphnia magna* population at 532 µg/L (NOEC = 180 µg/L).

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Daphnid	<i>Daphnia magna</i>	GF-3307	35 day	NOEC	180	µg/L, imm

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-3307: A laboratory study to determine the acute oral toxicity on the honey bees *Apis mellifera* L. (Hymenoptera: Apidae)

Comments of zRMS:	<p>The study was conducted in line with OECD 213 with a minor deviation.</p> <p>It was noted that in order to cover the range of the expected toxicity, actual spacing factor was 2.5 instead of 2.2 as required by the guideline. Since the validity criteria were met and the statistical analysis allowed for determination of the 95% confidence intervals for LD₅₀ (which was covered by the range of tested doses) in zRMS opinion this deviation is considered to have no impact on the outcome of the study.</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>oral 48h LD₅₀ = 55.46 µg product/bee</p>
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Reference:	KCP 10.3.1.1.1/1
Report:	Noël, E.; 2015; GF-3307: A laboratory study to determine the acute oral toxicity on the honey bees <i>Apis mellifera</i> L. (Hymenoptera: Apidae); SynTech Research France S.A.S., La Chapelle de Guinchay, France; Lab Study No. 014SRFR14C06; DAS Study No. 150736; 21 September 2015; Unpublished
Guideline(s):	OECD No. 213 (1998)
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable Yes
Duplication (if vertebrate study)	NA

COMPLIANCE

Guideline(s):	OECD No. 213 (1998)
US EPA Guideline(s):	Not Applicable
Deviations:	There was one intentional guideline deviation: in order to cover the range of the expected toxicity, actual spacing factor was 2.5 instead of 2.2 as required by the OECD guideline 213.
Dates of work:	21 July 2015 to 23 July 2015
GLP status:	Yes
Number of pages in final report:	93

MATERIALS AND METHODS

Test Item(s)

ISO Common name:	Not applicable
Test item (chemical/other name):	GF-3307; GF-3307 BLANK; GF-3307 (prothioconazole only); GF-3307 (XDE-777); prothioconazole; XDE-777
Purity:	GF-3307: 9.4% w/w prothioconazole + 4.8% w/w XDE-777 GF-3307 BLANK: Not applicable GF-3307 (prothioconazole only): 9.9% w/w prothioconazole GF-3307 (XDE-777 only): 5.2% w/w XDE-777 Prothioconazole: 98.0 % w/w XDE-777: 84.2% w/w
Description (physical state):	GF-3307, GF-3307 BLANK, GF-3307 (prothioconazole only), GF-3307 (XDE-777): Brown liquid Prothioconazole: White crystalline XDE-777 : White solid
Lot/batch no.:	GF-3307: ENBK-147245-050 GF-3307 BLANK:ENBK-144192-041 GF-3307 (prothioconazole only):ENBK-148646-019-P GF-3307 (XDE-777 only): ENBK-148646-019-T7 Prothioconazole: FL018728*1.0 XDE-777: XDE-777-01-02
CAS no.:	XDE-777: 517875-34-2 Prothioconazole: 178928-70-6

Test System

Organism (<i>Species</i>):	Honey bee (<i>Apis mellifera</i> L.)
Study type:	Laboratory study
Study design:	Acute oral toxicity test; duration 48 hrs; 3 replicates, each consisting of 10 bees in one cage per test concentration; assessment of mortality after 4, 24 and 48 hrs
Test concentrations:	GF-3307 BLANK: 9.90, 24.70, 61.80, 154.4 and 386.1 µg product/bee GF-3307: 11.50, 28.80, 72.0, 180.0 and 450.0 µg product/bee GF-3307 (prothioconazole only): 11.0, 27.40, 68.60, 171.3 and 428.4 µg product/bee GF-3307 (XDE-777 only): 10.50, 26.10, 65.30, 163.0 and 407.7 µg product/bee Prothioconazole at 1.10, 2.70, 6.80 and 16.90 µg a.s./bee + XDE-777 at 0.60, 1.40, 3.50 and 8.60 µg a.s./bee
Information on bee colony (health etc):	The bees used in the test were from a single, disease-free colony. The hive had not been treated for varroa mites or for disease during the year of the study. The bees were maintained in a clean holding cage.
Amount of treated diet consumed:	Each solution (100 µL per test unit) was entirely consumed within 6 hours.
Feeding method:	50% w/v sucrose solution <i>ad libitum</i> ; was given directly after treatment using syringes.
Environmental conditions:	Temperature: 23.4 - 26.7°C Relative Humidity: 56 - 70 % Photoperiod: organisms were kept in the dark during the study (red light was used for observations).
Reference substance:	0.10, 0.20 and 0.35 µg dimethoate / bee
Solvent substance (if applicable):	Acetone to dissolve the mix Prothioconazole + XDE-777
Adjuvant:	None

Methodology

GF-3307, GF-3307 BLANK, GF-3307 (XDE-777 only), GF-3307 (prothioconazole only) and prothioconazole + XDE-777 were dissolved in distilled water or acetone (prothioconazole + XDE-777) and dispersed in sucrose solution (500g/L) for acute toxicity test, at doses equivalent to:

- GF-3307 BLANK at 9.90, 24.70, 61.80, 154.4 and 386.1 µg product/bee
- GF-3307 at 11.50, 28.80, 72.0, 180.0 and 450.0 µg product/bee
- GF-3307 (prothioconazole only) at 11.0, 27.40, 68.60, 171.3 and 428.4 µg product/bee
- GF-3307 (XDE-777 only) at 10.50, 26.10, 65.30, 163.0 and 407.7 µg product/bee
- Prothioconazole at 1.10, 2.70, 6.80 and 16.90 µg a.s./bee + XDE-777 at 0.60, 1.40, 3.50 and 8.60 µg a.s./bee

A toxic reference item (400 g/L dimethoate) was applied at 3 doses to demonstrate the relative susceptibility of the test organisms and the sensitivity of the test system. Test included doses between 0.10-0.35 µg a.s./bee. Untreated sucrose solution control was included to assess the natural mortality of the test organisms. Direct treatment effects (mortality and other observed biological effects) were assessed after 4, 24 and 48 hours. Mortality data were analysed for significant differences compared to the untreated (GF-3307, GF-3307 BLANK, GF-3307 (XDE-777 only), GF-3307 (prothioconazole only)) or acetone (prothioconazole + XDE-777) control group using ANOVA plus Dunnett's ($p \leq 0.05$), after logarithmic transformation of the replicate mean data (n+1).

RESULTS AND DISCUSSION

Oral test has been carried out to evaluate potential adverse effects of GF-3307, GF-3307 BLANK, GF-3307 (XDE-777 only), GF-3307 (prothioconazole only) and prothioconazole + XDE-777 on the survival of honey bees *Apis mellifera* L. (Hymenoptera: Apidae) in laboratory conditions. The highest dose of the mix prothioconazole + XDE-777 (42.30 µg prothioconazole/bee + 21.60 µg XDE-777/bee) was not dissolved during application (precipitation in the sucrose solution); therefore, the results for that dose were excluded from the analysis.

The study was valid since average mortalities did not exceed 10% in the untreated sucrose solution control (actual value: 3.333 %) and 24-hour toxic reference LD₅₀ was in the range of 0.10 - 0.35 µg a.s./bee in the oral test (actual value: 0.140 µg a.s./bee; 95% confidence limits: 0.078 - 0.188 µg a.s./bee).

Table 1: Summary of *Apis mellifera* oral toxicity data – GF-3307 BLANK

Treatment µg GF-3307 BLANK /bee		Oral
Nominal	Actual	Mortality (%)
		48-hr
Control (0)	Not applicable	3.333
Acetone control	Not applicable	6.667
9.90	9.90	3.448
24.70	24.70	10.34
61.80	61.80	0.0
154.4	154.4	17.24
386.1	386.1	6.897
Oral 48-hr LD ₅₀		> 386.1
Oral LD ₅₀ (24-hr) value of the reference item: 0.140 µg dimethoate/bee		

Table 2: Summary of *Apis mellifera* oral toxicity data – GF-3307

Treatment µg GF-3307 /bee		Oral
Nominal	Actual	Mortality (%)
		48-hr
Control (0)	Not applicable	3.333
Acetone control	Not applicable	6.667
11.50	11.50	24.14*

Treatment µg GF-3307 /bee		Oral
Nominal	Actual	Mortality (%)
		48-hr
28.80	28.80	41.38*
72.0	72.0	51.72*
180.0	180.0	72.41*
450.0	450.0	79.31*
Oral 48-hr LD ₅₀		55.46
Oral LD ₅₀ (24-hr) value of the reference item: 0.140 µg dimethoate/bee		

* Significantly different from the control

Table 3:Summary of Apis mellifera oral toxicity data – GF-3307 (prothioconazole only)

Treatment µg GF-3307 (prothioconazole only)/bee		Oral
Nominal	Actual	Mortality (%)
		48-hr
Control (0)	Not applicable	3.333
Acetone control	Not applicable	6.667
11.0	11.0	6.897
27.40	27.40	10.34
68.60	68.60	17.24
171.3	171.3	27.59*
428.4	428.4	48.28*
Oral 48-hr LD ₅₀		>428.4
Oral LD ₅₀ (24-hr) value of the reference item: 0.140 µg dimethoate/bee		

* Significantly different from the control

Table 4:Summary of Apis mellifera oral toxicity data – GF-3307 (XDE-777 Only)

Treatment µg GF-3307 (XDE-777 only)/bee		Oral
Nominal	Actual	Mortality (%)
		48-hr
Control (0)	Not applicable	3.333
Acetone control	Not applicable	6.667
10.50	10.50	13.79
26.10	26.10	17.24
65.30	65.30	34.48*
163.0	163.0	62.07*
407.7	407.7	68.97*
Oral 48-hr LD ₅₀		127.7
Oral LD ₅₀ (24-hr) value of the reference item: 0.140 µg dimethoate/bee		

* Significantly different from the control

Table 5:Summary of Apis mellifera oral toxicity data – Prothioconazole + XDE 777

Treatment µg Prothioconazole + XDE 777/bee		Oral
Nominal	Actual	Mortality (%)
		48-hr
Control (0)	Not applicable	3.333
Acetone control	Not applicable	6.667
0.60 µg a.s XDE-777/bee + 1.10 µg a.s Prothioconazole/bee	0.60 µg a.s XDE-777/bee + 1.10 µg a.s Prothioconazole/bee	7.143
1.40 µg a.s XDE-777/bee + 2.70 µg a.s Prothioconazole /bee	1.40 µg a.s XDE-777/bee + 2.70 µg a.s Prothioconazole /bee	17.86
3.50 µg a.s XDE-777/bee + 6.80 µg a.s Prothioconazole /bee	3.50 µg a.s XDE-777/bee + 6.80 µg a.s Prothioconazole /bee	21.43
8.60 µg a.s XDE-777/bee + 16.90 µg a.s Prothioconazole /bee	8.60 µg a.s XDE-777/bee + 16.90 µg a.s Prothioconazole /bee	57.14*

Treatment µg Prothioconazole + XDE 777/bee		Oral
Nominal	Actual	Mortality (%)
		48-hr
Oral 48-hr LD50		XDE-777: 7.966 + Prothioconazole: 15.68
Oral LD ₅₀ (24-hr) value of the reference item: 0.140 µg dimethoate/bee		

* Significantly different from the acetone control

CONCLUSION

The study was valid since average mortalities did not exceed 10% in the untreated sucrose solution control (actual value: 3.333 %) and 24-hour toxic reference LD₅₀ was in the range of 0.10 - 0.35 µg a.s./bee in the oral test (actual value: 0.140 µg a.s./bee; 95% confidence limits: 0.078 - 0.188 µg a.s./bee).

Table 6: Summary of Apis mellifera oral toxicity data for all test items tested

Test organism / Exposure	Honey bee / Oral		
Assessment	Mortality after 48 hours [%]*		
	NOED	LOED	LD50 (95% confidence limits)
GF-3307 (µg f.p./bee)	< 11.50	11.50	55.46 (27.18 – 98.20)
GF-3307 BLANK (µg f.p./bee)	386.1	> 386.1	> 386.1
GF-3307 (prothioconazole only) (µg f.p./bee)	68.60	171.3	> 428.4
GF-3307 (XDE-777 only) (µg f.p./bee)	26.10	65.30	127.7 (74.08 – 239.8)
Prothioconazole + XDE-777 (µg a.s./bee)	XDE-777: 3.50 + Prothioconazole: 6.80	XDE-777: 8.60 + Prothioconazole: 16.90	XDE-777: 7.966 (4.385 - 31.42) + Prothioconazole: 15.68 (8.551 - 64.57)

* Based on the number of moribund and dead organisms; f.p.= formulated product; a.s.= active substance.

Common name	Species	Test item	Time-scale	End-point	Toxicity value	Units of test item
Honey bee	<i>Apis mellifera</i>	GF-3307	48-hr – oral	LD50	55.46	µg/bee
Honey bee	<i>Apis mellifera</i>	GF-3307 BLANK	48-hr – oral	LD50	> 386.1	µg/bee
Honey bee	<i>Apis mellifera</i>	GF-3307	48-hr – oral	LD50	> 428.4	µg/bee
Honey bee	<i>Apis mellifera</i>	GF-3307	48-hr – oral	LD50	127.7	µg/bee
Honey bee	<i>Apis mellifera</i>	Prothioconazole + XDE-777	48-hr – oral	LD50	15.68 + 7.966	µg/bee

A 2.3.1.1.1.2 Study 2 - GF-3307: Acute contact and oral effects on Honeybees (*Apis mellifera* L.) in the laboratory

Comments of zRMS:	<p>The study was conducted in line with OECD 213 and OECD 214 with minor deviations.</p> <p>It was noted that in the contact test a 5 µL droplet was chosen in deviation to the guideline recommendation of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test item. The study report provides an explanation that the testing facility's experience has proven that higher volumes are suitable and no adverse effects on the outcome of the study are to be expected. These findings were presented as a poster on the ICPBR Bee Protection Group meeting in Bologna, 2002. Additionally, the tested product was dispersed in carrier (tap water) with an addition of 0.5 % Adhäsit which was used to improve the adhesion of the droplet on the bee body. Adhäsit is non-toxic to honey bees.</p>
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	<p>In zRMS opinion these deviations had no impact on the outcome of the study since the validity criteria were met.</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>contact 72h LD₅₀ = 92.3 µg product/bee oral 72h LD₅₀ = 212.5 µg product/bee</p>
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Reference:	KCP 10.3.1.1.1/2; KCP 10.3.1.1.2/1
Report:	Schmitzer, S; 2014; GF-3307: Acute contact and oral effects on Honeybees (<i>Apis mellifera</i> L.) in the laboratory.; Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany ; Lab Study No. 90541035; DAS Study No. 140220 & 140213; 16 December 2014; Unpublished.
Guideline(s):	Acute oral toxicity test: OECD 213 (adopted 21 st September 1998) Acute contact toxicity test: OECD 214 (adopted 21 st September 1998)
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable Yes
Duplication (if vertebrate study)	NA

COMPLIANCE

Guideline(s):	Acute oral toxicity test: OECD 213 (adopted 21 st September 1998) Acute contact toxicity test: OECD 214 (adopted 21 st September 1998)
US EPA Guideline(s):	N/A
Deviations:	None.
Dates of work:	26 May 2014 to 25 September 2014
GLP status:	Yes
Number of pages in final report:	74

MATERIALS AND METHODS

Test Item GF-3307

ISO Common name:	Not applicable
Test item (chemical/other name):	GF-3307
Purity:	Prothioconazole: 9.4 % w/w XDE-777: 4.8 % w/w
Description (physical state):	Liquid
Lot/batch no.:	Lot No.: F1281-135-1 Batch No.: TSN307579
CAS no.:	Not applicable

Test Item GF-3307 BLANK

ISO Common name:	Not applicable
Test item (chemical/other name):	GF-3307 BLANK
Purity:	Blank Formulation without active ingredient (Prothioconazole or XDE-777)
Description (physical state):	Liquid
Lot/batch no.:	Lot No.: ENBK-144192-041
CAS no.:	Not applicable

Test System

Organism (<i>Species</i>):	Honey bee (<i>Apis mellifera</i>)
Study type:	Acute oral
Study design:	Acute contact and oral LD ₅₀ test; duration 48 hours up to 72 hours; 3 replicates, each consisting of 10 bees in one cage per test concentration; assessment of mortality after 4, 24 and 48 hours and additionally 72 hours (GF-3307 contact and oral test and GF-3307 Blank contact test), because of increasing mortality between 24 and 48 hours; reference item: dimethoate 400 g/L (nominal).
Test concentrations:	GF-3307: Oral [nominal]: 0 (control), 600, 300, 150, 75 and 37.5 µg GF-3307/bee Oral [measured]: 0 (control), 447.0, 324.0, 163.3, 79.6 and 41.3 µg GF-3307/bee Contact 0 (control), 300, 150, 75, 37.5, 18.8 and 9.4 µg GF-3307/bee GF-3307 BLANK: Oral [nominal]: 0 (control), 600, 300, 150, 75 and 37.5 µg GF-3307 Blank/bee Oral [measured]: 0 (control), 305.0, 230.0, 161.3, 79.4 and 40.1 µg GF-3307 Blank/bee Contact 0 (control), 300, 150, 75 and 37.5 and 18.8 µg GF-3307 Blank/bee
Information on bee colony (health etc):	The bees used in the test were 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:	GF-3307: Consumption of the treated diets resulted in calculated dosages ranging from 40.7 to 561.0 µg GF-3307/bee. GF-3307 BLANK: Consumption of the treated diets resulted in calculated dosages ranging from 39.0 to 324.0 µg GF-3307 Blank/bee.
Feeding method:	50 % w/v sucrose solution <i>ad libitum</i> ; was given directly after treatment using syringes; Fresh 50 % w/v sucrose solution was supplied after 48 hours, if appropriate.
Environmental conditions:	Temperature: 24-25°C Relative humidity: 51-86% 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 study: a single 5 µL droplet of GF-3307 or GF-3307 Blank in an appropriate carrier (tap water + 0.5 % Adhäsit) was placed on the dorsal bee thorax using a Burkard – Applicator or Multipette®, Eppendorf. For the control one 5 µL droplet of tap water containing 0.5 % Adhäsit was used.

Oral study: after mixing either GF-3307 or the GF-3307 Blank formulation 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

The contact test with GF-3307 was prolonged for a further 24 hours up to 72 hours due to increasing mortality between 24/48 hours.

At the end of the contact toxicity test (72 hours after application), mortality ranged between 100.0 and 10.0 % after treatment with 300, 150, 75, 37.5, 18.8 and 9.4 µg GF-3307/bee. There was no control mortality (water + 0.5 % Adhäsit) after 48 hours.

During the 4-hours assessment, behavioural abnormalities of the surviving bees (e.g. moribund or affected bees and/or apathy) were observed in all GF-3307 treated dose groups (except 9.4 µg/bee dose level). 24 hrs following treatment a few of the surviving bees still showed these behavioural abnormalities in these dose levels (150, 75, 37.5 and 18.8 µg GF-3307/bee). 48 hours following dosing, behavioural abnormalities occurred in the 150, 75 and 37.5 µg/bee dose levels and at the end of the study (72 hours) a few bees were affected, moribund or apathetic in the 150, 75 and 9.4 µg/bee dosing groups.

The oral test with GF-3307 was also prolonged for a further 24 hours up to 72 hours due to increasing mortality between 24/48 hours.

In the oral test, the maximum nominal dose level of GF-3307 (600 µg/bee) was not achieved, because the bees did not ingest the full volume of treated sugar solution even when offered over a period of 6 hours. Actual Oral doses of 447.0, 324.0, 163.3, 79.6 and 41.3 µg GF-3307 (led to mortality levels ranging from 76.7 to 20.0 % at the end of the test (72 hours after application). No mortality occurred in the control group (50 % w/v sucrose solution = 500 g sucrose/L tap water). A few single bees were behaving abnormal (affected and/or moribund) throughout the experimental time, but without a clear sign of a dose relationship.

Mortality and sublethal effects for the oral and contact studies are summarised below.

Table 1: Toxicity of GF-3307 to honey bees in an oral and contact toxicity test

Contact Test		Oral Test	
Dose Level µg/bee (nominal)	Mortality (% Mean) [72 hrs]	Dose Level µg/bee (measured based on consumption)	Mortality (% Mean) [72 hrs]
300.0	100.0	447.0	20.0
150.0	73.3	324.0	76.7
75.0	40.0	163.3	33.3
37.5	23.3	79.6	23.3
18.8	20.0	41.3	20.0
9.4	10.0	-	-
Water control	0.0	Water control	0.0

LD ₅₀ [µg/bee] at observation periods						
	Contact Test			Oral Test		
Dose Level [µg GF-3307/bee]	300, 150, 75, 37.5, 18.8 and 9.4			447.0, 324.0, 163.3, 79.6 and 41.3		
Observation Period	24 h	48 h	72 h	24 h	48 h	72 h
LD ₅₀ [µg GF-3307/bee]	121.2	96.5	92.3	300.3	224.0	212.5

The contact and oral LD₅₀ (24 h) values of the reference item (dimethoate) were calculated to be 0.21 and 0.14 µg a.i./bee, respectively.

Table 2: Sublethal effects of GF-3307 to honey bees in an oral and contact toxicity test

Treatment µg/bee				
Nominal	Consumed	Sublethal effects after 72 hrs (number of bees)		
		On back	Lethargic	Other
Contact:				
Control (0)	-	0	0	0
300.0	-	0	0	0
150.0	-	1	2	2
75.0	-	0	0	4
37.5	-	0	0	0
18.8	-	0	0	0
9.4	-	0	0	1
Oral:				
Control (0)	-	0	0	0
600.0	447.0	0	0	0
300.0	324.0	0	0	1
150.0	163.3	0	0	0
75.0	79.6	0	0	0
37.5	41.3	0	0	0

The contact test with the GF-3307 Blank formulation was prolonged for a further 24 hours up to 72 hours due to increasing mortality between 24/48 hours.

At the end of the contact toxicity test (72 hours after application), mortality ranged between 93.3 and 23.3 % following dosing of 300, 150, 75, 37.5 and 18.8 µg GF-3307 Blank/bee. The mortality in the control group (water + 0.5 % Adhäsit) was 0.0 % after 72 hours.

During the 4 and 24-hours assessment, behavioural abnormalities of some bees (e.g. moribund or affected bees and/or apathy) were observed in all GF-3307 Blank/bee dose groups. 48 hours after treatment only one bee in the 300.0 and 150.0 µg/bee dosing group was found to be moribund or affected, respectively. At the end of the test (72 hours following dosing) a few bees were behaving abnormal in these two highest dose groups. No further behavioural abnormalities occurred in the other GF-3307 Blank/bee groups.

In the oral test, the maximum nominal dose level of GF-3307 Blank (600 µg/bee) was not achieved, because the bees did not ingest the full volume of treated sugar solution even when offered over a period of 6 hours. Oral doses of 305.0 and 230.0 µg GF-3307 Blank/bee led to 40.0 and 26.7 % mortality at the end of the test (48 hours after application), respectively. No mortality occurred in the 161.3, 79.4 and 40.1 µg GF-3307 Blank/bee treatment groups. No mortality occurred in the control group (50 % w/v sucrose solution = 500 g sucrose/L tap water).

During the 4-hours assessment, behavioural abnormalities of some bees (e.g. moribund or affected bees) were observed in the 305.0 and 230.0 µg GF-3307 Blank/bee treatment groups. This was the only occurrence of treatment related behavioural abnormality during the trial.

Table 3: Toxicity of GF-3307 Blank to honey bees in an oral and contact toxicity test

Contact Test				Oral Test	
Dose Level µg Blank/bee (nominal)	Mortality (% Mean) [72 hrs]			Dose Level µg Blank/bee (measured based on consumption)	Mortality (% Mean) [48 hrs]
300.0	93.3			305.0	40.0
150.0	53.3			230.0	26.7
75.0	23.3			161.3	0.0
37.5	43.3			79.4	0.0
18.8	26.7			40.1	0.0
Water control	0.0			Water control	0.0
LD ₅₀ [µg Blank/bee] at observation periods					
	Contact Test			Oral Test	
Dose Level [µg Blank/bee]	300, 150, 75, 37.5 and 18.8			305.0, 230.0, 161.3, 79.4 and 40.1	
Observation Period	24 h	48 h	72 h	24 h	48 h
LD ₅₀ [µg Blank/bee]	199.6	157.6	138.9	> 305.0	> 305.0

The contact and oral LD₅₀ (24 h) values of the reference item (dimethoate) were calculated to be 0.17 and 0.13 µg a.i./bee, respectively.

Table 4: Sublethal effects of GF-3307 Blank to honey bees in an oral and contact toxicity test

Treatment µg/bee				
Nominal	Consumed	Sublethal effects after 72 hrs (number of bees)		
		On back	Lethargic	Other
Contact:				
Control (0)	-	0	0	0
300.0	-	1	0	0
150.0	-	0	0	3
75.0	-	0	0	0
37.5	-	0	0	0
18.8	-	0	0	0
Nominal	Consumed	Sublethal effects after 48 hrs (number of bees)		
		On back	Lethargic	Other
Oral:				
Control (0)	-	0	0	0
600.0	305.0	0	0	0
300.0	230.0	0	0	0
150.0	161.3	0	0	0
75.0	79.4	0	0	0
37.5	40.1	0	0	0

CONCLUSION

The acute contact and an oral toxicity of GF-3307 was tested in two sets of experiments.

In the first set, the toxicity of the product GF-3307 was assessed, whereas in the second test the intrinsic toxicity of the blank formulation components (without presence of the a.i. XDE-777 and prothioconazole) was investigated.

The contact toxicity of the product GF-3307 led to a 72 hour LD₅₀ value of 92.3 µg GF-3307/bee.

In the oral toxicity test with the product GF-3307 the LD₅₀ (72 h) value was 212.5 µg GF-3307/bee.

The contact toxicity of the Blank formulation of GF-3307 led to a LD₅₀ (72 h) value of 138.9 µg Blank/bee.

In the oral toxicity test with the Blank formulation the LD₅₀ (48 h) value was > 305.0 µg Blank/bee.

Comparison of the contact toxicity LD₅₀s of GF-3307 and GF-3307 blank suggest that the components of the formulation excluding the active ingredient contribute the majority of the toxicity observed in the GF-3307 test. In the oral toxicity tests, it appears that the components of the formulation contribute up to approximately half of the toxicity of GF-3307.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Honey bee	<i>Apis mellifera</i>	GF-3307	72 hr - contact	LD ₅₀	92.3	µg/bee
Honey bee	<i>Apis mellifera</i>	GF-3307	72 hr - oral	LD ₅₀	212.5	µg/bee
Honey bee	<i>Apis mellifera</i>	GF-3307 Blank	72 hr - contact	LD ₅₀	138.9	µg/bee
Honey bee	<i>Apis mellifera</i>	GF-3307 Blank	48 hr - oral	LD ₅₀	> 305.0	µg/bee

A 2.3.1.1.1.3 XDE-777 TGAI - Acute Oral and Contact Toxicity to Bumble Bees (*terrestris*) under Laboratory Conditions

Comments of zRMS:	The new active substance data for Bumble bees has not been evaluated in the current dossier by zRMS.
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Reference:	KCP 10.3.1.1.1/3
Report:	Cornement, M., Morgenthal, K. 2022. XDE-777 TGAI - Acute Oral and Contact Toxicity to Bumble Bees (<i>Bombus terrestris</i>) under Laboratory Conditions; Corteva Report No. 201076; IES; GLP, unpublished
Guideline(s):	OECD 2017
Deviations:	
GLP:	Yes
Acceptability:	
Duplication (if vertebrate study)	NA

COMPLIANCE

Guideline(s):	OECD, 2017. Test no. 247: Bumble bees, acute-oral toxicity. In OECD Guidelines for testing of chemicals and OECD, 2017. Test no. 246: Bumble bees, acute-contact toxicity. In OECD Guidelines for testing of chemicals
US EPA Guideline(s):	-
Guideline Deviations:	none
Dates of work:	October 06, 2020 to February 23, 2021
GLP status:	Yes
Number of pages in final report:	141

MATERIALS AND METHODS

Test Item(s)

Test item (common name):	XDE-777 TGAI
Purity:	82.8 % w/w of fenpicoxamid
Description (physical state):	Light orange solid
Lot/batch no.:	XDE-777-01-02 (TSN303159)

Test System

Organism (Species):	Bumble bee (<i>Bombus terrestris</i>)
Study type:	48-hour acute contact and oral toxicity test

Study design:	Assessment of survival and sublethal effects. 60 replicates per treatment in the contact test and 80 replicates per treatment in the oral test. 1 individual per replicate.
Age of test organism at initiation:	Adult worker bumble bees
Test doses (µg/bumble bee):	Contact: 0 (control), 0 (solvent control) and 300 µg a.i./bumble bee. Oral (nominal): 0 (control), 0 (solvent control) and 300 µg a.i./bumble bee. Oral (actual consumed): 0 (control), 0 (solvent control) and 272 µg a.i./bumble bee.
Information on bee colony:	The bumble bees used in the test were from healthy and queen-right colonies, obtained from a commercial bumblebee breeding company (Andermatt Biocontrol, Grossdietwil, Switzerland). The bumblebees were maintained in a clean cylindrical, latticed plastic cage.
Environmental conditions:	Temperature: Contact: 24.8 °C (range 23.7–25.3 °C) Oral: 24.9 °C (range 23.8–25.3 °C) Relative Humidity: Contact: 62.4 % (range 51.0–64.6 %) Oral: 62.5 % (range 53.4–63.8 %) Photoperiod: 24-hour darkness (except room lighting during treatment and observations). Feeding: 50 % w/v sucrose solution <i>ad libitum</i> given directly after treatment using glass vials.
Reference toxicant:	Dimethoate

Methodology

Oral Test: 80 replicates were established for the control, solvent control and test item treatment. 40 replicates were established for the reference item treatment. Each replicate consisted of one healthy, average-sized worker bumble bee. Before test start, bumble bees were transferred to Nicot cages, anesthetized with CO₂, weighed and acclimatized to test conditions for 8 to 24 hours. Afterwards, the bumble bees were starved for 2 hours, fed for 4 hours with 60 µL of sugar solution (50 % w/v) incorporating either the test or reference item at appropriate concentrations or no test item (control). At the end of the exposure period, the syringes with treated solution were replaced by 2 mL syringes with a shortened tip, filled with untreated sugar solution.

Contact Test: 60 replicates were established for the control, solvent control and test item treatment. 30 replicates were established for the reference item treatment. Each replicate consisted of one healthy, average-sized worker bumble bee. Before test start, bumble bees were transferred to Nicot cages and acclimatized to test conditions for 8 to 24 hours. Bumble bees were then anesthetized for approximately 5 seconds with CO₂, weighed and applied dorsally between the second and the third pairs of legs with 4 µL of solutions incorporating either the test or reference item at appropriate concentrations or no test item (control and solvent control).

The bumble bees remained in their individual cages with *ad libitum* access to untreated sugar solution until the end of the experiment. The mortality as well as sublethal effects (e.g. uncoordinated movements, moribund bees) were assessed at 4, 24 and 48 hours after dorsal application.

The concentrations of Fenpicoxamid as the active ingredients of the test item in test samples were determined by liquid chromatography coupled with positive ion electrospray tandem mass spectrometry (LC-MS/MS) using external standards calibration.

The NOED and LOED for mortality were determined by Fisher's exact binomial test (one-sided greater, $\alpha = 0.05$). A Fisher's exact binomial test (two-sided, $\alpha = 0.05$) was performed to test differences in mortality of the control and the solvent control. As no statistically significant mortality occurred in the test item treatments, the LD_x values were determined directly from the raw data.

RESULTS AND DISCUSSION

For the contact test, the 48 hours NOED and LOED values for mortality were determined to be ≥ 300 and > 300 $\mu\text{g a.i./bumble bee}$. The LD₁₀, LD₂₀ and LD₅₀ values at 48 hours were determined to be > 300 $\mu\text{g a.i./bumble bee}$.

For the oral test, the 48 hours NOED and LOED values for mortality when “non-feeders” are excluded were determined to be ≥ 272 and > 272 $\mu\text{g a.i./bumble bee}$, respectively. The LD₁₀, LD₂₀ and LD₅₀ values at 48 hours were determined to be > 272 $\mu\text{g a.i./bumble bee}$.

Study Validity

To demonstrate the validity of the study, the following conditions were fulfilled:

OECD Criteria	Required	Observed
Mean control mortality at the end of the test (Contact test)	$\leq 10\%$	0.0%
Mean solvent control mortality at the end of the test (Contact test)	$\leq 10\%$	0.0%
Mean control mortality at the end of the test (Oral test)	$\leq 10\%$	1.39%
Mean solvent control mortality at the end of the test (Oral test)	$\leq 10\%$	0.0%
Response to the reference toxicant (Contact test): Mean control mortality at the end of the test	$\geq 50\%$	100%
Response to the reference toxicant (Oral test): Mean control mortality at the end of the test	$\geq 50\%$	100%

Table 5: Analytical verification of treatment solution (Contact test)

Treatment $\mu\text{g a.i./bumble bee}$	% of nominal Fenpicoxamid
Control	<LOD
Solvent control	<LOD
300	99

LOD = 0.000698 mg a.i./L

Table 6: Analytical verification of treatment solution (Oral test)

Target dose $\mu\text{g a.i./bumble bee}$	Actual dose consumed $\mu\text{g a.i./bumble bee}$	% of nominal Fenpicoxamid
Control		<LOD
Solvent control		<LOD
300	272	96

LOD = 0.000698 mg a.i./L

Table 7: Mortality (Contact test)

Treatment $\mu\text{g a.i./bumble bee}$	Cumulative mortality			
	24-hour		48-hour	
	Mean No. dead	Mean %	Mean No. dead	Mean %
Control	0	0.0	0	0.0
Solvent control	0	0.0	0	0.0
300	1	1.67	1	1.67

Table 8: Mortality (Oral test – non-feeder excluded)

Treatment $\mu\text{g a.i./bumble bee}$	Actual dose consumed $\mu\text{g a.i./bumble bee}$	Cumulative mortality			
		24-hour		48-hour	
		Mean No. dead	Mean %	Mean No. dead	Mean %
Control		0	0.0	1	1.39
Solvent control		0	0.0	0	0.0
300	272	0	0.0	0	0.0

Table 9: *Sublethal effects (Contact test)*

Treatment µg a.i./bumble bee	Cumulative sublethal effects			
	24-hour		48-hour	
	Effects (n)	%	Effects (n)	%
Control	AN	0.0	AN	0.0
Solvent control	AN	0.0	AN	0.0
300	AN	0.0	AN	0.0

AN: All appeared normal; C: cramps; L: lethargic; M: moribund

Table 10: *Sublethal effects (Oral test)*

Treatment µg a.i./bumble bee	Actual dose consumed µg a.i./bumble bee	Cumulative sublethal effects			
		24-hour		48-hour	
		Effects (n)	%	Effects (n)	%
Control		AN	0.0	AN	0.0
Solvent control		AN	0.0	AN	0.0
300	272	AN	0.0	AN	0.0

AN: All appeared normal; C: cramps; L: lethargic; M: moribund

Table 11: *Effects of XDE-777 TGAI on the bumble bee, *Bombus terrestris**

Endpoint type		Endpoint value µg a.i./bumble bee	95% confidence limits µg a.i./bumble bee
48-h contact	NOED	≥ 300	N/A
	LOED, LD ₁₀ , LD ₂₀ and LD ₅₀	> 300	N/A
48-h oral (non-feeders excluded)	NOED	≥ 272	N/A
	LOED, LD ₁₀ , LD ₂₀ and LD ₅₀	> 272	N/A

N/A: Not applicable

CONCLUSION

For the contact test, the 48 hours NOED and LOED values for mortality were determined to be ≥ 300 and > 300 µg a.i./bumble bee. The LD₁₀, LD₂₀ and LD₅₀ values at 48 hours were determined to be > 300 µg a.i./bumble bee.

For the oral test, the 48 hours NOED and LOED values for mortality when “non-feeders” are excluded were determined to be ≥ 272 and > 272 µg a.i./bumble bee, respectively. The LD₁₀, LD₂₀ and LD₅₀ values at 48 hours were determined to be > 272 µg a.i./bumble bee.

Common name	Species	Test item	Exposure system	Time scale	Endpoint	Toxicity value	Units of test item
Bumble bee	<i>Bombus terrestris</i>	XDE-777 TGAI	Contact	48-hour	NOED	≥ 300	µg a.i./bee
Bumble bee	<i>Bombus terrestris</i>	XDE-777 TGAI	Contact	48-hour	LD ₅₀	> 300	µg a.i./bee
Bumble bee	<i>Bombus terrestris</i>	XDE-777 TGAI	Oral	48-hour	NOED	≥ 272	µg a.i./bee
Bumble bee	<i>Bombus terrestris</i>	XDE-777 TGAI	Oral	48-hour	LD ₅₀	> 272	µg a.i./bee

A 2.3.1.1.1.4 GF-3307 - Acute Oral and Contact Toxicity to Bumble Bees (*Bombus terrestris*) under Laboratory Conditions

Comments of zRMS:	The study was conducted in line with OECD 246 and 247 .
	All the validity criteria were met and the study is considered acceptable with the following

	<p>endpoint relevant for the risk assessment:</p> <p>contact 72h LD50 >500 µg GF-3307/bumble bee oral 72h LD50 > 453 µg GF-3307/bumble bee. The study was not used in the risk assessment.</p>
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Reference:	KCP 10.3.1.1.1/4
Report:	Cornement, M., Morgenthal, K. 2022. GF-3307 - Acute Oral and Contact Toxicity to Bumble Bees (<i>Bombus terrestris</i>) under Laboratory Conditions; Corteva Report No. 201076; IES; GLP, unpublished
Guideline(s):	OECD 2017
Deviations:	
GLP:	
Acceptability:	Acceptable
Duplication (if vertebrate study)	NA

COMPLIANCE

Guideline(s):	OECD. 2017. Test no. 247: Bumble bees, acute oral toxicity. In OECD Guidelines for testing of chemicals and OECD. 2017. Test no. 246: Bumble bees, acute contact toxicity. In OECD Guidelines for testing of chemicals
US EPA Guideline(s):	NA
Guideline Deviations:	none
Dates of work:	October 26, 2020 to April 24, 2021
GLP status:	Yes
Number of pages in final report:	193

MATERIALS AND METHODS

Test Item(s)

Test item (common name):	GF-3307
Purity:	9.7 % w/w of Prothioconazole and 4.7 % w/w of Fenpicoxamid
Description (physical state):	Brown liquid
Lot/batch no.:	MAR19CE01Q TSN400550

Test System

Organism (<i>Species</i>):	Bumble bee (<i>Bombus terrestris</i>)
Study type:	48-hour acute contact and oral toxicity test
Study design:	Assessment of survival and sublethal effects. 30 replicates per treatment in the contact test and 40 replicates per treatment in the oral test. 1 individual per replicate.
Age of test organism at initiation:	Adult worker bumble bees
Test doses (µg/bumble bee):	Contact: 0 (control), 21.3, 47.0, 103, 227 and 500 µg GF-3307/bumble bee. Oral (nominal): 0 (control), 21.3, 47.0, 103, 227 and 500 µg GF-3307/bumble bee.

	Oral (actual consumed): 0 (control), 21.1, 45.0, 99.4, 222 and 453 µg GF-3307/bumble bee.
Information on bee colony:	The bumblebees used in the test were from healthy and queen-right colonies, obtained from a commercial bumblebee breeding company (Andermatt Biocontrol, Grossdietwil, Switzerland). The bumblebees were maintained in a clean cylindrical, latticed plastic cage.
Environmental conditions:	Temperature: Contact: 24.4 °C (range 23.7 - 25.3 °C) Oral: 24.4 °C (range 23.7 - 25.3 °C) Relative Humidity: Contact: 62.4 % (range 54.1 - 64.4 %) Oral: 63.2 % (range 54.1 - 64.8 %) Photoperiod: 24-hour darkness (except room lighting during treatment and observations). Feeding: 50% w/v sucrose solution <i>ad libitum</i> given directly after treatment using 2 mL syringes.
Reference toxicant:	Dimethoate

Methodology

Oral Test: 40 replicates were established for the control, test item and reference item treatment. Each replicate consisted of one healthy, average sized worker bumble bee. Before test start, bumble bees were transferred to Nicot cages, anesthetized with CO₂, weighed and acclimatized to test conditions for 8 to 24 hours. Afterwards, the bumble bees were starved for 2 hours, fed for 4 hours with 40 µL of sugar solution (50 % w/v) incorporating either the test or reference item at appropriate concentrations or no test item (control). At the end of the exposure period, the syringes with treated solution were replaced by 2 mL syringes with a shortened tip, filled with untreated sugar solution.

Contact Test: 30 replicates were established for the control, test item and reference item treatment. Each replicate consisted of one healthy, average sized worker bumble bee. Each replicate consisted of one healthy, average sized worker bumble bee. Before test start, bumble bees were transferred to Nicot cages and acclimatized to test conditions for 8 to 24 hours. Bumble bees were then anesthetized for approximately 5 seconds with CO₂, weighed and applied dorsally between the second and the third pairs of legs with 2 µL of solutions incorporating either the test or reference item at appropriate concentrations or no test item (control and Etalfix pro control).

The bumble bees remained in their individual cages with *ad libitum* access to untreated sugar solution until the end of the experiment. The mortality as well as sublethal effects (e.g. uncoordinated movements, moribund bees) were assessed at 4, 24 and 48 hours after dorsal application.

The concentrations of Prothioconazole and Fenpicoxamid as the active ingredients of the test item GF-3307 in test samples were determined by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS) using external standards calibration.

The NOED for mortality was determined by Fisher's exact binomial test with Bonferroni correction ($\alpha = 0.05$, one-sided greater). In the contact test a Fisher's exact binomial test (two-sided, $\alpha = 0.05$) was performed to test differences in mortality of the control and the solvent control. As no statistically significant mortality occurred in the test item treatments, the LD_x values were determined directly from the raw data.

RESULTS AND DISCUSSION

For the contact test, the 48 hours NOED and LOED values for mortality were determined to be ≥ 500 and > 500 µg GF-3307/bumble bee. The LD₁₀, LD₂₀ and LD₅₀ values at 48 hours were determined to be > 500 µg GF-3307/bumble bee.

For the oral test, the 48 hours NOED and LOED values for mortality when "non-feeders" are excluded were determined to be ≥ 453 and > 453 µg GF-3307/bumble bee, respectively. The LD₁₀, LD₂₀ and LD₅₀ values at 48 hours were determined to be > 453 µg GF-3307/bumble bee.

Study Validity

To demonstrate the validity of the study, the following conditions were fulfilled:

OECD Criteria	Required	Observed
Mean control mortality at the end of the test (Contact test)	≤ 10 %	0.0 %
Mean control mortality at the end of the test (Oral test)	≤ 10 %	0.0 %
Response to the reference toxicant (Contact test): Mean control mortality at the end of the test	≥ 50 %	96.7 %
Response to the reference toxicant (Oral test): Mean control mortality at the end of the test	≥ 50 %	100 %

Table 12: *Analytical verification of treatment solution (Contact test)*

Treatment µg GF-3307/bumble bee	% of nominal	
	Prothioconazole	Fenpicoxamid
Control	<LOD	<LOD
21.3	62/53	62/53
47.0	103	86
103	105	88
227	100	90
500	89	84

LOD Prothioconazole = 0.0061 mg a.i./L

LOD Fenpicoxamid = 0.0030 mg a.i./L

Table 13: *Analytical verification of treatment solution (Oral test)*

Target dose µg GF-3307/bumble bee	Actual dose consumed µg GF-3307/bumble bee	% of nominal	
		Prothioconazole	Prothioconazole
Control		<LOD	<LOD
21.3	21.1	89	82
47.0	45.0	92	87
103	99.4	91	85
227	222	100	87
500	453	97	84

LOD Prothioconazole = 0.0061 mg a.i./L

LOD Fenpicoxamid = 0.0030 mg a.i./L

Table 14: *Mortality (Contact test)*

Treatment µg GF-3307/ bumble bee	Cumulative mortality			
	24-hour		48-hour	
	Mean No. dead	Mean %	Mean No. dead	Mean %
Control	0	0.0	0	0.0
21.3	0	0.0	0	0.0
47.0	0	0.0	0	0.0
103	0	0.0	0	0.0
227	1	3.33	1	3.33
500	0	0.0	0	0.0

Table 15: *Mortality (Oral test – non-feeder excluded)*

Treatment µg GF-3307/bumble bee	Actual dose consumed µg GF-3307/bumble bee	Cumulative mortality			
		24-hour		48-hour	
		Mean No. dead	Mean %	Mean No. dead	Mean %
Control		0	0.0	0	0.0
21.3	21.1	0	0.0	0	0.0
47.0	45.0	0	0.0	0	0.0
103	99.4	0	0.0	0	0.0
227	222	0	0.0	0	0.0
500	453	0	0.0	0	0.0

Table 16: *Sublethal effects (Contact test)*

Treatment µg GF-3307/bumble bee	Cumulative sublethal effects			
	24-hour		48-hour	
	Effects (n)	%	Effects (n)	%
Control	AN	0.0	AN	0.0
21.3	AN	0.0	AN	0.0
47.0	AN	0.0	AN	0.0
103	AN	0.0	AN	0.0
227	AN	0.0	AN	0.0
500	AN	0.0	AN	0.0

AN: All appeared normal; C: cramps; L: lethargic; M: moribund

Table 17: *Sublethal effects (Oral test)*

Treatment µg GF-3307/ bumble bee	Actual dose consumed µg GF-3307/ bumble bee	Cumulative sublethal effects			
		24-hour		48-hour	
		Effects (n)	%	Effects (n)	%
Control		AN	0.0	AN	0.0
21.3	21.1	AN	0.0	AN	0.0
47.0	45.0	AN	0.0	AN	0.0
103	99.4	AN	0.0	AN	0.0
227	222	AN	0.0	AN	0.0
500	453	AN	0.0	AN	0.0

AN: All appeared normal; C: cramps; L: lethargic; M: moribund

Table 18: *Effects of GF-3307 on the bumble bee, Bombus terrestris*

Endpoint type		Endpoint value µg GF-3307/bumble bee	95% confidence limits µg GF-3307/bumble bee
48-h contact	NOED	≥ 500	N/A
	LOED, LD ₁₀ , LD ₂₀ and LD ₅₀	> 500	N/A
48-h oral	NOED	≥ 453	N/A
	LOED, LD ₁₀ , LD ₂₀ and LD ₅₀	> 453	N/A

N/A: Not applicable

CONCLUSION

For the contact test, the 48 hours NOED and LOED values for mortality were determined to be ≥ 500 and > 500 µg GF-3307/bumble bee. The LD₁₀, LD₂₀ and LD₅₀ values at 48 hours were determined to be > 500 µg GF-3307/bumble bee.

For the oral test, the 48 hours NOED and LOED values for mortality when “non-feeders” are excluded were determined to be ≥ 453 and > 453 µg GF-3307/bumble bee, respectively. The LD₁₀, LD₂₀ and LD₅₀ values at 48 hours were determined to be > 453 µg GF-3307/bumble bee.

Common name	Species	Test item	Exposure system	Time scale	Endpoint	Toxicity value	Units of test item
Bumble bee	<i>Bombus terrestris</i>	GF-3307	Contact	48-hour	NOED	≥ 500	µg/bee
Bumble bee	<i>Bombus terrestris</i>	GF-3307	Contact	48-hour	LD ₅₀	> 500	µg/bee
Bumble bee	<i>Bombus terrestris</i>	GF-3307	Oral	48-hour	NOED	≥ 453	µg/bee
Bumble bee	<i>Bombus terrestris</i>	GF-3307	Oral	48-hour	LD ₅₀	> 453	µg/bee

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

A 2.3.1.1.2.1 Study 1 - GF-3307: Acute contact and oral effects on Honeybees (*Apis mellifera* L.) in the laboratory

Comments of zRMS:	Please see the zRMS opinion for the reference KCP 10.3.1.1.1/2 and KCP 10.3.1.1.2/1 above under acute oral toxicity to bees.
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Reference:	KCP 10.3.1.1.1/2; KCP 10.3.1.1.2/1
Report:	Schmitzer, S; 2014; GF-3307: Acute contact and oral effects on Honeybees (<i>Apis mellifera</i> L.) in the laboratory.; Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany ; Lab Study No. 90541035; DAS Study No. 140220 & 140213; 16 December 2014; Unpublished.
Guideline(s):	OECD 213 and OECD 214
Deviations:	Minor (see the commenting box under acute oral toxicity to bees)
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	NA

See Schmitzer, S. (2014) under acute oral toxicity to bees

A 2.3.1.1.2.2 Study 2 - GF-3307: A laboratory study to determine the acute contact toxicity on the honey bees *Apis mellifera* L. (Hymenoptera: Apidae)

Comments of zRMS:	<p>The study was conducted in line with OECD 214 with a minor deviation.</p> <p>It was noted that there was one intentional guideline deviation: in order to cover the range of the expected toxicity, actual spacing factor was 2.5 instead of 2.2 as required by OECD 213. Since the validity criteria were met and the statistical analysis allowed for determination of the 95% confidence intervals for LD₅₀ (which was covered by the range of tested doses) in zRMS opinion this deviation is considered to have no impact on the outcome of the study.</p> <p>It was also noted that 0.5 g adjuvant/L of the non-ionic surfactant AGRAL MAXX, non-toxic for honey bees, were added to treatments T202 to T221 (samples with the formulated product without the active substances, samples with the tested product, samples with only prothioconazole and samples with only fenpicoxamid) and R227 to R229 (reference item) in order to enable the application of the solution on the honey bee thorax. Treatments T222 to T226 (samples with technical prothioconazole and fenpicoxamid) were assessed with acetone as the control. However, the results for all treatments were corrected for the respective controls.</p>
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	<p>All the validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>LD₅₀ = 199.9 µg product/bee</p>
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Reference:	KCP 10.3.1.1.2/2
Report:	Noël, E.; 2015; GF-3307: A laboratory study to determine the acute contact toxicity on the honey bees <i>Apis mellifera</i> L. (Hymenoptera: Apidae); SynTech Research France S.A.S., La Chapelle de Guinchay, France; Lab Study No. 014SRFR14C07; DAS Study No. 150737; 21 September 2015; Unpublished
Guideline(s):	OECD No. 214 (1998)
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable Yes
Duplication (if vertebrate study)	NA

COMPLIANCE

Guideline(s):	OECD No. 214 (1998)
US EPA Guideline(s):	Not Applicable
Guideline Deviations:	There was one intentional guideline deviation: in order to cover the range of the expected toxicity, actual spacing factor was 2.5 instead of 2.2 as required by the OECD guideline 214.
Dates of work:	22 July 2015 to 24 July 2015
GLP status:	Yes
Number of pages in final report:	93

MATERIALS AND METHODS

Test Item(s)

ISO Common name:	Not applicable
Test item (chemical/other name):	GF-3307; GF-3307 BLANK; GF-3307 (prothioconazole only); GF-3307 (XDE-777 only); prothioconazole; XDE-777
Purity:	GF-3307: 9.4% w/w prothioconazole + 4.8% w/w XDE-777 GF-3307 BLANK: Not applicable GF-3307 (prothioconazole only): 9.9% w/w prothioconazole GF-3307 (XDE-777 only): 5.2% w/w XDE-777 Prothioconazole: 98.0 % w/w XDE-777: 84.2% w/w
Description (physical state):	GF-3307, GF-3307 BLANK, GF-3307 (prothioconazole only), GF-3307 (XDE-777 only): Brown liquid Prothioconazole: White crystalline XDE-777 : White solid
Lot/batch no.:	GF-3307: ENBK-147245-050 GF-3307 BLANK:ENBK-144192-041 GF-3307 (prothioconazole only):ENBK-148646-019-P GF-3307 (XDE-777 only): ENBK-148646-019-T7 Prothioconazole: FL018728*1.0 XDE-777: XDE-777-01-02
CAS no.:	XDE-777: 517875-34-2 Prothioconazole: 178928-70-6

Test System

Organism (<i>Species</i>):	Honey bee (<i>Apis mellifera</i> L.)
Study type:	Laboratory study
Study design:	Acute contact toxicity test; duration 48 hrs; 3 replicates, each consisting of 10 bees in one cage per test concentration; assessment of mortality after 4, 24 and 48 hrs
Test concentrations:	GF-3307 BLANK: 6.60, 16.50, 41.20, 103.0 and 257.4 µg product/bee GF-3307: 7.70, 19.20, 48.0, 120.0 and 300.0 µg product/bee GF-3307 (prothioconazole only): 7.30, 18.30, 45.7, 114.3 and 285.6 µg product/bee GF-3307 (XDE-777 only): 7.0, 17.4, 43.50, 108.8 and 271.8 µg product/bee- Prothioconazole: 0.70, 1.80, 4.50, 11.30 and 28.20 µg a.s./bee + XDE-777 at 0.40, 0.90, 2.30, 5.80 and 14.4 µg a.s./bee
Information on bee colony (health etc):	The bees used in the test were from a single, disease-free colony. The hive had not been treated for varroa mites or for disease during the year of the study. The bees were maintained in a clean holding cage.
Amount of treated diet consumed:	
Feeding method:	50% w/v sucrose solution <i>ad libitum</i> ; was given directly after treatment using syringes.
Environmental conditions:	Temperature: 23.4 - 26.7 °C Relative Humidity: 56 - 70 % Photoperiod: organisms were kept in the dark during the study (red light was used for observations).
Reference substance:	0.10, 0.20 and 0.30 µg dimethoate / bee
Solvent substance (if applicable):	Acetone to dissolve the mix Prothioconazole + XDE-777
Adjuvant:	Agral Maxx (0.5 g/L) to apply GF-3307, GF-3307 BLANK, GF-3307 (XDE-777 only), GF-3307 (prothioconazole only).

Methodology

GF-3307, GF-3307 BLANK, GF-3307 (XDE-777 only), GF-3307 (prothioconazole only) and prothioconazole + XDE-777 were dissolved in distilled water + adjuvant or in acetone (prothioconazole + XDE-777). 1 µL solution containing tested item at the suitable dose was applied on the dorsal side of the thorax of each honey bee.

- GF-3307 BLANK at 6.60, 16.50, 41.20, 103.0 and 257.4 µg product/bee
- GF-3307 at 7.70, 19.20, 48.0, 120.0 and 300.0 µg product/bee
- GF-3307 (prothioconazole only) at 7.30, 18.30, 45.7, 114.3 and 285.6 µg product/bee
- GF-3307 (XDE-777 only) at 7.0, 17.4, 43.50, 108.8 and 271.8 µg product/bee-
- Prothioconazole at 0.70, 1.80, 4.50, 11.30 and 28.20 µg a.s./bee + XDE-777 at 0.40, 0.90, 2.30, 5.80 and 14.4 µg a.s./bee

A toxic reference item (400 g/L dimethoate) was applied at 3 doses to demonstrate the relative susceptibility of the test organisms and the sensitivity of the test system. Test included doses between 0.10-0.30 µg a.s./bee. Distilled water control was included to assess the natural mortality of the test organisms. Direct treatment effects (mortality and other observed biological effects) were assessed after 4, 24 and 48 hours. Mortality data were analysed for significant differences compared to the adjuvant control (GF-3307, GF-3307 BLANK, GF-3307 (XDE-777 only), GF-3307 (prothioconazole only)) or acetone control (prothioconazole + XDE-777) control group using ANOVA plus Dunnett's ($p \leq 0.05$), after logarithmic transformation of the replicate mean data (n+1).

RESULTS AND DISCUSSION

Contact test has been carried out to evaluate potential adverse effects of GF-3307, GF-3307 BLANK, GF-3307 (XDE-777 only), GF-3307 (prothioconazole only) and prothioconazole + XDE-777 on the survival of honey bees *Apis mellifera* L. (Hymenoptera: Apidae) in laboratory conditions.

The study was valid since average mortalities did not exceed 10% in the untreated sucrose solution control (actual value: 3.333 %) and 24-hour toxic reference LD50 was in the range of 0.10 - 0.30 µg a.s./bee (actual value: 0.190 µg a.s./bee; 95% confidence limits: 0.068 - 0.227 µg a.s. /bee).

Table 1: Summary of *Apis mellifera* contact toxicity data – GF-3307 BLANK

Treatment µg GF-3307 BLANK /bee		Contact
Nominal	Actual	Mortality (%)
		48-hr
Control (0)	Not applicable	3.333
Acetone control	Not applicable	6.667
Adjuvant control	Not applicable	3.333
6.60	6.60	0.0
16.50	16.50	3.448
41.20	41.20	6.897
103.0	103.0	13.79
257.4	257.4	17.24
Contact 48-hr LD50		> 257.4
Contact LD ₅₀ (24-hr) value of the reference item: 0.190 µg dimethoate/bee		

Table 2: Summary of *Apis mellifera* contact toxicity data – GF-3307

Treatment µg GF-3307 /bee		Contact
Nominal	Actual	Mortality (%)
		48-hr
Control (0)	Not applicable	3.333
Acetone control	Not applicable	6.667
Adjuvant control	Not applicable	3.333
7.70	7.70	6.867
19.2	19.2	3.448
48.0	48.0	-3.448
120	120	10.34
300	300	86.21*
Contact 48-hr LD50		199.9
Contact LD ₅₀ (24-hr) value of the reference item: 0.190 µg dimethoate/bee		

* Significantly different from the adjuvant control

Table 3: Summary of *Apis mellifera* contact toxicity data – GF-3307 (prothioconazole only)

Treatment µg GF-3307 (prothioconazole only)/bee		Contact
Nominal	Actual	Mortality (%)
		48-hr
Control (0)	Not applicable	3.333
Acetone control	Not applicable	6.667
Adjuvant control	Not applicable	3.333
7.30	7.30	-3.44
18.30	18.30	3.448
45.70	45.70	6.897
114.3	114.3	10.34
285.6	285.6	13.79
Contact 48-hr LD ₅₀		> 285.6
Contact LD ₅₀ (24-hr) value of the reference item: 0.190 µg dimethoate/bee		

* Significantly different from the adjuvant control

Table 4: Summary of *Apis mellifera* contact toxicity data – GF-3307 (XDE-777 Only)

Treatment µg GF-3307 (XDE-777 only)/bee		Contact
Nominal	Actual	Mortality (%) 48-hr
Control (0)	Not applicable	3.333
Acetone control	Not applicable	6.667
Adjuvant control	Not applicable	3.333
7.0	7.0	3.448
17.40	17.40	3.448
43.50	43.50	13.79
108.8	108.8	31.03*
271.8	271.8	41.38*
Contact 48-hr LD50		> 271.8
Contact LD ₅₀ (24-hr) value of the reference item: 0.190 µg dimethoate/bee		

* Significantly different from the adjuvant control

Table 5: Summary of *Apis mellifera* contact toxicity data – Prothioconazole + XDE 777

Treatment µg Prothioconazole + XDE 777/bee		Contact
Nominal	Actual	Mortality (%) 48-hr
Control (0)	Not applicable	3.333
Acetone control	Not applicable	6.667
Adjuvant control	Not applicable	3.333
0.40 µg a.s XDE-777/bee + 0.70 µg a.s Prothioconazole/bee	0.40 µg a.s XDE-777/bee + 0.70 µg a.s Prothioconazole/bee	-7.143
0.90 µg a.s XDE-777/bee + 1.80 µg a.s Prothioconazole/bee	0.90 µg a.s XDE-777/bee + 1.80 µg a.s Prothioconazole/bee	0.0
2.30 µg a.s XDE-777/bee + 4.50 µg a.s Prothioconazole/bee	2.30 µg a.s XDE-777/bee + 4.50 µg a.s Prothioconazole/bee	3.571
5.80 µg a.s XDE-777/bee + 11.30 µg a.s Prothioconazole/bee	5.80 µg a.s XDE-777/bee + 11.30 µg a.s Prothioconazole/bee	17.86
14.40 µg a.s XDE-777/bee + 28.20 µg a.s Prothioconazole/bee	14.40 µg a.s XDE-777/bee + 28.20 µg a.s Prothioconazole/bee	57.14*
Contact 48-hr LD50		XDE-777: 12.18 + Prothioconazole: 23.84
Contact LD ₅₀ (24-hr) value of the reference item: 0.190 µg dimethoate/bee		

* Significantly different from the acetone control

CONCLUSION

The study was valid since average mortalities did not exceed 10% in the distilled water control (actual value: 3.333 %) and 24-hour toxic reference LD50 was in the range of 0.10 - 0.30 µg a.s./bee (actual value: 0.190 µg a.s./bee; 95% confidence limits: 0.068 - 0.227 µg a.s. /bee).

Table 6: Summary of *Apis mellifera* contact toxicity data for all test items tested

Test organism / Exposure	Honey bee / Contact		
Assessment	Mortality after 48 hours [%]*		
	NOED	LOED	LD50 (95% confidence limits)
GF-3307 (µg f.p./bee)	120	300	199.9 (158.9 - 239.7)
GF-3307 BLANK (µg f.p./bee)	257.4	> 257.4	> 257.4
GF-3307 (prothioconazole only) (µg f.p./bee)	285.6	> 285.6	> 285.6

GF-3307 (XDE-777 only) (µg f.p./bee)	43.50	108.8	> 271.8 (estimated: 367.7; 185.4 – 3035)
Prothioconazole + XDE-777 (µg a.s./bee)	XDE-777: 5.80 + Prothioconazole: 11.30	XDE-777: 14.40 + Prothioconazole: 28.20	XDE-777: 12.18 (8.530 – 23.49) + Prothioconazole: 23.84 (16.65 - 46.08)

* Based on the number of moribund and dead organisms; f.p.= formulated product; a.s.= active substance.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Honey bee	<i>Apis mellifera</i>	GF-3307	48-hr – Contact	LD50	199.9	µg/bee
Honey bee	<i>Apis mellifera</i>	GF-3307 BLANK	48-hr – Contact	LD50	> 257.4	µg/bee
Honey bee	<i>Apis mellifera</i>	GF-3307 (prothioconazole only)	48-hr – Contact	LD50	> 285.6	µg/bee
Honey bee	<i>Apis mellifera</i>	GF-3307 (XDE-777 only)	48-hr – Contact	LD50	> 271.8	µg/bee
Honey bee	<i>Apis mellifera</i>	Prothioconazole + XDE-777	48-hr – Contact	LD50	23.84 + 12.18	µg/bee

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

A 2.3.1.2.1 Study 1 - GF-3307 - Honey Bee (*Apis mellifera* L.) 22 Day Larval Toxicity Test (Repeated Exposure)

Comments of zRMS:	<p>The study was conducted in line with OECD 239 with minor deviations.</p> <p>It was noted that there were deviations > 2 hours from the recommended humidity ranges of 50-80% on day 15 and 19 (min. 34.1%). Other deviations in relative humidity and temperature were < 2 hours. It was also noted that for the toxic reference item groups only mortality was assessed and no other observations were conducted. No emergence boxes were used as from day 15 to enable the assignment of each emerged bee to the respective replicate. However, in zRMS opinion all these deviations are considered to have no impact on the outcome of the study since the validity criteria were met.</p> <p>It is noted that GF-3307 contains two active substances and in line with the requirements of the Central Zone the test concentrations of both substances should be verified in the respective chemical analyses or, as a minimum, the least stable active compound should be analysed. However, in the study only the concentration of fenpicoxamid were measured and no analyses of prothioconazole were performed. No explanation or justification of the substance selected for the analytical measurements was provided in the study report. However, based on the information from the area of environmental fate and behavior of both active substances it can be concluded that fenpicoxamid is the least stable substance. In the present study the measured concentrations of fenpicoxamid in the test item stock solutions and test item solutions were between 81 and 106 % of nominal. The measured concentrations of fenpicoxamid in the test item in the treated larval diet were within ± 20 % of nominal. However, in line with the recommendations in Appendix J EFSA Supporting publication 2019:EN-1673, the contribution of each active substance to the toxicity of the product to bees should be calculated in order to confirm that fenpicoxamid is the driver of the toxicity (contribution of > 90 %) to bees.</p> <p>For this reason the Applicant was requested to provide justification for selection of fenpicoxamid for chemical analyses, especially prothioconazole is also rapidly degraded in water and it thus expected to dissipate rapidly in the aqueous solutions used for preparation of the larvae diet.</p> <p>The following justification was provided by the Applicant and was considered acceptable by zRMS:</p> <p>Both fenpicoxamid and prothioconazole are unstable in water with a DT50 of 0.7 days (EFSA, 2018) and 0.8 – 1.0 days (EFSA, 2007), respectively. For chronic toxicity to honey bee larvae, fenpicoxamid is the toxic driver. In a 22 day larval toxicity test with GF-3308 (50g fenpicoxamid/L EC), adult emergence was reduced significantly and a</p>
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	<p>NOEDemergence of 0.437 µg fempicoxamid/larva was defined. For prothioconazole, no 22 day larval toxicity test but only a bee brood feeding test is available. In this feeding test, no adverse effects were observed at the highest tested concentration tested which was 0.47 g prothioconazole/L. Assuming consumption of 198 mg nectar per larva (Rortais et al., 2005), this would be roughly 90 µg prothioconazole/larva. Since GF-3307 contains 50g fempicoxamid/L and 100g prothioconazole/L, it is obvious that fempicoxamid is the toxic driver, given the toxicity of fempicoxamid and prothioconazole to honey bee larvae. Therefore, it is sufficient to determine the concentration of fempicoxamid in the larval diet.</p>				
	Species	Substance	Exposure System	Results	Reference
	<i>Apis mellifera</i>	GF-3308 (50 g/L fempicoxamid EC)	22 d, larval (OECD 239)	<p>NOEC = 55.6 µg prep/kg-diet period (2.84 µg a.s./kg-diet, based on 5.1% fempicoxamid)</p> <p>NOEDemergence = 8.56 µg prep/larva (0.437 µg a.s./larva, based on 5.1% fempicoxamid)</p>	Verge/2020/2018/DAS# 190305
	<i>Apis mellifera</i>	Prothioconazole SC 480 (formulation)	Bee brood feeding test (to Oomen et al.)	No adverse effects of brood development, mortality and behaviour after feeding honeybee colonies sugar syrup at 0.47 g prothioconazole/L	Schmitzer & Ehmke (2014) KCA 8.3.1.3/01, M-478670-01-1
<p>As it can be confirmed that fempicoxamid is the driver of the toxicity to bees, then, since the measured concentrations of fempicoxamid in the present study were within 80-120% of nominal, the zRMS is of the opinion that the endpoints can be based on nominal concentrations as provided in the study report.</p> <p>EC₅₀ = 190 mg product/kg food NOEC = 43 mg product/kg food</p> <p>ED₅₀ = 28.1 µg product/larvae NOED = 6.62 µg product/larvae</p>					

Reference:	KCP 10.3.1.2/1
Report:	Oberrauch, S.; 2018; GF-3307 - Honey Bee (<i>Apis mellifera</i> L.) 22 Day Larval Toxicity Test (Repeated Exposure); Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH, Eutinger Str. 24, D-75223 Niefern-Öschelbronn, Germany; Lab Study No. S17-04700; DAS Study No. 171043; 15 Jan 2018; Unpublished
Guideline(s):	OECD Guidance Document 239 on Honey bee (<i>Apis mellifera</i>) Larval Toxicity Test, Repeated Exposure (2016)
Deviations:	Minor (see the commenting box above)
GLP:	Yes

Acceptability:	Acceptable Yes
Duplication (if vertebrate study)	NA

COMPLIANCE

Guideline(s):	OECD Guidance Document 239 on Honey bee (<i>Apis mellifera</i>) Larval Toxicity Test, Repeated Exposure (2016)
US EPA Guideline(s):	None
Deviations:	For the toxic reference item groups mortality but no other observations were assessed. No emergence boxes were used as from day 15 to enable the assignment of each emerged bee to the respective replicate.
Dates of work:	10 July 2017 to 12 Oct 2017
GLP status:	Yes
Number of pages in final report:	107

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-3307
Content of a.i., analysed:	1) Fenpicoxamid: 4.7 % w/w, 48 g/L 2) Prothioconazole: 9.3 % w/w, 96 g/L
Description (physical state):	liquid / brown
Lot/batch no.:	ENBK-169144-026 (TSN314279)

Test System

Organism (<i>Species</i>):	Honey bee (<i>Apis mellifera</i>)
Study type:	Chronic Larval – repeated exposure
Study design:	Dose-response test; duration 22 days; 3 or more replicates, each starting with at least 12 synchronised 1 st instar larvae per concentration per replicate; assessment of mortality and behavioural effects daily after administration of the test item on days 3, 4, 5, and 6 and additionally on days 7, 8, 15 and 22. Visual assessment of uneaten food on day 8 prior to transfer of plate 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), 17.2, 43.0, 108, 269 and 672 mg product/kg diet equivalent to 2.65, 6.62, 16.6, 41.4 and 66.5 µg product/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:

Samples of the test item stock solution, the test item solutions and of the respective solvent were taken directly after preparation. Samples of the test item treated larval diet of each test item group (T1-T5) and of the larval diet of the control group (C) were taken directly from the prepared diet. The test item stock solution was prepared freshly at each application day. The larval diet was prepared freshly in advance and divided into aliquots using a multi stepper pipette. The aliquots were subsequently stored deep-frozen ($\leq -18\text{ }^{\circ}\text{C}$, with deviations $> -18\text{ }^{\circ}\text{C}$ of maximum 7h 20 min and deviations above $0\text{ }^{\circ}\text{C}$ of maximum 35 min) until use. On each feeding day the required amount of diet was warmed in the incubator before feeding. Fenpicoxamid was analysed in the test item solutions and solvent 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 ± 1 hours after preparation) of the test item in the larval diet.

The analytical verification of Fenpicoxamid resulted in recoveries of 81 to 106 % (solutions) and 88 to 110 % (diet) of the nominal values. Thus, the concentration of the test item in the larval diet was confirmed. The concentrations of Fenpicoxamid in the homogeneity samples taken from the top and bottom of the treated diet of the highest and lowest test item group were equivalent to recoveries of 90 to 111 %. The measured recovery rate of Fenpicoxamid in the aged larval diet was 47 %.

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: 33.2 - 35.2 $^{\circ}\text{C}$; mean 34.2-34.8 $^{\circ}\text{C}$

Relative Humidity:

66.8 - 100.0 %; mean 99-99.5% (day 1 to day 8),

45.7 - 85.4 %; mean 82.4% (day 8 to day 15),

34.1 - 75.0 %; mean 63.5% (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-3307 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 the cumulative mortality was 2.1 % in the control, 6.3 % in the solvent control, 95.8 % in the dimethoate reference item group and 8.3 % in the fenoxycarb reference item group. On day 22, the adult emergence rate in the control and solvent control group was 85.4 and 81.3 %, 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 T3 with a corresponding concentration of 108 mg product/kg diet (Cochran-Armitage test, one sided greater, $\alpha = 0.05$). Therefore, the NOEC for adult emergence on day 22 was determined as 43.0 mg product/kg diet, equivalent to a NOED of 6.62 μg product/larva per developmental period.

Table 1: Toxicity of GF-3307 to honey bee larvae in a chronic exposure toxicity test

Nominal Treatment		Chronic larval exposure toxicity		
mg product/kg diet	μg product/larva per developmental period	Mortality (%) (Corrected Mortality (%))		Emergence (%)
		Day 8	Day 15	Day 22
Control (0)		2.1 (n.a.)	12.5 (n.a.)	85.4
Solvent Control (0)		6.3 (n.a.)	16.7 (n.a.)	81.3
17.2	2.65	4.2 (2.1)	8.3 (-4.8)	87.5
43.0	6.62	2.1 (0.0)	8.3 (-4.8)	91.7
108	16.6	16.7* (14.9)	29.2* (19.1)	70.8*
269	41.4	52.1* (51.1)	75.0* (71.4)	25.0*
672	66.5	100.0* (100.0)	100.0* (100.0)	0.0*
Reference item (7.39 μg dimethoate/larva per developmental period, nominal)		95.8 (95.5)	---	---
Reference item (0.0493 μg fenoxycarb/larva per developmental period, nominal)		8.3 (6.3)	31.3 (17.5)	0.0
22-day NOED, nominal treatment		6.62 μg product/larva per developmental period, equivalent to 43.0 mg product/kg diet		

* Significantly different compared to control (Cochran-Armitage test, one sided greater, $\alpha = 0.05$)

n.a. not applicable

Table 2: Uneaten food, developmental and behavioural effects in the chronic exposure larval toxicity test for GF-3307

Nominal Treatment		Chronic larval exposure toxicity		
mg product/kg diet	µg product/larva per developmental period	Uneaten food observed on day 8	Behavioural effects (day)	Developmental effects (day)
Control (0)		yes	none	none
Solvent Control (0)		yes	none	none
17.2	2.65	yes	none	none
43.0	6.62	yes	none	none
108	16.6	yes	none	none
269	41.4	yes	none	none
672	66.5	--- ^a	none	none
Reference item (7.39 µg dimethoate/larva per developmental period)		yes	none	none
Reference item (0.0493 µg fenoxycarb/larva per developmental period)		yes	none	none

^a All larvae were dead on D8

CONCLUSION

In a repeated exposure larval toxicity test with GF-3307 and a duration of 22 days, the NOEC for adult emergence was determined as 43.0 mg product/kg diet, equivalent to a NOED of 6.62 µg product/larva per developmental period.

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-3307	22 day	NOEC	43.0	µg product/larva per developmental period
Honey bee	<i>Apis mellifera</i>	GF-3307	22 day	NOED	6.62	mg product/kg diet

A 2.3.1.2.2 Study 2 - GF-3307: Assessment of Effects on the Adult Honey Bee, *Apis mellifera* L, in a 10 Day Chronic Feeding Test under Laboratory Conditions

Comments of zRMS:	<p>The study was conducted in line with the OECD (2016): Proposal for a new guideline for the testing of chemicals: Honey bee (<i>Apis mellifera</i> L.), chronic oral toxicity test 10 day feeding test in the laboratory, October 2016 with no deviations but was evaluated according to OECD 245 (2017) HONEY BEE (<i>APIS MELLIFERA</i> L.), CHRONIC ORAL TOXICITY TEST (10-DAY FEEDING).</p> <p>All the validity criteria were met.</p> <p>It is noted that GF-3307 contains two active substances and in line with the requirements of the Central Zone the test concentrations of both substances should be verified in the respective chemical analyses or, as a minimum, the least stable active compound should be analysed. However, in the study only the concentrations of fenpicoxamid were measured and no analyses of prothioconazole were performed. No explanation or justification of the substance selected for the analytical measurements was provided in the study report. However, based on the information from the area of environmental fate and behavior of both active substances it can be concluded that fenpicoxamid is the least stable substance. In the present study the actual concentrations of fenpicoxamid (corrected for recovery) in the feeding solutions, prepared on each application day, were in the range from 79 to 110 % of the nominal concentrations. The mean recoveries of fenpicoxamid in the feeding solutions were in the range from 97 to 106 % of nominal. However, in line with the recommendations in Appendix J EFSA Supporting publication 2019:EN-1673, the contribution of each active substance to the toxicity of the product to bees should be calculated in order to confirm that fenpicoxamid is the driver of the toxicity (contribution of > 90 %) to bees.</p>
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	<p>It is confirmed that fenpicoxamid is the driver of the toxicity to bees, then, since the measured concentrations of fenpicoxamid in the present study were within 80-120% of nominal, the zRMS is of the opinion that the endpoints can be based on nominal concentrations as provided in the study report.</p> <p>Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>LC₅₀ > 15.0 mg product/kg food NOEC ≥ 15 mg product/kg food</p> <p>LDD₅₀ > 418 ng product/bee/day correspond to 0.418 µg product/bee/day NOEDD ≥ 418 ng product/bee/day</p>
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Reference:	KCP 10.3.1.2/2
Report:	Vergé, E., Kästel, A.; 2018; GF-3307 - Assessment of Effects on the Adult Honey Bee, <i>Apis mellifera</i> L., in a 10 Day Chronic Feeding Test under Laboratory Conditions; Eurofins Agrosience Services EcoChem GmbH / Eurofins Agrosience Services Ecotox GmbH, Eutingen Straße 24, D - 75223 Niefern-Öschelbronn, Germany; Lab Study No. S17-00198; DAS Study No. 170077; 10 January 2018; Unpublished
Guideline(s):	OECD guideline proposal 2016
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable Yes
Duplication (if vertebrate study)	NA

COMPLIANCE

Guideline(s):	OECD guideline proposal 2016
US EPA Guideline(s):	-
Deviations:	None
Dates of work:	21 August 2017 to 12 October 2017
GLP status:	Yes
Number of pages in final report:	98

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-3307
Content of a.i., analysed:	fenpicoxamid 4.7% w/w prothioconazole 9.3 % w/w
Description (physical state):	liquid / brown
Lot/batch no.:	ENBK-169144-026 (TSN314279)

Test System

Organism (Species):	Honey bee (<i>Apis mellifera</i>)
Study type:	Chronic oral

Study design:	Dose-response test; duration 10 days; minimum 3 4 replicates for each treatment group, each replicate consisting of 10 bees in one cage per test concentration; assessment of mortality, food consumption and behavioural effects daily.
Test concentrations:	0 (control), 0.38, 0.96, 2.40, 6.00 and 15.0 mg product/kg food (equivalent to 0.0179, 0.0451, 0.113, 0.282 and 0.705 mg fenpicoxamid/kg food)
Information on bee colony (health etc):	The bees used in the test were from disease-free colonies. The hives had not been treated for <i>Varroa</i> mites or for disease in the last 4 weeks. The bees were maintained in a clean holding cage at a temperature of approximately 33°C and 50 to 70% humidity.
Amount of treated diet consumed:	Consumption of the treated diets ranged from 24.3 to 29.1 mg of diet. Calculated daily dosages ranged from 9.8 to 417.7 ng product/bee.
Feeding method:	During holding/acclimation and after administration of the test dosages, bees were provided <i>ad libitum</i> a 500 g/L (w/v) sucrose solution in water. The bees for the definitive test were housed in cages containing pre-weighed feeders (syringes) containing approximately 3 - 5 mL 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: 32.4 – 32.9 °C Relative humidity: 52.4 – 62.1 % Photoperiod: The environmental chamber was kept dark except when room lighting was used during observation periods.
Reference substance:	Dimethoate: 0.9 mg a.i./kg diet
Solvent substance (if applicable):	Deionized water

Methodology

Honey bees were exposed to a 50 % (w/v) aqueous sucrose solution containing five concentrations of GF-3307 by continuous and *ad libitum* feeding over a period of 10 days. The control group was fed with 50 % (w/v) untreated aqueous sucrose. Mortality and behavioural abnormalities were assessed daily during the 10 day exposure period. The chronic effects of GF-3307 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), after 10 days of continuous feeding 2.5 % mortality was observed.

In the test item treatment groups no statistically significant mortality was observed after 10 days.

Single apathetic, affected or hyperactive bees were observed in the control and all test item treatment groups on various observation dates. In the three highest treatment groups (2.40, 6.00 and 15.0 mg product/kg food) many affected and apathetic bees were additionally observed on assessment six and ten. In the reference item group a lot of affected and apathetic bees were observed from assessment three until ten.

The overall mean daily consumption of feeding solution over the entire test period was 28.2 mg/bee/day in the control group. At the concentrations of 0.38, 0.96, 2.40, 6.00 and 15.0 mg product/kg food the

overall mean daily consumption of feeding solution was 25.9, 24.6, 29.1, 24.3 and 27.8 mg/bee/day, respectively. The food consumption was not statistically significantly different compared to the control group at any test item group. In the toxic reference item group, the overall mean daily consumption of feeding solution was 18.4 mg/bee/day.

At the end of the 10 day test period, the accumulated uptake of the test item at the concentrations of 0.38, 0.96, 2.40, 6.00 and 15.0 mg product/kg food was 98.4, 237, 698, 1460 and 4180 ng product/bee/day, respectively. The corresponding daily mean uptake was therefore 9.84, 23.7, 69.8, 146 and 418 ng product/bee, respectively.

The actual concentrations of fenpicoxamid in the feeding solutions T1 to T5, prepared on each application day, were in the range from 79 to 110 % of the nominal concentrations.

The actual concentrations of fenpicoxamid in the spent diet, determined from 1DAA1 to 1DAA9, were in the range from 3 to 36 % of the nominal concentrations.

Table 1: Toxicity of GF-3307 to honey bees in the chronic oral toxicity test

Treatment		Oral 10 day test									
Nominal mg/kg diet	Measured daily mean uptake ng /bee	Cumulative Mortality (%)									
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Control (0)	0	0.0	0.0	0.0	0.0	2.5	2.5	2.5	2.5	2.5	2.5
0.38	9.8	0.0	0.0	0.0	0.0	0.0	2.5	2.5	2.5	5.0	5.0
0.96	23.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2.40	69.8	0.0	0.0	0.0	0.0	0.0	0.0	2.5	2.5	2.5	2.5
6.00	146.0	0.0	0.0	0.0	0.0	2.5	5.0	5.0	5.0	5.0	5.0
15.0	417.7	0.0	0.0	0.0	0.0	0.0	0.0	7.5	15.0	15.0	15.0
Reference Item		0.0	0.0	7.5	22.5	55.0	70.0	80.0	82.5	87.5	97.5
10 day LDD ₁₀		n.d.					n.d.				
10 day LDD ₅₀		> 418 ng product/bee/day					> 19.6 ng fenpicoxamid/bee/day ¹				
10 day NOEDD		≥ 418 ng product/bee/day					≥ 19.6 ng fenpicoxamid/bee/day ¹				
10 day LC ₁₀		n.d.					n.d.				
10 day LC ₅₀		> 15 mg product/kg diet					> 0.705 mg fenpicoxamid/kg food ¹				
10 day NOEC		≥ 15.0 mg product/kg diet					≥ 0.705 mg fenpicoxamid/kg food ¹				

¹ calculated with the analysed content of a.i. of 4.7 % w/w

n.d.: not determined

Table 2: Effect of GF-3307 on diet consumption in honey bees in the chronic oral toxicity test

Treatment		Oral 10 day test									
Nominal mg/kg diet	Measured daily mean uptake ng /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
Control (0)	0	16.8	28.1	26.3	29.3	31.5	19.4	32.0	38.5	28.6	32.1
0.38	9.8	25.7	10.6	21.0	23.5	30.1	31.6	27.6	27.8	30.1	31.2
0.96	23.7	14.7	19.8	22.2	25.2	28.0	34.1	17.4	33.2	28.3	23.6
2.40	69.8	17.3	13.8	31.8	28.0	29.8	39.8	32.3	38.9	33.3	26.1
6.00	146.0	20.3	10.9	16.7	28.3	23.1	34.4	23.2	23.7	39.2	23.7
15.0	417.7	26.7	11.5	31.8	29.7	25.6	35.0	31.6	29.5	38.4	18.9
Reference Item		26.8	20.5	26.1	11.5	20.0	9.5	11.6	22.8	4.2	18.8
10 day NOEC, diet consumption		≥ 15.0 mg product/kg food					≥ 0.705 mg fenpicoxamid/kg food ¹				

¹ calculated with the analysed content of a.i. of 4.7 % w/w

Table 3:Effect of GF-3307 on weight of surviving bees in honey bees in the chronic oral toxicity test

Treatment		Oral 10 day test			
Nominal mg/kg diet	Measured daily mean ng/bee	Mean weight surviving bees (mg)			
		Replicate 1	Replicate 2	Replicate 3	Replicate 4
Control (0)	0	105.6	115.3	112.0	103.5
0.38	9.8	117.4	103.8	118.5	120.8
0.96	23.7	99.8	123.2	96.8	96.0
2.40	69.8	118.0	108.1	98.1	112.8
6.00	146.0	114.9	113.3	102.1	107.5
15.0	417.7	120.7	107.0	115.0	102.7
10 day NOEC, weight surviving bees		≥ 15.0 mg product/kg food		≥ 0.705 mg fenpicoxamid/kg food ¹	

¹ calculated with the analysed content of a.i. of 4.7 % w/w

Table 4:Sublethal effects of GF-3307 to honey bees in the chronic oral toxicity test

Treatment		Oral 10 day test									
Nominal mg/kg diet	Measured daily mean ng/bee	Behavioural abnormalities									
		E 1	E 2	E 3	E 4	E 5	E 6	E 7	E 8	E 9	E 10
Control (0)	0	0	0	1ap, 1a	0	0	1ap	1ap	1ap	0	1a
0.38	9.8	1ap	0	0	0	0	2ap	0	0	0	0
0.96	23.7	0	1ap	0	0	0	2ap, 2a	0	0	0	0
2.40	69.8	1ap	0	0	0	0	2ap, 2a	0	0	0	3ap
6.00	146.0	0	0	0	0	0	6ap, 1a	0	1a	0	6ap
15.0	417.7	2ap	1ap	1ap	1ap	2ap	5ap, 1a	1h	1a	0	5ap
Reference Item		0	0	8ap, 2a	18ap, 6a	8ap, 10a	12a	8a	7a	5a	1a

a = affected

ap = apathetic/lethargic

h = hyperactive

CONCLUSION

It can be concluded that the continuous *ad libitum* feeding of honey bees in the laboratory over a period of 10 consecutive days with the test item GF-3307 at the treatment levels of 0.38, 0.96, 2.40, 6.00 and 15.0 mg product/kg food caused no adverse effects regarding mortality, behavioural abnormalities, food consumption and weight of bees.

The LC₅₀ and LDD₅₀ after 10 days of continuous exposure were determined to be > 15.0 mg product/kg food and > 418 ng product/bee/day, respectively.

The LOEC based on mortality after 10 days of continuous exposure could not be determined. Therefore the NOEC was determined to be ≥ 15.0 mg product/kg food. The corresponding NOEDD, based on the actual consumption of the respective feeding solutions, was determined to be ≥ 418 ng product/bee/day. The LOEC based on overall mean consumption of feeding solution after 10 days of continuous exposure could not be determined. Therefore, the NOEC was considered to be ≥ 15.0 mg product/kg food.

The LOEC based on the weight of surviving bees after 10 days of continuous exposure could not be determined. Therefore, the NOEC was determined to be ≥ 15.0 mg product/kg food.

The study is considered valid because:

- The mean mortality in the control was ≤ 15% at the end of the test.
- The mean mortality in the reference item group was ≥ 50 % at the end of the test.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item	Toxicity value	Units of test item
Honey bee	<i>Apis mellifera</i>	GF-3307	10 days	LC ₁₀	n.d.	mg product/kg diet	n.d.	mg a.i./kg diet
Honey bee	<i>Apis mellifera</i>	GF-3307	10 days	LC ₅₀	> 15.0	mg product/kg diet	> 0.705	mg a.i./kg diet
Honey bee	<i>Apis mellifera</i>	GF-3307	10 days	LDD ₁₀	n.d.	ng/bee/day	n.d.	ng a.i./bee/day
Honey bee	<i>Apis mellifera</i>	GF-3307	10 days	LDD ₅₀	> 418	ng/bee/day	> 19.6	ng a.i./bee/day
Honey bee	<i>Apis mellifera</i>	GF-3307	10 days	LOEC	n.d.	mg product/kg diet	n.d.	mg a.i./kg diet
Honey bee	<i>Apis mellifera</i>	GF-3307	10 days	LOEDD	n.d.	ng/bee/day	n.d.	ng a.i./bee/day
Honey bee	<i>Apis mellifera</i>	GF-3307	10 days	NOEC	≥ 15.0	mg product/kg diet	≥ 0.705	mg a.i./kg diet
Honey bee	<i>Apis mellifera</i>	GF-3307	10 days	NOEDD	≥ 418	ng product/bee/day	≥ 19.6	ng a.i./bee/day
Honey bee	<i>Apis mellifera</i>	GF-3307	10 days	Food consumption LOEC	n.d.	mg product/kg diet	n.d.	mg a.i./kg diet
Honey bee	<i>Apis mellifera</i>	GF-3307	10 days	Food consumption NOEC	≥ 15.0	mg product/kg diet	≥ 0.705	mg a.i./kg diet
Honey bee	<i>Apis mellifera</i>	GF-3307	10 days	Weight bees LOEC	n.d.	mg product/kg diet	n.d.	mg a.i./kg diet
Honey bee	<i>Apis mellifera</i>	GF-3307	10 days	Weight bees NOEC	≥ 15.0	mg product/kg diet	≥ 0.705	mg a.i./kg diet

¹ calculated with the analysed content of a.i. of 4.7 % w/w
n.d.: not determined

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-3307 (Fenpicoxamid + Prothioconazole): Brood Development of the Honeybee (*Apis mellifera* L.) in a Semi-Field Tunnel Study in *Phacelia tanacetifolia* in Germany 2017

Comments of zRMS:	<p>The semi-field study on effects of GF-3307 on honeybee brood has been submitted in support of the application GF-3307 in cereals.</p> <p>The study was conducted in compliance with the OECD guideline 75/EPPO 170, without major deviations. The following validity criteria was met.</p> <ul style="list-style-type: none"> - Significant effects seen on the bee brood in the toxic standard group such as: significantly increased brood termination rate of eggs during the first brood cycle, significantly reduced brood indices and compensation indices of eggs during the first brood cycle, and clearly increased pupal mortality and pupal malformations observed during the daily assessments of mortality, demonstrated sufficient sensitivity of the test system. - The mean brood termination rate of eggs in the control was < 50% in both brood cycles. Actual value at the last assessment day: 8.04% at the end of the first brood cycle, and 19.52% at the end of the second brood cycle. <p>The study was conducted in tunnels assembled on a field of flowering <i>Phacelia tanacetifolia</i> in Ispringen /Baden-Württemberg in Germany.</p> <p>The trial consisted of four treatment groups: two groups (T1 and T2) consisting of</p>
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	<p>GF-3307 treatment, each replicated five times (T1a – T1e, T2a – T2e), one group (C) consisting of five replicates of a water treated control (Ca – Ce) and one group consisting of an Insegar (active substance: fenoxycarb) treated reference group.</p> <p>The application rates of GF-3307 were 50 g fenpicoxamid a.i./ha plus 100 g prothioconazole a.i./ha (corresponding to 1 L product/ha) in treatment group T1 and 100 g fenpicoxamid a.i./ha plus 200 g prothioconazole a.i./ha (corresponding to 2 L product/ha) in the treatment group T2. The spray volume was 300 L/ha.</p> <p>The investigated parameters and timing of observations were in line with recommendations of OECD 75.</p> <p>The colonies were kept inside the tunnels and exposed to the crop from 4DBA to 7DAA and were then brought to a monitoring site for further observation until up to 42DAA. The condition of the colonies and the development stage of the bee brood were assessed twice before application (7DBA, 1DBA; DBA = Days before application), once during exposure (4DAA; DAA = Days after application) and 8 times after exposure during further monitoring (9DAA, 15DAA, 21DAA, 26DAA, 31DAA, 38DAA, 42DAA, 60DAA, 90DAA. In total, 12 assessments were carried out.</p> <p>The development of brood in individually marked cells 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 application (1DBA = BFD0; BFD = brood area fixing day) and lasted until BFD+22. The second brood cycle started at the completion of the first brood cycle (21DAA = BFD+22 of the first brood cycle = BFD0 of the 2nd brood cycle) and lasted until 42DAA (=BFD+21 of the 2nd brood cycle).</p> <p>The results on full flowering Phacelia during daily honeybee flight activity had effects as follows:</p> <p>There was no adverse effect of the test item treatments T1 and T2 on the mortality of adult worker bees, worker bee pupae, male adult bees and male pupae, foraging activity, colony size, amount of brood (total number of brood cells or number of cells with eggs, larvae or pupae) and storage of nectar and pollen.</p> <p>There was a slight adverse effect on the behaviour of worker bees in test item treatment T1 during the monitoring period outside tunnels and a slight transient effect on honeybee behaviour in T2 on the day of application (0 DAA).</p> <p>There was no effect of the test item treatment on the termination rates, brood indices and compensation indices of eggs during the 1st brood cycle (1DBA to 21DAA) and no effect on the young larvae and old larvae during the 1st and 2nd brood cycle (1DBA to 42DAA).</p> <p>There was a slight effect on the termination rates, brood index and compensation index of eggs on BFD+17 and BFD+21 of the 2nd brood cycle.</p> <p>There was no effect of the test item treatments T1 or T2 on the weight or malformations of pupae sampled from combs during the 1st brood cycle (17DAA). The slightly increased mean values in T1 and T2 resulted mainly from unusual high values in replicates T1e and T2e. Comparison to the other replicates in the respective treatment group (T1 or T2) identified data in T1e and T2e as outliers at all assessment dates except BFD+5 in T2 (Grubbs' test, $p \leq 0.05$), i.e. observations in these replicates did not match the observations in the other replicates within the same group. However, T1e and T2e were included in the evaluations and calculations of mean values. In addition these results could not be observed for young or old larvae.</p> <p>Furthermore, according to Pistorius et al. 2012 and Becker, Lückmann & Pistorius, 2015 the artificial tunnel conditions ('caging effect') are one of the major factors influencing the brood termination in honeybee colonies and the observed effect on the termination</p>
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	<p>rates (treatment T1 and treatment T2 \pm 34 %) was slightly above the natural termination rate 21.7 %, respectively 29.2 % for control data and 34.7 % for historical data) and could therefore be regarded as not biologically relevant.</p> <p>The results of the semi-field study with regard to the brood development of the honey bees did not indicate unacceptable adverse effects on adult bees or bee brood development at an application at up to 2 L GF-3307/ha equivalent to 100 g fenpicoxamid plus 200 g prothioconazole/ha.</p>
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Reference:	KCP 10.3.1.5/1
Report:	Kleinhenz, M.; 2018; GF-3307 (Fenpicoxamid + Prothioconazole): Brood Development of the Honeybee (<i>Apis mellifera</i> L.) in a Semi-Field Tunnel Study in <i>Phacelia tanacetifolia</i> in Germany 2017; Eurofins Agrosience Services EcoChem GmbH / Eurofins Agrosience Services Ecotox GmbH, Niefern-Öschelbronn, Germany; Lab Study No. S17-02707; DAS Study No. 170673 ; 24 May 2018; Unpublished
Guideline(s):	OECD Guidance Document No. 75 (2007) plus recommendations by Pistorius et al. (2012); OEPP/EPPO Guideline No. 170(4) (2010); EC Guidance Document 7029/VI/95 rev. 5 (1997)
Deviations:	
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	NA

COMPLIANCE

Guideline(s):	OECD Guidance Document No. 75 (2007) plus recommendations by Pistorius et al. (2012); OEPP/EPPO Guideline No. 170(4) (2010); EC Guidance Document 7029/VI/95 rev. 5 (1997)
US EPA Guideline(s):	Not applicable
Deviations:	-
Dates of work:	30 June 2017 to 21 February 2018
GLP status:	Yes
Number of pages in final report:	312

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-3307
Content of a.i., analysed:	Fenpicoxamid: 4.8% w/w (nominal), 4.7% w/w (analysed) Prothioconazole: 9.6% w/w (nominal), 9.3% w/w (analysed)
Description (physical state):	Brown liquid
Lot/batch no.:	ENBK-169144-026 (TSN314279)

Test System

Organism (*Species*):

Honey bee (*Apis mellifera*)

For the test, 23 normally developed, healthy and queen-right (one egg-laying queen) bee colonies with one body including 10 combs were used. The colonies were as homogeneous as possible and originated from one breeding line (sister queens, reared at the test facility in 2017, were used).

The following criteria for the colonies were fulfilled at the first colony assessment (7DBA):

- Colony sizes ranged from approx. 6500 to 8500 bees for the hives a-e intended for biological observations (actual: 6045 to 8125 honeybees/colony) and from approx. 10000 to 13000 bees in the replicates “s” intended for sampling (actual: 8515 to 11570 honeybees/colony);
- At least 4-6 brood combs with all brood stages (actual: 4-8 combs for replicates a-e, 2-8 combs for replicates “s” intended for sampling);
- At least 2 honey and pollen combs (actual: 5-9 combs);
- Queen rightness of the colonies was confirmed by direct sight on the queen in all colonies except Rd. Hive Rd was in an overall good condition and all brood stages present on 7DBA. Queen presence was confirmed during a short inspection on 5DBA (before transport to the field site) and during the next colony assessment (7DAA).

Food source:

Full flowering *Phacelia tanacetifolia* served as food supply, no additional feeding throughout the study. Each tunnel was provided with a water supply.

Study type:

Effects on honeybee brood development

Study design:

Tunnel test under semi-field conditions

Test concentrations:

Treatment T1: 100 g prothioconazole/ha + 50 g fenpicoxamid/ha (target)

Treatment T2: 200 g prothioconazole/ha + 100 g fenpicoxamid/ha (target)

Dosing method:

Spray application on *Phacelia tanacetifolia* plants during honeybee flight. Direct exposure of adults to the crop, indirect exposure by consumption of nectar and pollen collected from the treated crop. The test item solutions were prepared shortly before application. The application was carried out with a calibrated portable boom sprayer that simulates a commercial application.

During the application air temperature, humidity, wind speed and cloud cover data were recorded directly in the field with field equipment (GLP record).

The following conditions were met for application:

- *Phacelia tanacetifolia* was in bloom (BBCH 64-65),
- At application, ≥ 10 honeybees / m² target (actual: 12.5 to 16.5 honeybees / m², mean per treatment group) were actively foraging in the crop inside the tents (replicates a-e),
- Wind speed was less than 2 m/s inside the tunnels (actual: ≤ 1.7 m/s),
- Air temperature did not exceeded 30 °C (actual: 24.7 to 29.7°C),
- The amount actually sprayed was within $\pm 10\%$ tolerance of the target spray volume in all tunnels.

During the application the water containers were taken out of the tunnels. The colonies were covered with a plastic sheet for the duration of spraying until the end of application to avoid direct contamination of the whole colony.

After the application of each plot the volume applied was determined by measuring the remaining spray solution compared to the prepared spray solution.

Environmental conditions:

For the entire exposure and monitoring period the following climatic data were recorded by a rain gauge placed at the field site or at the monitoring site, and by an EAS weather station in Nief-ern-Öschelbronn at a distance of 5.6 km to the field site and 5.0 km to the monitoring site:

- air temperature (daily minimum / maximum / mean)
- relative air humidity (daily minimum / maximum / mean)
- precipitation (daily sum)

During application and assessments, the following climatic data were recorded at the field site (GLP record):

- cloud cover (only during application and mortality/flight activity assessment)
- wind speed (only during application)
- air temperature (only during application)

Precipitation was measured continuously by a rain gauge placed at the field site, approx. 50 m away from the nearest tunnel tent (GLP data).

Perception during the study:

1. Field phase in the tunnels (4DBA to 7DAA): Daily rainfall from 0 to 7.4 mm (sum) was noted;

4DBA: 0.2 mm, 3 DBA: 0 mm, 2 DBA: 0 mm, 0DBA/0DAA: 2.8 mm - rainfall from 18:30 – 18:37, i.e. 10.5 hours after the end of the last application was noted.

On days; 1 DAA and 2 DAA no rainfall occurred.

The heavy rainfall during the period inside the tunnels was recorded on DAA 3: 6.2 mm and DAA 4: 7.4 mm - rainfall mostly during the night until 7:13h in the morning and 1.0 mm from 14:00 to 14:03h.

In addition the rainfall occurred on: on day DAA 5: 3 mm (rainfall after 17:26h) and on 7 DAA: 2.6 mm (after 16:09h)

Relative humidity (RH) from 28.7% to 97.5%, temperatures from 10.1°C to 33.1°C.

2. At the monitoring site (8DAA to 90DAA):

Daily rainfall from 0 to 36 mm (sum); DAA 14: 3 mm, DAA 14: 3.0 mm, DAA 15: 23.0 mm, DAA 14: 1 mm, DAA 18: 3.5 mm, DAA 19: 28 mm, DAA 20: 4 mm, DAA 21: 3 mm, DAA 24: 5 mm, DAA 25: 1.5 mm, DAA 26: 7 mm, DAA 34: 36 mm, DAA 35: 6 mm, DAA 36: 15 mm, DAA 37: 2 mm, DAA 43: 1.2 mm, DAA 52: 0.2 mm, DAA 54: 2.4 mm, DAA 55: 6.2 mm

RH from 25.9% to 100%, temperatures from 2.8°C to 34.5°

C: Tap water

R: Insegar 25 WG (250 g fenoxycarb/kg)

Control:

Reference substance:

Test units:

Group/size/replicates:

Tunnels with an area of 100 m², containing 82.72 m² of *Phacelia tanacetifolia*, each with one colony; tunnels equipped with a water supply.

The trial consisted of four treatment groups: two groups (T1 and T2) consisting of GF-3307 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 watertreated control (Ca – Ce) plus one water-treated replicate for sampling (Cs) and one group consisting of an Insegar (active substance: fenoxycarb) treated reference group

Statistic:	<p>The software “SAS®”, version 9.3, was used for the statistical analysis of mortality, foraging activity data, colony data (number of bees, food cells and brood cells), brood termination rates, brood indices and compensation indices resulting from the photographic assessments and pupae weights per replicate.</p> <p>No statistical evaluation of dead male bees and male pupae was carried out due to their rare occurrence during this study.</p> <p>For all tests, the significance level was set as $\alpha = 0.05$.</p> <p>For the pre-application period all tests were conducted in a two-sided approach whereas for the data assessed after application one-sided tests (“upper” for mortality data and brood termination rates, “lower” for flight activity, brood index, compensation index, colony assessment data and pupae weights) were conducted. For evaluation of mortality, the number of dead bees on the linen sheets, in the dead bee traps and on the hive floor (bottom drawer) were summarized per replicate.</p> <p>Test item treatments T1 and T2:</p> <p>Data of the test item treatments T1 and T2 and the control were checked for normality using Shapiro-Wilks test. If the distribution of the data fitted the normal distribution very well (Shapiro-Wilks test, $p \geq 0.2$) then Bartlett’s test was used to check for homoscedasticity of data, in the other cases Levene’s test was used.</p> <p>If logarithmic transformation (data of mortality, flight intensity, colony assessments, pupae weights) or square-root transformation (brood index, compensation index, brood termination rates) of data solved problems with normality or homoscedasticity, then the transformed data was used for analysis to enable use of tests with higher statistical power.</p> <p>If normality and also homoscedasticity were proven Dunnett’s t-test was used for analysis of the data. If normality was met but homoscedasticity was disturbed, the Bonferroni-Holms corrected t-test (same as Welch test) was used for analysis. If data were not normal, the Bonferroni-Holms corrected U-test was used.</p> <p>Reference item treatment R:</p> <p>Data of the reference item treatment and corresponding data of the control were tested for normality using Shapiro-Wilks Test and for homoscedasticity using the Folded F-Test.</p> <p>Transformed data (logarithmic or square-root transformation as described above) were used for analysis if these data allowed the use of tests with higher power.</p> <p>Student’s t-Test (pooled) was used for data meeting normal distribution and homoscedasticity. In case of no homoscedasticity but proven normality Satterthwaite’s t-test was used. In case of no normal distribution of data, the Mann-Whitney Exact Test was used.</p>
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Methodology

The objective of the study was to determine the potential effects of GF-3307 (a.i.: fenpicoxamid + prothioconazole) on the honeybee (*Apis mellifera* L.) 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-3307, a.i.: fenpicoxamid + prothioconazole), one toxic reference item group R (Insegar; a.i.: fenoxycarb) and a water-treated control C, applied during daily bee flight at the beginning of full flowering of *Phacelia tanacetifolia* at

BBCH 64-65. The application rates of GF-3307 were 50 g fenpicoxamid a.i./ha plus 100 g prothioconazole a.i./ha in treatment group T1 and 100 g fenpicoxamid a.i./ha plus 200 g prothioconazole a.i./ha in the treatment group T2. The spray volume was 300 L/ha.

Commercial bee colonies were placed in the tunnel tents at beginning of flowering (BBCH 62-63). The mortality, foraging activity and behaviour of the bees were examined before (4DBA to 0DBA; DBA = days before application) and after application (0DAA to 42DAA; DAA = days after application). Photographic assessments of the brood development of single, individually marked cells (target: ≥ 200 cells containing eggs, ≥ 200 cells containing young larvae and ≥ 200 cells containing old larvae) were conducted over two brood cycles (1st cycle: BFD0, BFD+5, BFD+10, BFD+16, BFD+22; 2nd cycle: BFD0 (=BFD+22 of the 1st cycle), BFD+5, BFD+10, BFD+17, BFD+21). The condition of the colonies was assessed twice before the application (7DBA and 1DBA), over two brood cycles (4DAA, 9DAA, 15DAA, 21DAA, 26DAA, 31DAA, 38DAA, 42DAA) plus two additional assessments (60DAA, 90DAA) until start of overwintering of the colonies. Additionally the weight and malformations of pupae collected from combs during the 1st brood cycle was evaluated.

The influence of GF-3307 was evaluated by comparing data of the assessments of the test item treatment groups T1 and T2 and the reference item group R to the control C, and by comparing the pre-application data to the post-application data.

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 whole *Phacelia* plants and forager bees (for preparation of pollen and nectar) were taken once before (1DBA) and three times after application (0DAA, 1DAA, 6DAA) in Cs, T1s and T2s for subsequent residue analysis.

RESULTS AND DISCUSSION

Mortality

Adult worker bees:

Mean pre-application mortality (mean number of dead worker bees per day) during the period from 4DBA to 0DBA was 57.4 in the control, 36.0 in T1, 57.0 in T2 and 50.1 in R. These slight differences were not statistically significant.

On the day of application, after completion of the application (0DAA), mean mortality in all treatment groups was very similar (38.0, 26.0, 45.4 and 29.0 dead honeybees in C, T1, T2 and R) and not significantly different from the control. On the following days during exposure to the treated crop in the tunnels (1DAA to 7DAA), worker bee mortality in T1 and T2 was on the same level or lower than control mortality on all days. Mean post-application mortality over the period from 0DAA to 7DAA was 63.2, 38.3, 56.6 and 49.5 dead adult worker honeybees/day in C, T1, T2 and R (not significant). There were no significant differences in T1, T2 or R compared to the control on any day during this period.

During further observation at the monitoring site (8DAA to 42DAA), there were no statistically significant differences of T1, T2 or R to the control on any day except one record in reference item group R on 36DAA. Mean daily mortality over the duration of the monitoring period (8DAA to 42DAA) was 34.2, 33.2, 22.4 and 43.4 dead adult worker honeybees/day in C, T1, T2 and R (not significant).

Overall, there was no effect of the test item treatments T1 or T2 on adult worker honeybee mortality.

The results are presented below:

Table 1. 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-3307 at 50 g fenpicoxamid/ha plus 100 g prothioconazole/ha)	T2 (GF-3307 at 100 g fenpicoxamid/ha plus 200 g prothioconazole/ha)	R (Insegar)
03 Jul 2017	4DBA	14.6 ± 7.7	13.0 ± 10.1	11.8 ± 4.1	14.8 ± 8.2
04 Jul 2017	3DBA	67.6 ± 27.6	50.6 ± 28.3	62.6 ± 24.5	59.4 ± 21.4
05 Jul 2017	2DBA	74.4 ± 20.5	46.0 ± 16.0	87.2 ± 73.5	72.2 ± 49.7
06 Jul 2017	1DBA	61.8 ± 29.0	34.0 ± 5.7	58.2 ± 30.3	49.4 ± 38.1
07 Jul 2017	0DBA	68.6 ± 14.2	36.4 ± 13.4	65.4 ± 40.0	54.8 ± 20.5
Mean pre-exposure period (4DBA to 0DBA)		57.4 ± 17.5	36.0 ± 12.2	57.0 ± 30.9	50.1 ± 25.2
07 Jul 2017	0DAA	38.0 ± 8.6	26.0 ± 4.1	45.4 ± 20.9	29.0 ± 16.1
08 Jul 2017	1DAA	11.0 ± 5.2	7.6 ± 3.4	15.6 ± 8.5	12.2 ± 4.8
09 Jul 2017	2DAA	68.8 ± 24.7	43.8 ± 9.7	70.4 ± 40.2	61.8 ± 14.8
10 Jul 2017	3DAA	60.0 ± 20.8	40.2 ± 14.4	55.4 ± 19.2	47.4 ± 15.3
11 Jul 2017	4DAA	57.4 ± 15.9	31.2 ± 13.9	43.4 ± 21.8	48.6 ± 18.8
12 Jul 2017	5DAA	90.6 ± 17.2	49.6 ± 20.2	64.8 ± 38.2	70.8 ± 14.0
13 Jul 2017	6DAA	54.8 ± 25.2	33.2 ± 5.9	50.6 ± 20.5	49.0 ± 25.6
14 Jul 2017	7DAA	125.2 ± 57.9	74.8 ± 21.5	107.2 ± 38.6	77.2 ± 14.4
Mean exposure period (0DAA to 7DAA)		63.2 ± 16.0	38.3 ± 7.9	56.6 ± 22.3	49.5 ± 13.3
Mean post-exposure period (8DAA to 42DAA)		34.2 ± 13.5	33.2 ± 18.0	22.4 ± 9.3	43.4 ± 35.6

DBA/DAA = Days before/after application

There were no statistically significant differences of adult worker bee mortality in the test item treatments T1 and T2 or the reference item treatment R (except R on 36DAA) compared to the control.

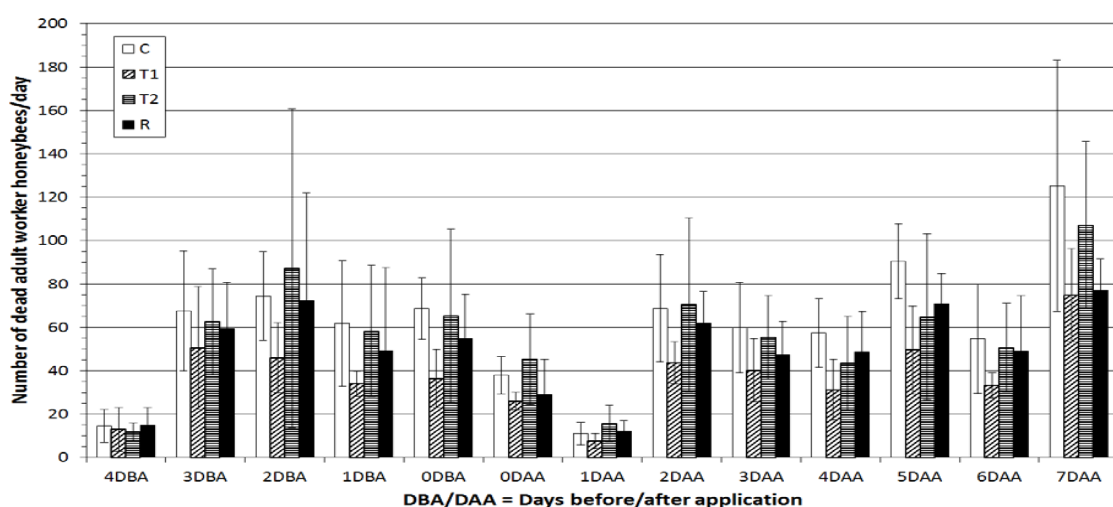


Figure 1. Mortality of adult worker bees during the exposure period in the tunnels

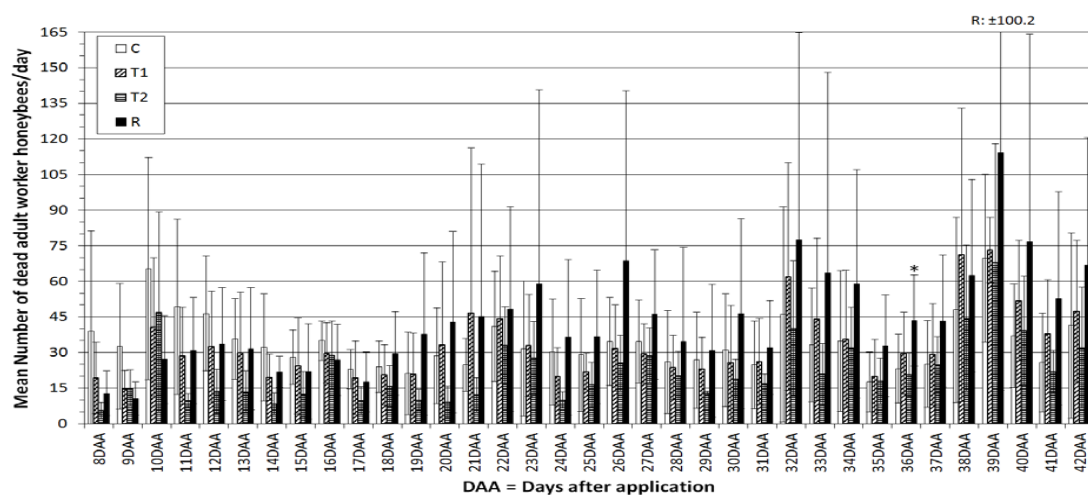


Figure 2. Mortality of adult worker bees during the post-exposure period outside the tunnels at the monitoring site

Worker larvae and pupae:

Mortality of worker pupae was on a low level in the control, T1 and T2 throughout the study period. Before application (4DBA to 0DBA), daily pupae mortality was 0.1, 0.4, 0.5 and 0.4 dead worker pupae/day in C, T1, T2 and R (not significant). On the following days during exposure to the treated crop in the tunnels (0DAA to 7DAA), mean daily mortality of worker pupae was 0.1 in the control, 0.1 in T1, 0.2 in T2 and 0.5 dead worker pupae/day in R. None of these slight differences of T1 or T2 to the control were statistically significant. Mean pupae mortality in R over the entire exposure period (0DAA to 7DAA) was significantly different from the control (one-sided Student's t-test (pooled), $p \leq 0.05$). During further observation at the monitoring site (8DAA to 42DAA), there were no statistically significant differences of daily worker pupae mortality in T1 or T2 compared to the control on any day. Mean daily worker pupae mortality during this period was in the range from 0.0 to 1.0 (mean: 0.2) in C, from 0.0 to 0.6 (mean: 0.2) in T1 and from 0.0 to 0.6 (mean: 0.1) dead worker pupae/day in T2 (not significant).

In the reference item group R, worker pupae mortality clearly increased to up to a maximum of 83.2 dead pupae/day on 10DAA. Pupae mortality was significantly higher in R than in the control on most days during the monitoring period (10DAA to 42DAA, except 36DAA and 37DAA) and also the mean daily pupae mortality during this period was statistically significantly different from the control (25.3 dead pupae/day in R compared to 0.2 dead pupae/day in the control). This effect was expected from this reference item, confirming suitability of the test design and exposure of the bees and brood to the treated crop.

Overall, there was no effect of the test item treatments T1 or T2 on worker pupae mortality during the whole observation period up to 42DAA.

The results are presented below:

Table 2. Mean daily mortality of worker pupae per treatment group

Date	Timing	Mortality (mean number of dead worker pupae/day)			
		C (tap water)	T1 (GF-3307 at 50 g fenpicoxamid/ha plus 100 g prothioconazole/ha)	T2 (GF-3307 at 100 g fenpicoxamid/ha plus 200 g prothioconazole/ha)	R (Insegar)
03 Jul 2017	4DBA	0.4 ± 0.5	0.4 ± 0.9	0.2 ± 0.4	1.2 ± 1.6
04 Jul 2017	3DBA	0.0 ± 0.0	0.2 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
05 Jul 2017	2DBA	0.2 ± 0.4	1.0 ± 1.7	1.0 ± 1.7	0.8 ± 0.8
06 Jul 2017	1DBA	0.0 ± 0.0	0.4 ± 0.9	1.4 ± 3.1	0.0 ± 0.0
07 Jul 2017	0DBA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Mean pre-exposure period (4DBA to 0DBA)		0.1 ± 0.2	0.4 ± 0.3	0.5 ± 1.1	0.4 ± 0.5
07 Jul 2017	Sum 0DAA	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.4	0.4 ± 0.5
08 Jul 2017	1DAA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.4
09 Jul 2017	2DAA	0.0 ± 0.0	0.2 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
10 Jul 2017	3DAA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
11 Jul 2017	4DAA	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.4	0.4 ± 0.5
12 Jul 2017	5DAA	0.2 ± 0.4	0.2 ± 0.4	0.0 ± 0.0	0.8 ± 1.1
13 Jul 2017	6DAA	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.4	1.0 ± 1.7
14 Jul 2017	7DAA	0.2 ± 0.4	0.2 ± 0.4	0.6 ± 1.3	1.0 ± 1.4
Mean exposure period (0DAA to 7DAA)		0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.4	0.5* ± 0.3
Mean post-exposure period (8DAA to 42DAA)		0.2 ± 0.3	0.2 ± 0.1	0.1 ± 0.1	25.3* ± 8.1

DBA/DAA = Days before/after application

* Statistically significant different from the control (one-sided Student's t-test, method: pooled, $p \leq 0.05$).

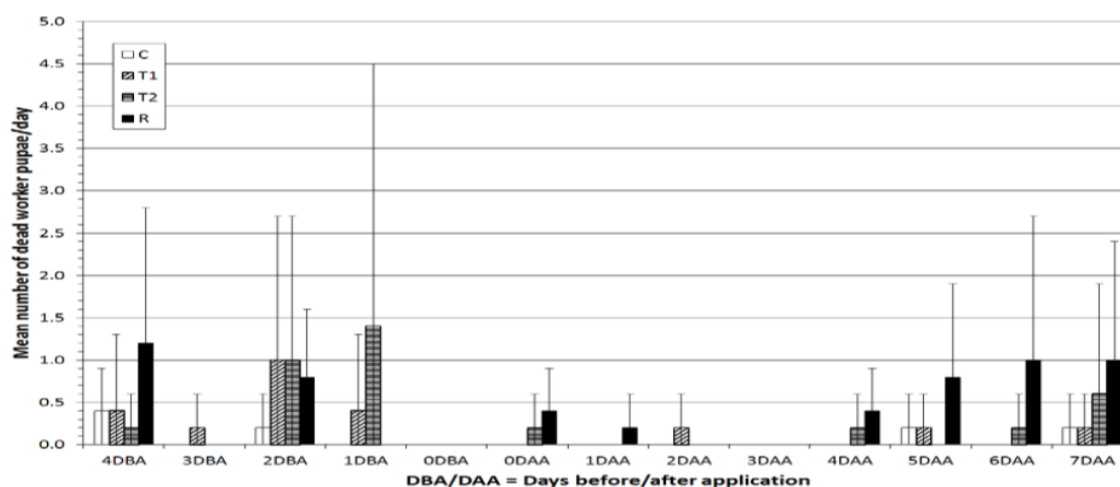


Figure 3. Mortality of worker pupae during the exposure period in the tunnels

Mortality of male bees and male pupae:

Mortality of male bees and male pupae was on a very low level throughout the study in all treatment groups (C, T1, T2 and R). Mean pre-application mortality of male bees and male pupae per day was 1.5 in C, 1.6 in T1, 1.4 in T2 and 1.5 in R. During the post-application exposure period in the tunnels (0DAA to 7DAA), the daily mean of dead male bees and pupae was lower in T1, T2 and R than in the control: there were 0.7 dead male bees and pupae/day in the control, 0.1 in test item treatment T1, 0.2 in T2 and 0.3 in R. At the monitoring site (8DAA to 42DAA), there were on average 0.2 dead male bees and pupae per day in the control, 0.1 in T1, 0.2 in T2 and 0.1 in R.

Overall, there was no effect of the test item treatments T1 and T2 on the mortality of male adult bees and male pupae.

Foraging Activity

During the pre-application period (4DBA to 0DBA), daily foraging activity (number of foraging bees/m²/10-15 sec) was in the range from 11.7 to 25.0 (mean: 18.9) in the control, from 10.5 to 26.1 (mean: 19.0) in T1, from 9.7 to 24.0 (mean: 18.7) in T2 and from 12.7 to 24.3 (mean: 19.1) in R. None of these differences to the control were statistically significant.

On the day of application (0DAA), mean foraging activity over several assessments on this day was 19.0 forager bees/m² in the control, 20.0 in T1, 20.3 in T2 and 24.1 in R. None of these differences to the control were statistically significant.

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 15.7 forager bees/m² in C, 15.3 in T1, 16.3 in T2 and 16.0 in R. None of these differences to the control were statistically significant.

Overall, test item treatments T1 and T2 had no adverse effect on honeybee foraging activity.

The results are provided below:

Table 3. Mean daily foraging activity per treatment group

Date	Timing	Foraging activity (mean number forager bees/m ² /10-15 sec)			
		C (tap water)	T1 (GF-3307 at 50 g fenpicoxamid/ha plus 100 g prothioconazole/ha)	T2 (GF-3307 at 100 g fenpicoxamid/ha plus 200 g prothioconazole/ha)	R (Insegar)
03 Jul 2017	4DBA	11.7 ± 5.0	10.5 ± 3.3	9.7 ± 5.3	12.7 ± 7.4
04 Jul 2017	3DBA	23.4 ± 7.1	21.1 ± 1.8	22.1 ± 5.7	21.3 ± 5.5
05 Jul 2017	2DBA	25.0 ± 5.1	26.1 ± 10.0	24.0 ± 3.1	24.3 ± 3.5
06 Jul 2017	1DBA	21.7 ± 2.4	20.9 ± 8.6	22.9 ± 5.1	21.7 ± 2.4
07 Jul 2017	0 DBA	12.5 ± 3.2	16.5 ± 3.9	14.6 ± 2.7	15.5 ± 2.2
Mean pre-exposure period (4DBA to 0DBA)		18.9 ± 2.5	19.0 ± 3.8	18.7 ± 3.6	19.1 ± 3.1
07 Jul 2017	Mean 0DAA	19.0 ± 2.3	20.0 ± 5.2	20.3 ± 2.9	24.1 ± 2.0
08 Jul 2017	Mean 1DAA	18.3 ± 2.9	18.4 ± 3.3	20.3 ± 3.9	17.1 ± 1.1
09 Jul 2017	2DAA	14.5 ± 3.0	14.8 ± 3.8	13.9 ± 3.3	16.1 ± 3.3
10 Jul 2017	3DAA	19.2 ± 4.5	15.2 ± 2.9	21.4 ± 3.5	16.9 ± 3.6
11 Jul 2017	4DAA	12.1 ± 3.3	13.9 ± 3.7	14.1 ± 5.5	12.2 ± 6.6
12 Jul 2017	5DAA	7.3 ± 3.5	7.9 ± 3.7	7.2 ± 3.5	7.1 ± 5.3
13 Jul 2017	6DAA	30.9 ± 6.7	26.7 ± 7.1	27.7 ± 4.0	27.5 ± 4.6
14 Jul 2017	7DAA	4.5 ± 4.5	5.4 ± 4.6	5.1 ± 3.1	7.1 ± 5.5
Mean exposure period (0DAA to 7DAA)		15.7 ± 2.3	15.3 ± 2.4	16.3 ± 1.9	16.0 ± 1.4

DBA/DAA = Days before/after application

There were no statistically significant differences of foraging activity in any treatment group (T1, T2, R) compared to the control.

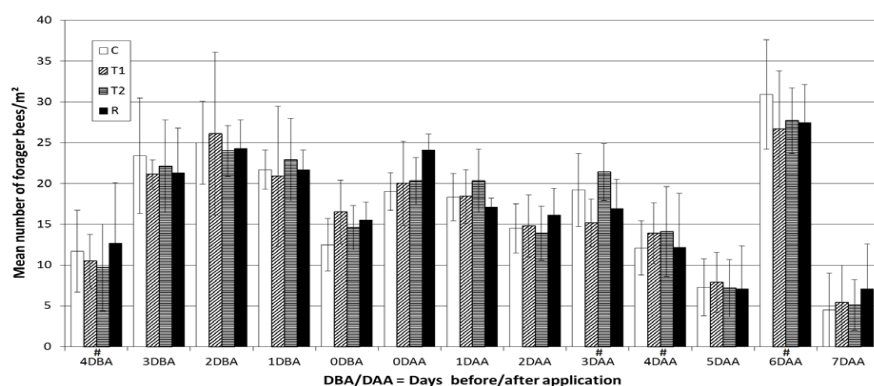


Figure 4. Foraging activity in the tunnels [mean number of forager bees/m²]
There were no statistically significant differences to the control on any day during this period.
= 2nd assessment on these days (during more favourable weather conditions)

Behaviour

In all treatment groups except for T2, a few bees with locomotion problems were observed during the pre-application period (4DBA to 0DBA).

In the control, a few bees with unusual behaviour were observed during the post-application period in the tunnels from 0DAA to 7DAA (7 observations in total, i.e. 7 bees in the 5 tunnels per treatment group showed unusual behaviour at the assessments during this period) and several observations of cramping bees and bees with locomotion problems were recorded during the monitoring period from 8DAA to 42DAA (183 observations in total).

In T1, there were 10 observations of bees with unusual behaviour during the exposure period (0DAA to 7DAA) and 298 observations during the monitoring period from 8DAA to 42DAA (bees with locomotion problems, trembling bees, cramping bees and inactive bees).

In T2, there were 18 observations of bees with unusual behaviour on the day of application (0DAA), 6 observations during further exposure (1DAA to 7DAA) and 93 observations during the monitoring period (mainly bees with locomotion problems and cramping bees).

In the reference item group R, there were 6 observations of unusual behaviour during the exposure period (0DAA to 7DAA) and 672 observations during the monitoring period (8DAA to 42DAA).

Overall, there was a slight adverse effect on the behaviour of worker bees in test item treatment T1 during the monitoring period and a slight transient effect on honeybee behaviour in T2 on the day of application.

Condition of the Colonies

The mean colony sizes per treatment were on the same level in all treatment groups (C, T1, T2, R) at the first assessment at start of the study (7DBA).

Mean colony size in the test item treatments T1 and T2 was essentially very similar to that in the control, and there were no statistically significant differences on any day during the entire observation period from 7DBA to 90DAA.

Mean colony size in the reference item treatment group R was noticeably smaller than control colony size and was significantly different from the control on all assessments from 26DAA to 90DAA. Moreover, two colonies in the reference item group declined and died before the last assessment before start of the overwintering period (90DAA).

There was no effect of the test item treatment T1 or T2 on the total number of brood cells or presence of the different brood stages during the study. The total amount of brood was always comparable to the control and minor fluctuations were within the range of natural variability observed during the study (e.g., the total amount of brood in T1 was lower than the control on 31DAA and 42DAA but was very similar on 9DAA, 21DAA, 38DAA and 90DAA and even higher than the control on 4DAA, 15DAA and 26DAA).

No biologically relevant negative effect of the test item treatments T1 and T2 on the storage of nectar and pollen could be discerned.

The results are presented below:

Colony size

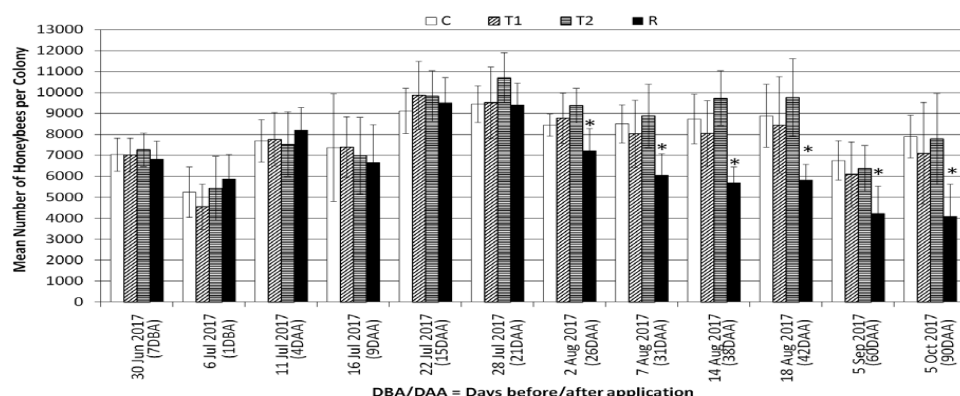


Figure 5. Mean colony size (number of bees per hive) per treatment group

* Statistically significantly different from the control (Student's t-test (method: pooled), one-sided, $p \leq 0.05$)

Amount of brood

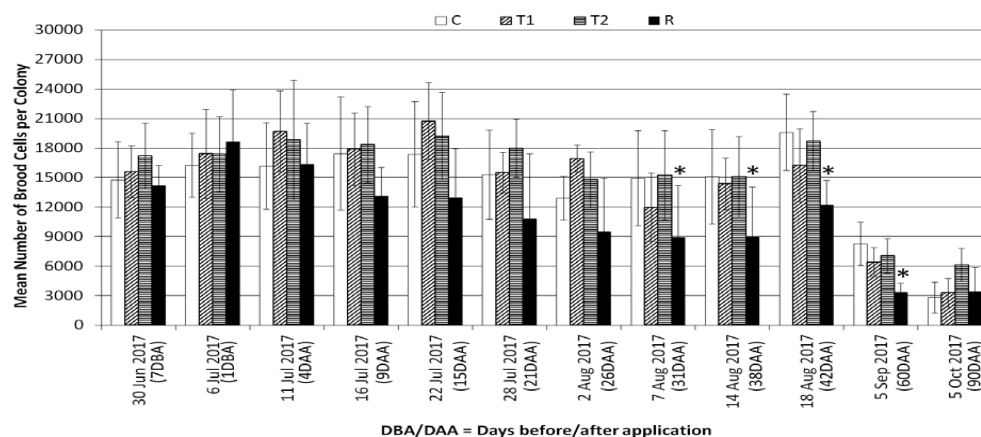


Figure 6. Mean number of brood cells (all brood stages) per treatment group

* Statistically significantly different from the control (Student's t-test (method: pooled) or Mann-Whitney Exact test, one-sided, $p \leq 0.05$)

Photographic Evaluation of Brood Development in Individual Cells

1st brood cycle (1DBA to 21DAA): 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 one of the 5 replicates in each test item treatment due to poor performance of eggs (T1e, to a lesser extent also T2e, though neither of them was statistically significantly different from the control) and young or old larvae (T2e), whereas the other 4 replicates performed very well. Since brood was always present in these hives, the unusual observations in T1e and T2e are not considered an effect of the test item but rather due to poor adaptation of these colonies to the confined situation in the tunnels.

A clear impact of the reference item treatment R on the brood indices, compensation indices and termination rates of eggs and old larvae was observed during the 1st brood cycle, confirming suitability of the test design and exposure of the bee colonies to the treated crop.

Overall, there was no effect of the test item treatment on the termination rates, brood indices and compensation indices of eggs, young larvae and old larvae during the 1st brood cycle.

The results are presented below:

Mean Brood Index of cell initially containing eggs - 1st brood cycle

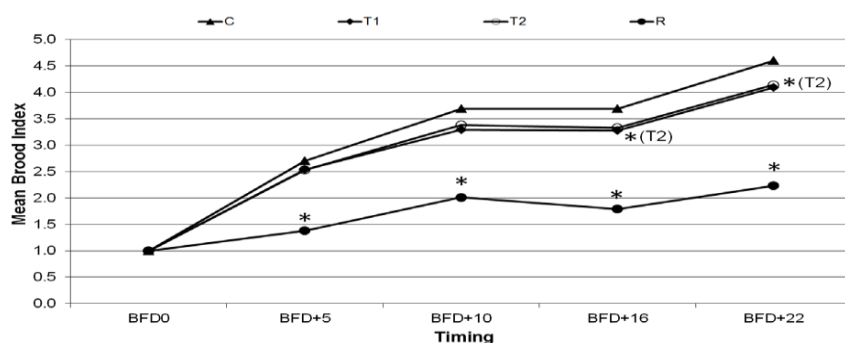


Figure 7. Development of the brood index of cells initially containing eggs (1st brood cycle)

* Statistically significantly different from the control (one-sided Bonferroni U (Holms) Exact test or Satterthwaite t-test, one-sided, $p \leq 0.05$)

Mean Compensation Index of cell initially containing eggs - 1st brood cycle

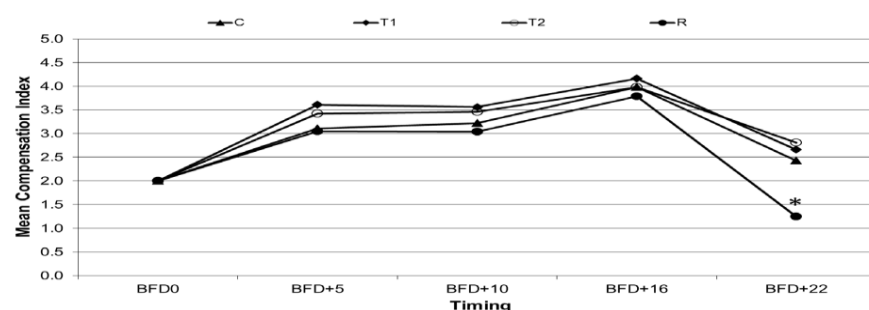


Figure 8. Development of the compensation index of cells initially containing eggs (1st brood cycle)

* Statistically significantly different from the control (Satterthwaite t-test, one-sided, $p \leq 0.05$).

Mean Brood Index of cells initially containing young larvae - 1st brood cycle

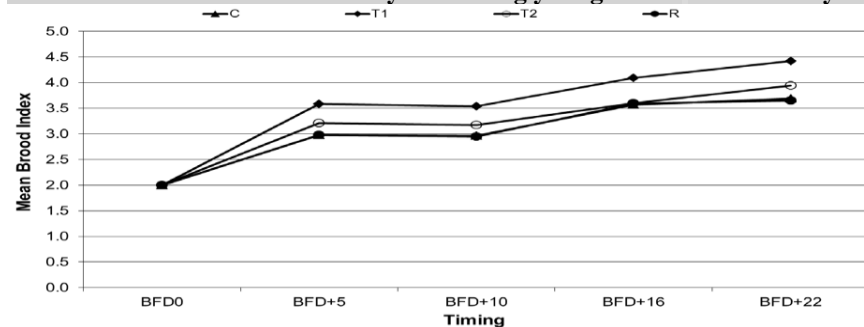


Figure 9. Development of the brood index of cells initially containing young larvae (1st brood cycle)

There were no statistically significant differences between the test item treatments T1 and T2 or the reference item treatment R compared to the control.

Mean Compensation Index initially of cell containing young larvae – 1st brood cycle

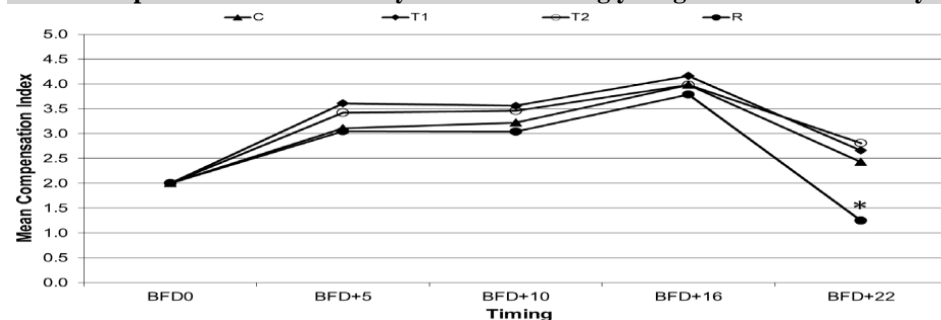


Figure 10. Development of the compensation index of cells initially containing young larvae (1st brood cycle)

* significantly different from the control (Student's t-test (method: pooled), one-sided, $p \leq 0.05$)

Mean Brood Index of cell initially containing old larvae - 1st brood cycle

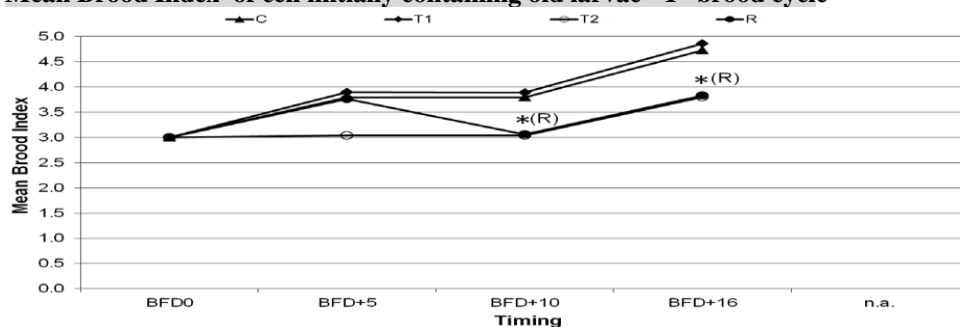


Figure 11. Development of the brood index of cells initially containing old larvae (1st brood cycle)
* Statistically significantly different from the control (Student's t-test (pooled), one-sided, $p \leq 0.05$)
n.a. = not applicable (development of old larvae expected to be complete until BFD+16)

Mean Termination Rate of cells initially containing eggs - 1st brood cycle

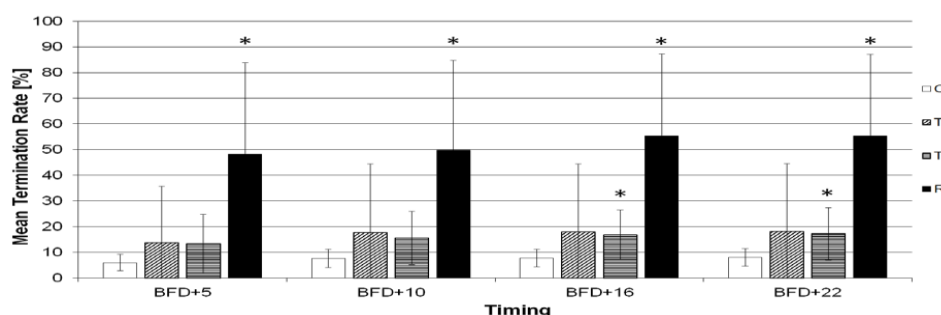


Figure 12. Termination rates of cells initially containing eggs (1st brood cycle)
* Statistically significantly different from the control (one-sided Bonferroni U (Holms) Exact test or Satterthwaite t-test, one-sided, $p \leq 0.05$).

Mean Termination Rate of cells initially containing young larvae - 1st brood cycle

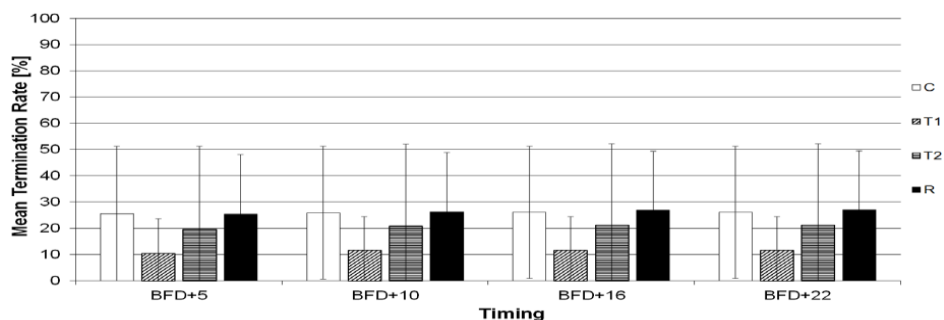


Figure 13. Termination rates of cells initially containing young larvae (1st brood cycle)
There were no statistically significant differences between the test item treatments T1 and T2 or the reference item treatment R compared to the control

Mean Termination Rate of cells initially containing old larvae - 1st brood cycle

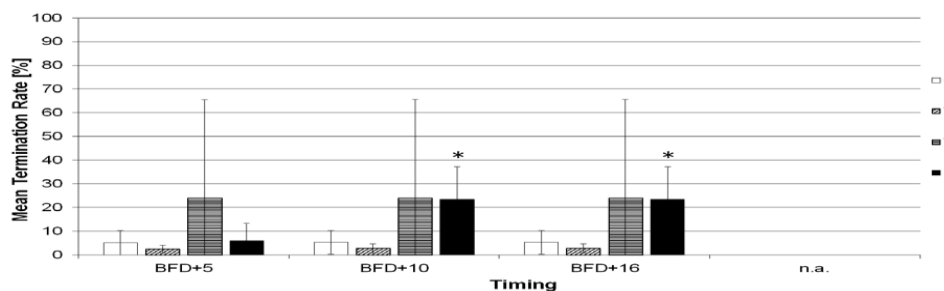


Figure 14. Termination rates of cells initially containing old larvae (1st brood cycle)
* Statistically significantly different from the control (one-sided Student's t-test (pooled), $p \leq 0.05$)
n.a. = not applicable (development of old larvae expected to be complete until BFD+16)

2nd brood cycle

2nd brood cycle (21DAA to 42DAA): there were slight differences of the brood indices, compensation indices and termination rates of eggs in T1 and T2 compared to the control (statistically significant on BFD+17 and BFD+21). There were no statistically significant differences of these indices and termination rates for young larvae or old larvae in T1 and T2 compared to the control on any day of the 2nd brood cycle.

Overall, there was a slight effect of the test item treatments T1 or T2 on the termination rates, brood index and compensation index of eggs, though not on young larvae and old larvae during the 2nd brood cycle.

The results are presented below:

Mean Brood Index of cell initially containing eggs- 2nd brood cycle

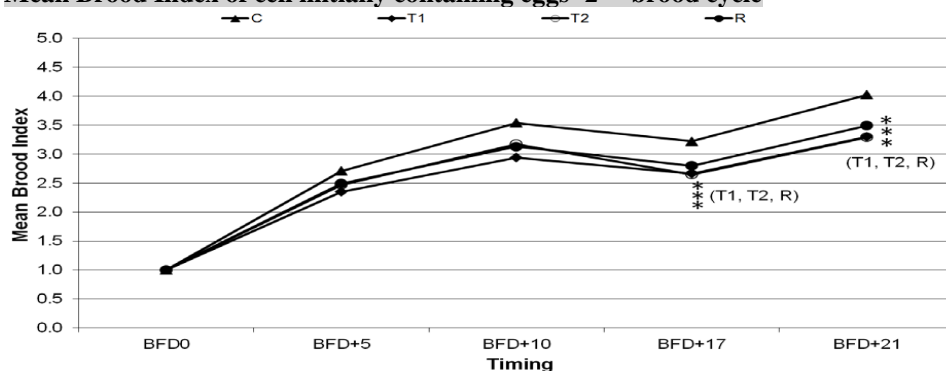


Figure 15. Development of the brood index of cells initially containing eggs (2nd brood cycle)

* Statistically significantly different from the control (one-sided Dunnett's t-test or Student's t-test (pooled), $p \leq 0.05$)

Mean Compensation Index of cell initially containing eggs - 2nd brood cycle

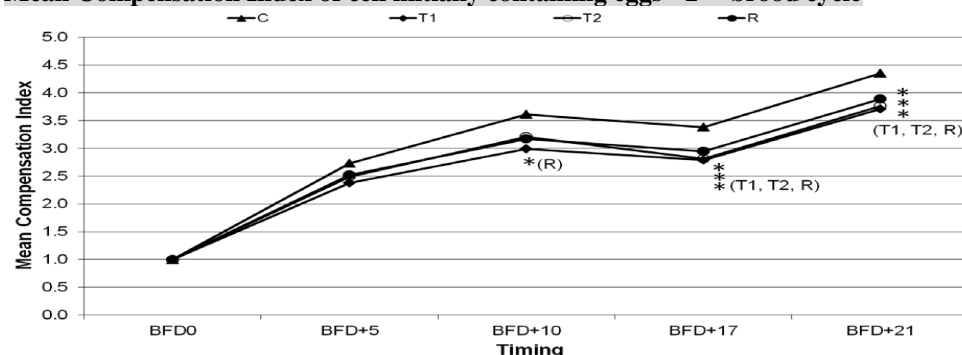


Figure 16 Development of the compensation index of cells initially containing eggs (2nd brood cycle)

* Statistically significantly different from the control (one-sided Dunnett's t-test or Student's t-test (pooled), $p \leq 0.05$)

Mean Termination Rate of cells initially containing eggs - 2nd brood cycle

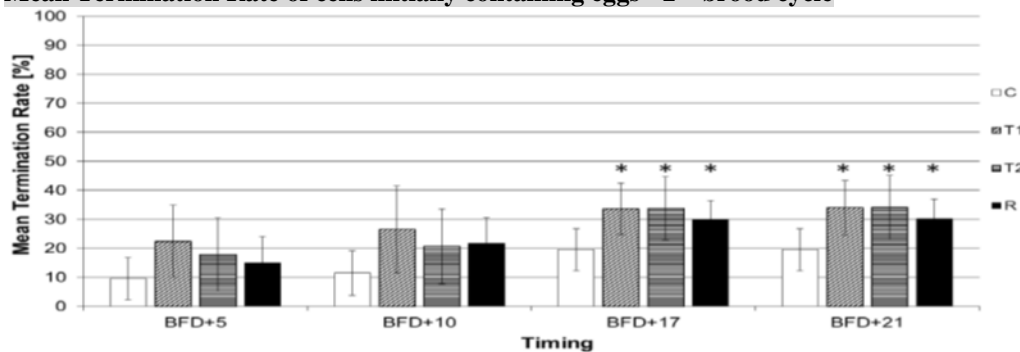


Figure 17. Termination rates of cells initially containing eggs (2nd brood cycle)

* Statistically significantly different from the control (one-sided Dunnett's t-test or Student's t-test (pooled), $p \leq 0.05$)

Mean Brood Index of cell initially containing young larvae - 2nd brood cycle

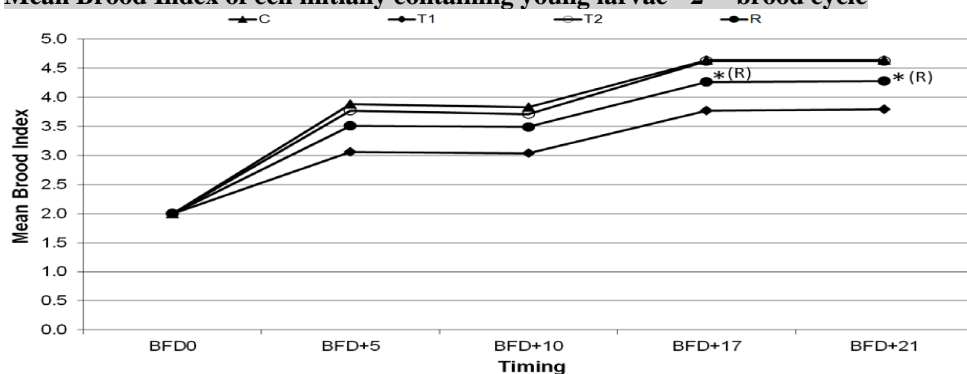


Figure 18. Development of the brood index of cells initially containing young larvae (2nd brood cycle)

* Statistically significantly different from the control (one-sided Student's t-test (pooled), $p \leq 0.05$)

Mean Compensation Index of cell initially containing young larvae - 2nd brood cycle

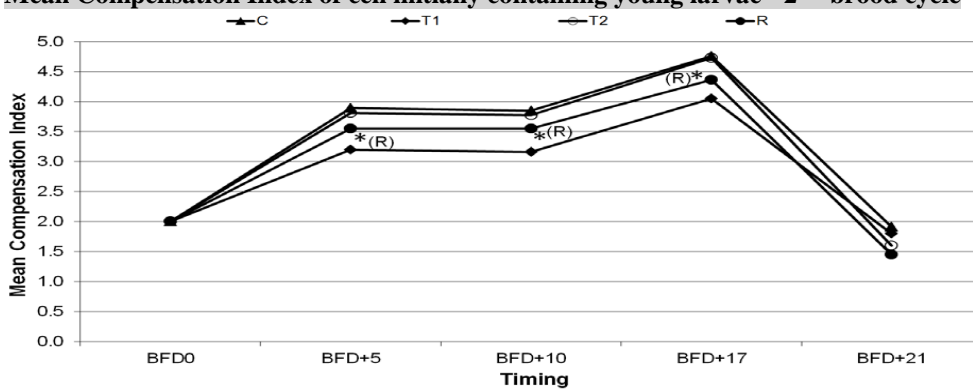


Figure 19. Development of the compensation index of cells initially containing young larvae (2nd brood cycle)

* Statistically significantly different from the control (one-sided Student's t-test (pooled) or Satterthwaite t-test, $p \leq 0.05$)

Mean Termination Rate of cells initially containing young larvae - 2nd brood cycle

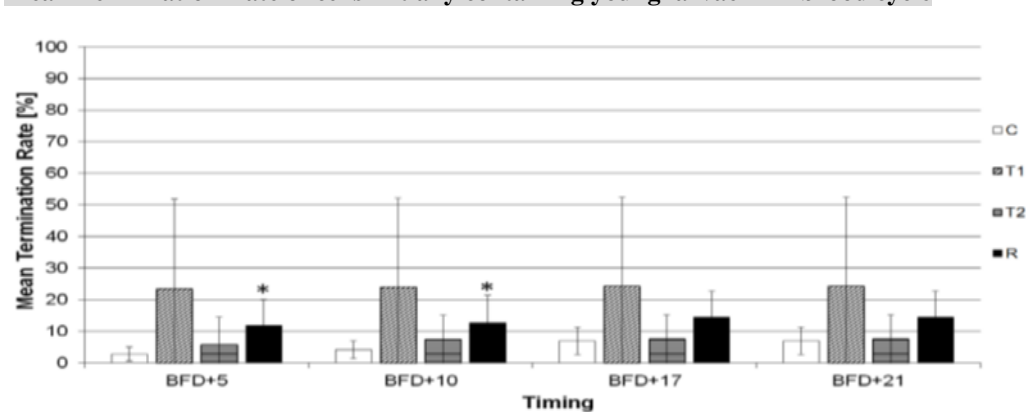


Figure 20. Termination rates of cells initially containing young larvae (2nd brood cycle)

* Statistically significantly different from the control (one-sided Student's t-test (pooled), $p \leq 0.05$)

Mean Brood Index of cell initially containing old larvae- 2nd brood cycle

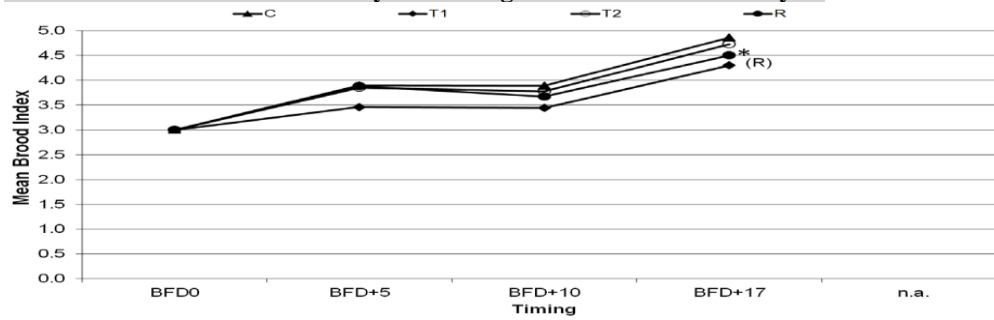


Figure 20. Development of the brood index of cells initially containing old larvae(2nd brood cycle)
* Statistically significantly different from the control (one-sided Student's t-test (pooled), $p \leq 0.05$)
n.a. = not applicable (development of old larvae expected to be complete until BFD+17)

Mean Compensation Index of cell initially containing old larvae - 2nd brood cycle

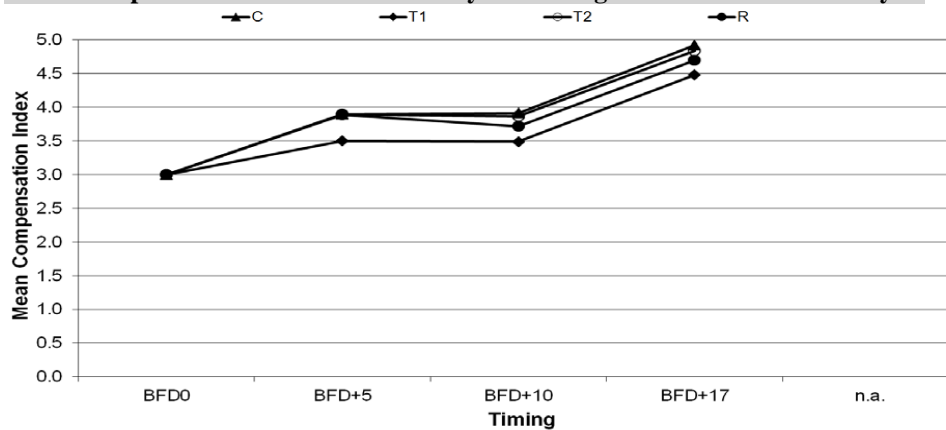


Figure 21. Development of the compensation index of cells initially containing old larvae (2nd brood cycle).
There were no statistically significant differences of T1, T2 or R compared to the control.
n.a. = not applicable (development of old larvae expected to be complete until BFD+17)

Mean Termination Rate of cell containing old larvae - 2nd brood cycle

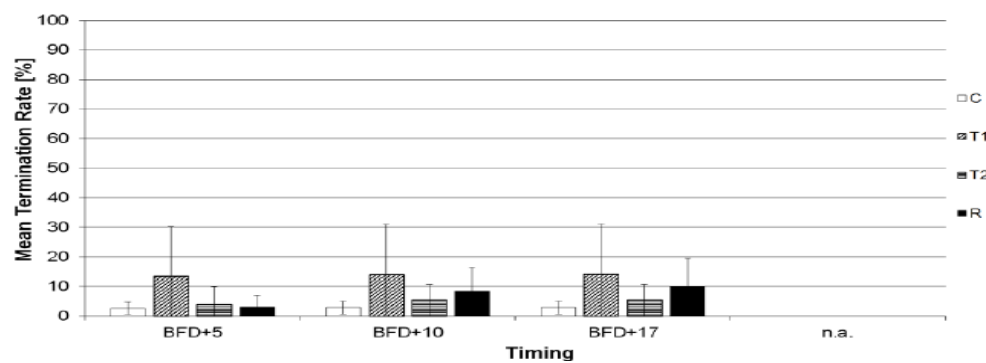


Figure 22: Termination rates of cells initially containing old larvae (2nd brood cycle)
There were no statistically significant differences of T1, T2 or R compared to the control.
n.a. = not applicable (development of old larvae expected to be complete until BFD+17)

Determination of Weight and Assessment of Morphological Abnormalities of Pupae

The weight of pupae collected from combs on 17DAA ranged from 0.1087 to 0.1561 g (mean: 0.1324 g) in the control, from 0.1083 to 0.1452 g (mean: 0.1296 g) in T1, from 0.1190 to 0.1506 g (mean: 0.1330 g) in T2 and from 0.1146 to 0.1445 g (mean: 0.1301 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 except two pupae in T2d where only one eye was pigmented. In the reference item treatment no malformations were recorded but the overall condition of most pupae was poor (decaying condition, rather brownish appearance and in some cases mould growing on some pupae).

Analytical Results

Analyses of residues of GF-3307 in *Phacelia* plants and in nectar and pollen prepared from forager bees resulted in the following residue levels:

Untreated and Control samples:

There were no detectable residues of fenpicoxamid, prothioconazole-desthio or prothioconazole in any of the samples of nectar, pollen and plants of the control group C taken at any date before and after application.

In samples taken before application in T1 and T2, quantifiable residues of fenpicoxamid, prothioconazole-desthio and prothioconazole were detected in plant samples in T1 and T2, in pollen from forager bees in T2 and in T1 (prothioconazole only) as described below. Prothioconazole-desthio in plants was not confirmed by re-analysis of A-samples and analysis of R-samples.

Since no residues were found in the control group C and these samples were taken one day before the applications, and since fenpicoxamid had never been used on this field and prothioconazole had not been used at least during the past 3 years, it is obvious that these values resulted from later contamination either during sampling/sample bagging, during sample preparation or during residue analysis in the laboratory.

Phacelia tanacetifolia plants:

Residues in *Phacelia* plant samples collected in test item treatment T1 ranged from 0.0251 to 0.0272 mg fenpicoxamid/kg before application and from 0.0156 to 0.0347 mg fenpicoxamid/kg after application, from not detectable (<LOD) to 0.0140 mg prothioconazole-desthio/kg before application and from 0.312 to 0.688 mg prothioconazole-desthio/kg after application, and from 0.0165 to 0.0434 mg prothioconazole/kg before application and 0.0943 to 0.552 mg prothioconazole/kg after application.

Residues in *Phacelia* plant samples collected in test item treatment T2 ranged from 0.0343 to 0.0480 mg fenpicoxamid/kg before application and from 0.0570 to 0.0595 mg fenpicoxamid/kg after application, from not detectable (<LOD) to 0.0149 mg prothioconazole-desthio/kg before application and from 0.509 to 1.06 mg prothioconazole-desthio/kg after application, and from 0.0298 to 0.0681 mg prothioconazole/kg before application and 0.141 to 1.92 mg prothioconazole/kg after application.

Pollen from forager bees:

In pollen from forager bees collected in test item treatment T1, there were no quantifiable residues of fenpicoxamid before application and 0.0172 to 7.23 mg fenpicoxamid/kg after application, no detectable residues of prothioconazole-desthio before application and 0.0902 to 2.47 mg/kg after application. Residues of prothioconazole in pollen of treatment group T1 were 0.00154 mg before application and 0.00230 to 11.1 mg prothioconazole/kg after application.

In pollen from forager bees collected in test item treatment T2, residues were 0.00901 to 0.0597 mg fenpicoxamid/kg before application and 0.1960 to 22.3 mg fenpicoxamid/kg after application, 0.00822 to 0.0155 mg prothioconazole-desthio/kg before application and 0.326 to 8.39 mg prothioconazole-desthio/kg after application, and 0.00823 to 0.103 mg prothioconazole/kg before application and 0.0186 to 34.0 mg prothioconazole/kg after application.

Nectar from forager bees:

In the test item treatments T1 and T2, there were no detectable residues of fenpicoxamid, prothioconazole-desthio or prothioconazole in nectar from forager bees collected before the application.

After the application in T1, residues in nectar ranged from not detectable (<LOD) to 0.205 mg fenpicoxamid/kg, from 0.00136 to 0.0543 mg prothioconazole-desthio/kg and from not detectable (<LOD) to 0.140 mg prothioconazole/kg.

After the application in T2, residues in nectar ranged from not detectable (<LOD) to 0.287 mg fenpicoxamid/kg, from 0.00131 to 0.428 mg prothioconazole-desthio/kg and from non-quantifiable (<LOQ) to 0.466 mg prothioconazole/kg.

Table 4 : Effects of GF-3307 on honey bee brood under semi-field conditions – tunnel test

Treatment	Untreated control	GF-3307		Toxic standard
Rate ¹	-	100 g prothioconazole/ha plus 50 g fenpicoxamid/ha	200 g prothioconazole/ha plus 100 g fenpicoxamid/ha	1200 g product/ha
Brood termination rate, 1 st brood cycle				
Eggs:	8.04	18.20	17.23*	55.30*
Young larvae:	26.12	11.60	21.18	27.04
Old larvae:	5.33	2.75	24.01	23.42*
Brood termination rate, 2 nd brood cycle				
Eggs:	19.52	33.92*	34.10*	30.21*
Young larvae:	7.11	24.30	7.56	14.44
Old larvae:	2.81	14.07	5.43	10.04
Dead worker bees ²				
Exposure:	63.2	38.3	56.6	49.5
Monitoring:	34.2	33.2	22.4	43.4
Dead pupae ²				
Exposure:	0.1	0.1	0.2	0.5*
Monitoring:	0.2	0.2	0.1	25.3*

¹ Delivered in 300 L/ha of water

² Over the post-application period (exposure period in the tunnels 8 days (0DAA to 7DAA), further monitoring over 35 days (8DAA to 42DAA); mean value per hive per day (5 replicates per treatment)

* statistically significantly different from the control (one-sided Dunnett's t-test, Bonferroni-U (Holms) Exact test, Satterthwaite t-test or pooled t-test, p≤0.05)

CONCLUSION

One application of GF-3307 at rates of 50 g fenpicoxamid a.i./ha plus 100 g prothioconazole a.i./ha (treatment T1) or 100 g fenpicoxamid a.i./ha plus 200 g prothioconazole a.i./ha (treatment T2), applied at full flowering and during daily honeybee flight activity, had effects as follows:

There was no adverse effect of the test item treatments T1 and T2 on the mortality of adult worker bees, worker bee pupae, male adult bees and male pupae, foraging activity, colony size, amount of brood (total number of brood cells or number of cells with eggs, larvae or pupae) and storage of nectar and pollen.

There was a slight adverse effect on the behaviour of worker bees in test item treatment T1 during the monitoring period outside tunnels and a slight transient effect on honeybee behaviour in T2 on the day of application (0DAA).

There was no effect of the test item treatment on the termination rates, brood indices and compensation indices of eggs during the 1st brood cycle (1DBA to 21DAA) and no effect on the young larvae and old larvae during the 1st and 2nd brood cycle (1DBA to 42DAA). There was a slight effect on the termination rates, brood index and compensation index of eggs on BFD+17 and BFD+21 of the 2nd brood cycle.

There was no effect of the test item treatments T1 or T2 on the weight or malformations of pupae sampled from combs during the 1st brood cycle (17DAA).

Residue analysis:

Analyses of residues of GF-3307 in *Phacelia* plants and in nectar and pollen prepared from forager bees resulted in the following residue levels:

Untreated and Control samples:

There were no detectable residues of fenpicoxamid, prothioconazole-desthio or prothioconazole in any of the samples of nectar, pollen and plants of the control group C taken at any date before and after application.

Residues of the analytes in samples of *Phacelia* plants and pollen from forager bees taken before application in T1 and/or T2 are considered as due to later contamination during sampling or sample processing/analysis in the laboratory.

Phacelia tanacetifolia plants:

Residues in *Phacelia* plant samples collected in test item treatment T1 ranged from 0.0251 to 0.0272 mg fenpicoxamid/kg before application and from 0.0156 to 0.0347 mg fenpicoxamid/kg after application, from not detectable (<LOD) to 0.0140 mg prothioconazole-desthio/kg before application and from 0.312 to 0.688 mg prothioconazole-desthio/kg after application, and from 0.0165 to 0.0434 mg prothioconazole/kg before application and 0.0943 to 0.552 mg prothioconazole/kg after application.

Residues in *Phacelia* plant samples collected in test item treatment T2 ranged from 0.0343 to 0.0480 mg fenpicoxamid/kg before application and from 0.0570 to 0.0595 mg fenpicoxamid/kg after application, from not detectable (<LOD) to 0.0149 mg prothioconazole-desthio/kg before application and from 0.509 to 1.06 mg prothioconazole-desthio/kg after application, and from 0.0298 to 0.0681 mg prothioconazole/kg before application and 0.141 to 1.92 mg prothioconazole/kg after application.

Pollen from forager bees:

In pollen from forager bees collected in test item treatment T1, there were no quantifiable residues of fenpicoxamid before application and 0.0172 to 7.23 mg fenpicoxamid/kg after application, no detectable residues of prothioconazole-desthio before application and 0.0902 to 2.47 mg/kg after application. Residues of prothioconazole in pollen of treatment group T1 were 0.00154 mg before application and 0.00230 to 11.1 mg prothioconazole/kg after application.

In pollen from forager bees collected in test item treatment T2, residues were 0.00901 to 0.0597 mg fenpicoxamid/kg before application and 0.1960 to 22.3 mg fenpicoxamid/kg after application, 0.00822 to 0.0155 mg prothioconazole-desthio/kg before application and 0.326 to 8.39 mg prothioconazole-desthio/kg after application, and 0.00823 to 0.103 mg prothioconazole/kg before application and 0.0186 to 34.0 mg prothioconazole/kg after application.

Nectar from forager bees:

In the test item treatments T1 and T2, there were no detectable residues of fenpicoxamid, prothioconazole-desthio or prothioconazole in nectar from forager bees collected before the application.

After the application in T1, residues in nectar ranged from not detectable (<LOD) to 0.205 mg fenpicoxamid/kg, from 0.00136 to 0.0543 mg prothioconazole-desthio/kg and from not detectable (<LOD) to 0.140 mg prothioconazole/kg. After the application in T2, residues in nectar ranged from not detectable (<LOD) to 0.287 mg fenpicoxamid/kg, from 0.00131 to 0.428 mg prothioconazole-desthio/kg and from non-quantifiable (<LOQ) to 0.466 mg prothioconazole/kg.

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

A 2.3.1.6.1.1 Determination of Residues of Fenpicoxamid and Prothioconazole in Nectar, Pollen and Plants of Winter Oilseed Rape after One Application of GF-3307 in a Semi-Field Residue Study in Central and Southern Europe in 2020

Comments of zRMS:	<p>The method of determination of residues of Fenpicoxamid and Prothioconazole and amount of residues of both active substances in Nectar, Pollen and Plants of Winter Oilseed Rape after One Application of GF-3307 in a Semi-Field Residue Study provided in Central and Southern Europe in 2020 was submitted.</p> <p>However, the applicant did not provide the summary of this residue study from which the trials were taken in order to analyze residues of active substances in nectar and pollen after application of GF-3307 in flowering oilseed rape.</p> <p>The Applicant is kindly reminded that detailed summaries of all studies used in evaluation must be presented in dRR.</p> <p>However, the study was generated for application of GF-3307 in flowering oilseed rape and as being not relevant for the intended use pattern of GF-3307 in application in sugar beet and then was not considered in the risk assessment.</p>
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Reference:	CP 10.3.1.6/1
Report:	Appeltauer, A. — 2021 — Determination of Residues of Fenpicoxamid and Prothioconazole in Nectar, Pollen and Plants of Winter Oilseed Rape after One Application of GF-3307 in a Semi-Field Residue Study in Central and Southern Europe in 2020; DAS Study No. 200670; Eurofins Agroscience Services Ecotox GmbH, Germany, GLP, unpublished
Acceptability:	Yes
Duplication (if vertebrate study)	NA

Risk Assessment Analytical Method Summary

Material and Methods

Method Principle

Residues of fenpicoxamid, prothioconazole and prothioconazole desthio were extracted/determined from samples of pollen from forager bees, nectar from forager bees and whole plants (winter oil seed rape) by extraction (pollen, whole plant) or dilution (nectar) with acetonitrile/water (50/50, v/v) + 0.1 % formic acid and no liquid/liquid partition for nectar or liquid/liquid partition by addition of magnesium sulphate, sodium chloride and sodium citrate followed by subsequent centrifugation for pollen and whole plant samples. No clean-up / purification was performed for nectar and purification of an aliquot of the acetonitrile extract by dispersive SPE with primary/secondary amine (PSA) and graphitized carbon black (GCB) for pollen and whole plant samples. The final sample was analysed for fenpicoxamid, prothioconazole and prothioconazole desthio by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70–110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table:1 Recovery results from method validation of Fenpicoxamid (m/z 615/239Q) using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Nectar	0.001	99	2	5	
Nectar	0.01	101	6	5	
Pollen	0.001	99	14	5	
Pollen	0.01	97	5	5	
Whole Plant	0.001	96	4	5	
Whole Plant	0.01	97	3	5	

Table:2 Recovery results from method validation of Prothioconazole (m/z 342/58Q) using the analytical method*

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Nectar	0.001	87	7	5	
Nectar	0.01	92	6	5	
Pollen	0.001	102	14	5	
Pollen	0.01	93	5	5	
Whole Plant	0.001	77	9	5	
Whole Plant	0.01	84	7	5	

*Only used for method verification, transition was changed for sample analysis due to response fluctuations when using bipolar mode for longer sequences.

Table:3 Recovery results from method validation of Prothioconazole-desthio (m/z 312/70Q) using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Nectar	0.001	98	2	5	
Nectar	0.01	98	3	5	
Pollen	0.001	107	11	5	
Pollen	0.01	93	2	5	
Whole Plant	0.001	98	3	5	
Whole Plant	0.01	98	2	5	

Table:4 Procedural recovery results of Fenpicoxamid (m/z 615/239Q) using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Nectar	0.001	90	2	5	
Nectar	0.01	109	5	5	
Nectar	10	99	3	5	
Pollen	0.001	91	19	8	
Pollen	0.01	100	7	5	
Pollen	50	92	3	5	
Whole Plant	0.001	88	4	5	

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Whole Plant	0.01	106	3	8	
Whole Plant	4	104	3	5	

Table:5 Procedural recovery results of Prothioconazole (m/z 344/189Q) (m/z 344/154Q) using the analytical method*

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Nectar	0.001	102	8	5	
Nectar	0.01	98	3	5	
Nectar	40	100	3	5	
Pollen	0.001	97	12	8	
Pollen	0.01	94	8	5	
Pollen	50	93	2	5	
Whole Plant	0.001	77	4	5	
Whole Plant	0.01	89	5	8	
Whole Plant	4	91	4	5	

*Mass transition 344/154 m/z for whole plant only

Table:6 Procedural recovery results of Prothioconazole-desthio (m/z 312/70Q) using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Nectar	0.001	110	1	5	
Nectar	0.01	93	5	5	
Nectar	40	91	3	5	
Pollen	0.001	94	13	7	
Pollen	0.01	95	4	5	
Pollen	50	85	3	5	
Whole Plant	0.001	83	7	5	
Whole Plant	0.01	99	1	8	
Whole Plant	4	99	6	5	

Table:7 Characteristics for the analytical method used for determination of residues of Fenpicoxamid, Prothioconazole and Prothioconazole-desthio in Pollen

Analyte	Fenpicoxamid	Prothioconazole	Prothioconazole-desthio
Matrix	Pollen	Pollen	Pollen
Technique	LC-MS/MS	LC-MS/MS	LC-MS/MS
Specificity	m/z 615/239Q m/z 615/515C blank value <30% LOQ	m/z 344/189Q m/z 344/154C blank value <30% LOQ	m/z 312/70Q m/z 312/125C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r ² ≥0.9984 8 data points	linear regression analysis with 1/x weighting r ² ≥0.9994 8 data points	linear regression analysis with 1/x weighting r ² ≥0.9998 8 data points

Calibration range	Concentration range of 0.01–5 ng/mL (equivalent sample concentration 0.0003–0.15 mg/kg)	Concentration range of 0.01–5 ng/mL (equivalent sample concentration 0.0003–0.15 mg/kg)	Concentration range of 0.01–5 ng/mL (equivalent sample concentration 0.0003–0.15 mg/kg)
Limit of quantitation	0.001 mg/kg	0.001 mg/kg	0.001 mg/kg
Validation Range	0.001–50 mg/kg	0.001–50 mg/kg	0.001–50 mg/kg

Table:8 Characteristics for the analytical method used for determination of residues of Fenpicoxamid, Prothioconazole and Prothioconazole-desthio in Nectar

Analyte	Fenpicoxamid	Prothioconazole	Prothioconazole-desthio
Matrix	Nectar	Nectar	Nectar
Technique	LC-MS/MS	LC-MS/MS	LC-MS/MS
Specificity	m/z 615/239Q m/z 615/515C blank value <30% LOQ	m/z 344/189Q m/z 344/154C blank value <30% LOQ	m/z 312/70Q m/z 312/125C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.9998$ 8 data points	linear regression analysis with 1/x weighting $r \geq 0.9998$ 8 data points	linear regression analysis with 1/x weighting $r \geq 0.9997$ 8 data points
Calibration range	Concentration range of 0.01–5 ng/mL (equivalent sample concentration 0.0003–0.15 mg/kg)	Concentration range of 0.01–5 ng/mL (equivalent sample concentration 0.0003–0.15 mg/kg)	Concentration range of 0.01–5 ng/mL (equivalent sample concentration 0.0003–0.15 mg/kg)
Limit of quantitation	0.001 mg/kg	0.001 mg/kg	0.001 mg/kg
Validation Range	0.001–10 mg/kg	0.001–10 mg/kg	0.001–10 mg/kg

Table:9 Characteristics for the analytical method used for determination of residues of Fenpicoxamid, Prothioconazole and Prothioconazole-desthio in Whole Plant

Analyte	Fenpicoxamid	Prothioconazole	Prothioconazole-desthio
Matrix	Whole Plant	Whole Plant	Whole Plant
Technique	LC-MS/MS	LC-MS/MS	LC-MS/MS
Specificity	m/z 615/239Q m/z 615/515C blank value <30% LOQ	m/z 344/154Q m/z 344/189C blank value <30% LOQ	m/z 312/70Q m/z 312/125C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.9991$ 8 data points	linear regression analysis with 1/x weighting $r \geq 0.9990$ 8 data points	linear regression analysis with 1/x weighting $r \geq 0.9999$ 8 data points
Calibration range	Concentration range of 0.03–5 ng/mL (equivalent sample concentration 0.0003–0.05 mg/kg)	Concentration range of 0.03–5 ng/mL (equivalent sample concentration 0.0003–0.05 mg/kg)	Concentration range of 0.03–5 ng/mL (equivalent sample concentration 0.0003–0.05 mg/kg)
Limit of quantitation	0.001 mg/kg	0.001 mg/kg	0.001 mg/kg
Validation Range	0.001–4 mg/kg	0.001–4 mg/kg	0.001–4 mg/kg

Conclusion

This method was successfully validated for the determination of fenpicoxamid, prothioconazole and

~~prothioconazole-dethio in nectar, pollen and whole plants from winter oilseed rape.~~

~~quantifiable (<LOQ) to 0.466 mg prothioconazole/kg.~~

~~A 2.3.1.6.2~~ Study 1 - GF-3307 (Fenpicoxamid + Prothioconazole): Brood Development of the Honeybee (*Apis mellifera* L.) in a Semi-Field Tunnel Study in *Phacelia tanacetifolia* in Germany 2017
Assessment of Side-Effects on the GF-3307 (Fenpicoxamid and Prothioconazole): Brood Development of the Honey Bee (*Apis mellifera* L.) in a Colony Feeding Test in Germany 2020;

<p>Comments of zRMS:</p>	<p>The colony feeding study has been submitted in support of the current application GF-3307 in cereals and for extended use in sugar beet. The study was previously peer reviewed during commenting period process and no comments were given to this study. The study is considered acceptable for extended uses in sugar beet.</p> <p>The study was conducted in accordance with the OEPP/EPPO Bulletin No. 22 (Oomen et al., 1992) with partly integration of the OECD Guidance Document No. 75 (2007) with major deviations and is considered to be valid by the zRMS.</p> <p>The following validity criteria were met:</p> <ul style="list-style-type: none"> - A detectable effect of both toxic reference items was found (e. g. mean brood termination rate of egg cells during 1st brood cycle in R2 $\geq 70\%$ and a significant increase in worker mortality in R1 and pupal mortality in R2 during the feeding phase). - The mean brood termination rate for eggs over all replicates in the control group was $\leq 30\%$ at the end of both brood cycles <p>The feeding study was conducted in near Pforzheim (Baden-Württemberg) in Germany. The trial was conducted at one field site with honey bee colonies having free access to nectar and pollen sources.</p> <p>The study consisted of eight treatment groups: a control group, five test item groups T1, T2, T3, T4 and T5 (a.s.: Fenpicoxamid and Prothioconazole), two toxic reference item groups R1 (dimethoate) and R2 (fenoxycarb). The test item / reference items were applied as feeding solution in sugar solution (Apiinvert®) and the colonies of the untreated control were fed with pure Apiinvert®.</p> <p>All colonies were fed during 9 consecutive days at rates of 4, 20, 50, 100, 500 mg product/L in treatment group T1, T2, T3, T4 and T5, respectively, and at rates of 11.21 mg a.i./L and 84 mg a.s./L in the toxic reference item group R1 and R2, respectively.</p> <p>The mortality, behaviour of the bees, condition of the colonies, hive weight, brood development and morphological abnormalities of pupae were examined.</p> <p>The influence of the test item GF-3307 was evaluated by comparing the data of the five test item treatment groups to the control group C. The magnitude of residues in pollen, nectar, honey, pupae, larvae and worker jelly under field conditions were determined.</p> <p>Assessments were carried out for up to 358 days after beginning of feeding; the development of brood in individually marked cells was evaluated over two brood cycles</p> <p>The investigated parameters and timing of observations were in line with recommendations of OECD 75.</p> <p>The results on honeybee flight activity had effects as follows:</p> <ul style="list-style-type: none"> • No effect on mortality of worker bees, pupae and larvae, behaviour of worker bees, development on eggs, young larvae and old larvae in individual cells, mean weight of pupae, mean number of food cells, total amount of brood and colony size were observed for GF-3307 up to the highest concentration of 500 mg GF-3307/L sugar solution. • Slight, but significant effects on adult mortality and mean brood termination rate
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	<p>at 50 mg GF-3307/L were observed, but they were assumed to be caused by high variability of data since they were not dose related.</p> <ul style="list-style-type: none"> It should be noted that the loss of queens observed in several colonies was assumed by the study author to be caused by disturbance of the colonies during assessment. The residues determined in pollen, nectar honey, worker jelly, larvae and pupae declined rapidly in all matrices inside the colony.
Reference:	KCP 10.3.1.6/2
Report:	Gonsoir, G. 2021; GF-3307: Assessment of Side-Effects on the GF-3307 (Fenpicoxamid and Prothioconazole): Brood Development of the Honey Bee (<i>Apis mellifera</i> L.) in a Colony Feeding Test in Germany 2020; Eurofins Agrosience Services Ecotox GmbH, D-75223 Niefern-Öschelbronn Germany; Lab Study No. S20-02058; DAS Study No. 200660; 14 April 2021; Unpublished
Guideline(s):	OEPP/EPPO Bulletin No. 22 (Oomen et al. 1992), OECD guidance document No.75 (2007)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	NA

COMPLIANCE

Guideline(s):	OEPP/EPPO Bulletin No. 22 (Oomen et al. 1992), OECD guidance document No.75 (2007)
US EPA Guideline(s):	-
Deviations:	no deviations from the guidelines
Dates of work:	27 April 2020 to 23 August 2021
GLP status:	Yes
Number of pages in final report:	668

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-3307 (Fenpicoxamid and Prothioconazole)
Content of a.i., analysed:	101 g/L for Prothioconazole and 49 g/L for Fenpicoxamid
Description (physical state):	liquid / brown
Lot/batch no.:	MAR19CE01Q

Test System

Organism (<i>Species</i>):	Honey bee (<i>Apis mellifera</i>)
Study type:	Honeybee effect study, Colony feeding test
Study design:	Oomen study under field conditions

Test concentrations:	The test item, Fenpicoxamid and Prothioconazole (formulation GF-3307), was applied via feeding solution at 4 mg product/L sugar solution in T1, 20 mg product/L sugar solution in T2, 50 mg product/L sugar solution in T3, 100 mg product/L sugar solution in T4 and 500 mg product/L sugar solution in T5. Test item was provided via Apiinvert® sucrose solution for 9 consecutive days, 500 mL per colony and day.
Dosing method:	Each day freshly prepared test feeding solution was offered to the hives; amounts not consumed by colonies were removed and weighed.
Environmental conditions:	Until the end of the second brood cycle (5DBF to 43DAF) rainfall (sum of 89.1 mm; measured by a rain gauge), relative air humidity from minimum 29.0% to maximum 99.4% and minimum and maximum temperatures of -0.7 and 27.0 °C (recorded by a data logger) were recorded. From the end of the second brood cycle until beginning of overwintering (44DAF to 153DAF) rainfall (sum of 146.6 mm; measured by a weather station), relative air humidity from minimum 21.6% to maximum 99.7% and minimum and maximum temperatures of 2.8 and 35.9 °C (recorded by a data logger) were recorded. During overwintering (154DAF to 358DAF) rainfall (sum of 283.0 mm), relative air humidity from minimum 20.7% to maximum 100% and minimum and maximum temperatures of -13.7 and 26.0 °C were recorded (weather station).
Reference substance:	Dimethoate, BAS 152 11 I Fenoxycarb, Insegar

Aim of the study

The aim of the study was to determine potential effects of GF-3307 (active substance: Fenpicoxamid and Prothioconazole) on the honey bee mortality and behaviour, condition of the colonies, hive weight, honey bee brood development over two brood cycles, overwintering success, morphological abnormalities and weight of pupae and *Varroa* infestation of the colonies. The aim of the study was also to determine the magnitude of residues in pollen, nectar, honey, pupae, larvae and worker jelly under field conditions. Additionally, samples of honeybees and honey from bee colonies were taken for the analysis of bee diseases and bee viruses at the beginning of the study, before and after overwintering. The effect of the test item was examined on commercial honey bee colonies (*Apis mellifera carnica* L.; Hymenoptera, Apidae) under field conditions.

Methodology

The study consisted of eight treatment groups: five test item groups (T1, T2, T3, T4 and T5) consisting of GF-3307 treatment, each replicated five times (a – e) plus one additional replicate for residue sampling and sampling of pupae for the assessment of morphological abnormalities (T1s, T2s, T3s, T4s and T5s), one control group (C) consisting of five replicates (Ca – Ce) plus one replicate for residue sampling and sampling of pupae for the assessment of morphological abnormalities (Cs). One reference item treated group was treated with active substance dimethoate (R1) and another reference item group was treated with active substance: fenoxycarb (R2), each replicated two times (a – b,) plus one additional replicate for sampling of pupae for the assessment of morphological abnormalities (R1s and R2s). Each replicate comprised one colony. The test item and reference items were applied as feeding solution in sugar solution (Apiinvert®) and the colonies of the untreated control were fed with pure Apiinvert® (500 mL per colony and day). All colonies were fed during 9 consecutive days at rates of 4, 20, 50, 100, 500 mg product/L in treatment group T1, T2, T3, T4 and T5, respectively, and at rates of 11.21 mg a.i./L and 84 mg a.i./L in the toxic reference item group R1 and R2, respectively. Each day freshly prepared test feeding solution was offered to the hives; amounts not consumed by colonies were removed and

weighed.

The colonies were placed on the test site 5 days before start of feeding and remained there over the whole duration of the study. The colonies were free-flying, with access to natural nectar sources.

Assessments of mortality and behaviour were carried out daily from 4DBF (DBF = Days before feeding) to 43DAF (DAF = Days after start of feeding).

The condition of the colonies and the development stage of the bee brood were assessed twice before application (7/6DBF, 0DBF), 12 times during exposure and during further monitoring (10DAF, 22DAF, 33DAF, 44DAF, 58DAF, 70DAF, 84DAF, 98DAF, 113DAF, 126DAF, 140DAF, 153DAF and 358DAF). In total, 15 assessments were carried out.

The development of brood in individually marked cells (target: ≥ 200 cells containing eggs, ≥ 200 cells containing young larvae and ≥ 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 first feeding was performed (0DBF = BFD0; BFD = brood area fixing day) and lasted until BFD+22. The second brood cycle started on 22 DAF (last day of brood cycle one; BFD0 for the 2nd cycle) and lasted until 44 DAF (BFD+22).

RESULTS AND DISCUSSION

Table 2: GF-3307 (Fenpicoxamid and Prothioconazole) on honey bee brood in a colony feeding test

Treatment	Untreated control	GF-3307 (Fenpicoxamid and Prothioconazole)					Toxic standard	
							Dimethoate	Insegar
Rate	–	4 mg-/kg-sugar solution ^a	20 mg-/kg-sugar solution ^a	50 mg-/kg-sugar solution ^a	100 mg-/kg-sugar solution ^a	500 mg-/kg-sugar solution ^a	11.21 mg a.i./kg-sugar-solution ^a	84 mg a.i./kg-sugar-solution ^a
Dead worker bees [†]	67.3 ± 16.6	69.6 ± 31.5	59.5 ± 16.2	89.9 ± 10.6	70.9 ± 30.3	56.0 ± 9.8	408.7 ± 389.8	49.1 ± 11.6
Dead worker larvae and pupae [†]	0.3 ± 0.2	0.3 ± 0.4	0.1 ± 0.0	0.4 ± 0.6	0.8 ± 0.8	0.1 ± 0.1	0.3 ± 0.4	0.3 ± 0.1

^a density of Apimvert ®: 1.352 g/cm³ (non-GLP record)

[†] over the exposure period (1DAF to 8DAF)

Overall test item treatments T1, T2, T3, T4 and T5 had no effect on the mortality of worker bees during the entire exposure and monitoring period. In the reference item group R1 significant effects could be seen starting at 2DAF and lasting until 18DAF.

Test item treatments T1, T2, T3, T4 and T5 had no significant effect on the mortality of worker pupae and larvae bees during the whole exposure period. During the first brood cycle some significant effects were observed in the reference item treatment group R1. In R2 significant effects of treatment were observed through the exposure time, confirming suitability of the test design.

Mortality of Queens

Dead queens, hives without brood or with unmarked queens were found in T3e (10DAF, 15 May 2020), R1a (10DAF, 15 May 2020), R1b (11DAF, 16 May 2020), T4d (13DAF, 18 May 2020), T3e (19DAF, 24 May 2020), R1a (22DAF, 27 May 2020), T4d (24DAF, 29 May 2020), R1a (33DAF 07 Jun 2020), T5d (58DAF, 02 Jul 2020), T3d (98DAF, 11 Aug 2020), T4c (113DAF, 26 Aug 2020), and T4e (141DAF, 23 Sep 2020).

It can be assumed that the loss of queens was caused by the disturbance during colony assessments, samplings and photographic assessments.

Details to the mean mortality of adult worker bees can be found in Table 1.

Mortality:

Adult worker bees

Mean pre-exposure mortality (mean number of dead worker bees per day) during the period from 4DBF to 0DBF was 32.7 in the control, 38.4 in T1, 24.1 in T2, 50.5 in T3, 35.9 in T4, 34.2 in T5, 36.8 in R1 and 34.5 in R2.

Table 1. Mean daily mortality of adult worker bees per treatment group

Treatment group (dead worker bees/colony) mean \pm STD	C	T1	T2	T3	T4	T5	R1	R2
Mean pre-exposure period (4DBF to 0DBF)	32.7 \pm 14.4	38.4 \pm 7.2	24.1 \pm 7.5	50.5 \pm 21.8	35.9 \pm 14.9	34.2 \pm 16.8	36.8 \pm 28.3	34.5 \pm 9.2
Mean exposure during feeding (1DAF to 8DAF)	67.3 \pm 16.6	69.6 \pm 31.5	59.5 \pm 16.2	89.9 \pm 10.6	70.9 \pm 30.3	56.0 \pm 9.8	408.7 \pm 389.8	49.1 \pm 11.6
Mean exposure period 1 st brood cycle (1DAF to 22DAF)	39.7 \pm 9.5	45.4 \pm 24.6	35.3 \pm 8.2	55.9 \pm 10.7	41.4 \pm 15.6	33.8 \pm 4.5	241.2 \pm 220.2	28.2 \pm 4.7
Mean exposure period (1DAF to 43DAF)	30.8 \pm 7.6	39.1 \pm 22.7	28.6 \pm 5.2	43.9 \pm 12.7	32.7 \pm 4.2	31.9 \pm 9.5	135.8 \pm 120.8	23.4 \pm 6.2

DBF/DAF = Days before/after start of feeding, STD = standard deviation

The daily mean mortality of adult worker bees did not statistically differ during the feeding period (1DAF to 8DAF) in T1, T2, T3, T4, T5 and R2 when compared to the control. For R1 significant differences were detected on four out of 10 days. During the first brood cycle (1DAF to 22DAF), the mortality of adult worker bees did not significantly differ to the control in the test and reference item treatment groups of T1, T2, T4, T5 and R2. Regarding treatment T3, significances were detected on three out of 22 days. However, no significant differences were determined for mean mortality of adult worker bees in T1, T2, T3, T4, T5 and R2 and R1. For reference treatment R1 significant differences were detected on 11 out of 22 days. Overall test item treatments T1, T2, T3, T4 and T5 had no effect on the mortality of worker bees during the entire exposure and monitoring period. In the reference item group R1 significant effects could be seen starting at 2DAF and lasting until 18DAF.

Worker larvae and pupae

Details to the mean mortality of worker larvae and pupae is shown in Table 2.

Before start of feeding (4DBF to 0DBF), the mean daily worker pupae and larvae mortality was 0.5 dead pupae and larvae /colony/day in the control, 1.1 in T1, 0.3 in T2, 0.7 in T3, 0.3 in T4, 0.7 in T5, 1.5 in R1 and 0.6 in R2.

Table 2. Mean daily mortality of worker larvae and pupae per treatment group

Treatment group (dead worker larvae and pupae/colony) mean \pm STD	C	T1	T2	T3	T4	T5	R1	R2
Mean pre-exposure period (4DBF to 0DBF)	0.5 \pm 0.4	1.1 \pm 1.6	0.3 \pm 0.4	0.7 \pm 0.4	0.3 \pm 0.3	0.7 \pm 0.7	1.5 \pm 1.0	0.6 \pm 0.3
Mean exposure during feeding (1DAF to 8DAF)	0.3 \pm 0.2	0.3 \pm 0.4	0.1 \pm 0.0	0.4 \pm 0.6	0.8 \pm 0.8	0.1 \pm 0.1	0.3 \pm 0.4	0.4 \pm 0.1
Mean exposure period 1 st brood cycle (1DAF to 22DAF)	0.3 \pm 0.1	0.5 \pm 0.3	0.2 \pm 0.1	0.7 \pm 0.9	0.8 \pm 1.0	0.1 \pm 0.1	5.0 \pm 6.7	178.2* \pm 47.8
Mean exposure period (1DAF to 43DAF)	0.3 \pm 0.2	0.3 \pm 0.2	0.2 \pm 0.1	0.5 \pm 0.5	0.6 \pm 0.7	0.1 \pm 0.1	4.0 \pm 5.2	167.1* \pm 33.9

DBF/DAF = Days before/after start of feeding, STD = standard deviation

* Statistically significantly higher than control

During the feeding period (1DAF to 8DAF), no statistically significant differences were detected for any day and any treatment. For reference item group R2 mean daily worker pupae and larvae mortality of the first brood cycle (1DAF to 22DAF) and the mean of the exposure period (1DAF to 43DAF) were significantly higher compared to the control. For reference item treatment R1 the daily mean values were statistically significant different compared to the control on five out of 43 days (1DAF to 43DAF). In the reference item treatment R2 statistically significant differences compared to the control were found on 32 out of 43 days.

Overall test item treatments T1, T2, T3, T4 and T5 had no significant effect on the mortality of worker pupae and larvae bees during the whole exposure period. During the first brood cycle some significant effects were observed in the reference item treatment group R1. In R2 significant effects of treatment were observed throughout the exposure time, confirming suitability of the test design

Mortality of Queens

Colony T3e was found queenless during the colony assessment / beekeeper check on 15 May 2020. The queen of colony R1b was found dead during mortality assessments on 16 May 2020. The queen of colony T4d was found dead on 18 May 2020. The missing queens were replaced with sister queens on 19 May 2020. The queen of colony T3e was found dead on 24 May 2020 and replaced with a young queen from the same breeding line (but no sister queen) on 26 May. An unmarked, hatched queen was found dead in colony T4d on 29 May 2020. Colony T4c was found without eggs or open brood during a colony assessment on 26 Aug 2020 and considered queenless. A new queen was introduced on 02 Sep 2020. An unmarked queen was found in colony T4c on 23 Sep 2020 and replaced with a young marked queen. An absence of eggs and open brood was observed in colony R1a on 15 May 2020, 27 May 2020, 7 Jun 2020, 02 Jul 2020, in colony T5d on 02 Jul 2020 after swarming, in colony T3d on 11 Aug 2020. The absence of eggs or larvae on and after 126DAF (08 Sep 2020) can be attributed to colonies going into overwintering mode, where the queens stop laying eggs. It can be assumed that the loss of queens was caused by the disturbance during colony assessments, samplings and photographic assessments.

Behaviour of the Honey Bees

In all treatment groups (C, T1, T2, T3, T4, T5, R1 and R2), only few bees with unusual behaviour were observed during the pre-feeding period (4DBF to 0DBF). During both brood cycles (1DAF to 43 DAF), abnormal behaviour as cramping, locomotion problems, trembling and inactive bees were observed in control, T2, T3, T4, T5 and R2 at very low levels. Maximal number of impacted bees per hive/category was observed in treatment group T3 on 7DAF, when 29 bees showed locomotion problems (T3e: 29, T3d: 10). This only occurred once, in two (T3e, T3d) out of 5 replicates. Therefore, observed abnormal behaviour was not regarded as test item related. Clustering was observed only at single events (31DAF: Ce; 6, 20 and 36DAF: T3e; 29DAF: T4c; 36DAF: T5e). In the reference item group R1, elevated numbers of bees with unusual behaviour as cramping, locomotion problems, trembling and inactivity were observed from 2DAF up to 15DAF, which is a known effect of the reference item. Maximum number of bees per hive/category was observed on 4DAF with 261 inactive bees and on 8DAF with 243 bees showing locomotion problems in replicate R1a.

Overall, test item treatments T1, T2, T3, T4 and T5 had no effect on the behaviour of worker bees.

1st Brood Cycle

There was no effect observed in the test item treatments T1, T2, T3, T4 and T5 on the brood indices, compensation indices and termination rates of eggs, young larvae or old larvae on any assessment day of the 1st brood cycle (BFD0 to BFD+22 = 0DBF to 22DAF). Clear effects could be seen in the reference item group R1 and R2 regarding the brood indices, compensation indices and termination rates of eggs. The mean brood indices of cells initially containing eggs were 1.00 in all treatment groups at the first assessment (BFD0) and they were 4.49 in C, 4.56 in T1, 4.49 in T2, 4.12 in T3, 4.27 in T4, 4.70 in T5, 1.60 in R1 and 0.87 in R2 at the end of the development cycle (BFD+22). None of the differences of any test item treatment group compared to the control were statistically significant on any observation day. Statistically significant differences were observed only in the reference item group R2 on BFD+16 and BFD+22 (Student's t-Test (pooled), left-sided, $p \leq 0.05$). The mean compensation indices of cells initially containing eggs were 1.00 in all treatment groups at the first assessment (BFD0) and they were 4.71 in C, 4.77 in T1, 4.72 in T2, 4.44 in T3, 4.63 in T4, 4.83 in T5, 1.67 in R1 and 2.03 in R2 at the end of the development cycle (BFD+22). None of the differences of any test item treatment group compared to the control were statistically significant on any observation day. Statistically significant differences were observed only in the reference item group R2 on BFD+16 and BFD+22 (Student's t-Test (pooled), left-sided, $p \leq 0.05$).

The results are provided below:

Eggs

Mean Brood index of cells initially containing eggs - 1st brood cycle

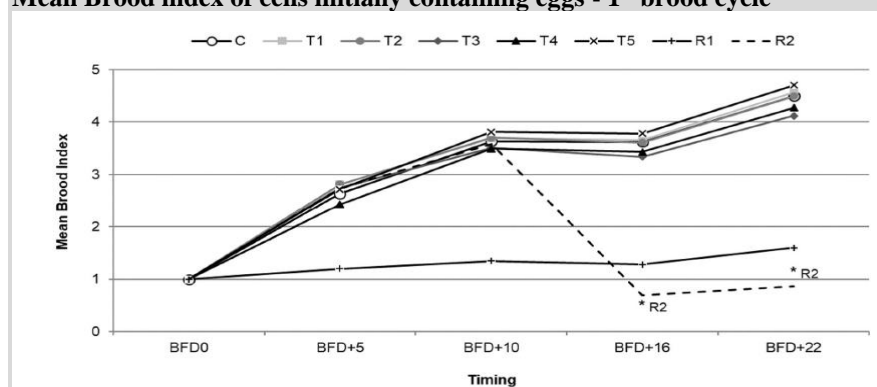


Figure 1. Development of cells initially containing eggs: Brood index (1st brood cycle)
* statistically significant different than control group (pooled t-Test, left sided; $p \leq 0.05$)

Mean compensation index of cells initially containing eggs – 1st brood cycle

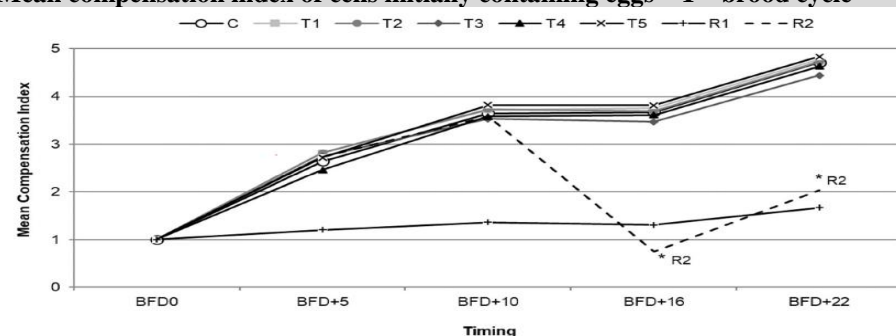


Figure 2. Development of cells initially containing eggs: Compensation index (1st brood cycle)
* statistically significant different than control group (pooled t-Test, left sided; $p \leq 0.05$)

The mean termination rates of cells initially containing eggs were 10.12% in C, 8.88% in T1, 10.14% in T2, 17.66% in T3, 14.59% in T4, 6.01% in T5, 67.96% in R1 and 82.67% in R2 at the end of the development cycle (BFD+22). None of the differences of any test item treatment group compared to the control were statistically significant on any observation day. Statistically significant differences were observed in the reference item group R1 on BFD+10, BFD+16 and BFD+22 and in R2 on BFD+16 and BFD+22 (Student's t-Test (pooled), right-sided, $p \leq 0.05$).

Mean termination rates of cells initially containing eggs - 1st brood cycle

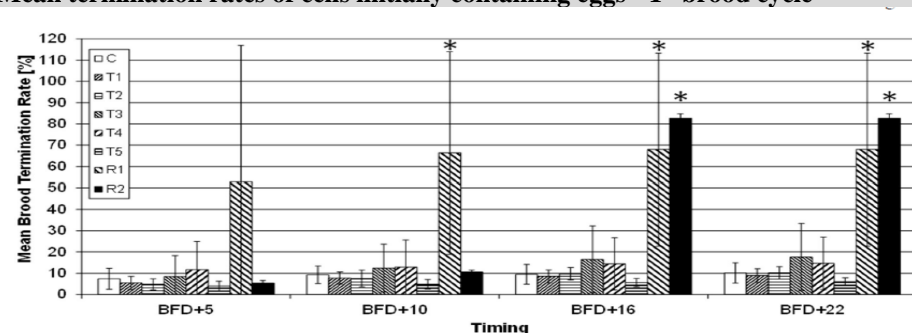


Figure 3. Termination rates of cells initially containing eggs (1st brood cycle)
* statistically significant different than control group (pooled t-Test, right sided; $p \leq 0.05$)

Young Larvae

The mean brood indices of cells initially containing young larvae were 2.00 in all treatment groups at the first assessment (BFD0) and they were 4.72 in C, 4.57 in T1, 4.65 in T2, 4.12 in T3, 4.66 in T4, 4.79 in T5, 2.96 in R1 and 4.74 in R2 at the end of the development cycle (BFD+22). Differences of the brood indices of young larvae compared to the control were statistically significant in T3 (BFD+22) and in R1 (BFD+5) (Bonferroni-Holms corrected U-Test or Mann-Whitney U-Test, left-sided, $p \leq 0.05$).

Mean Brood Index of cells initially containing young larvae - 1st brood cycle

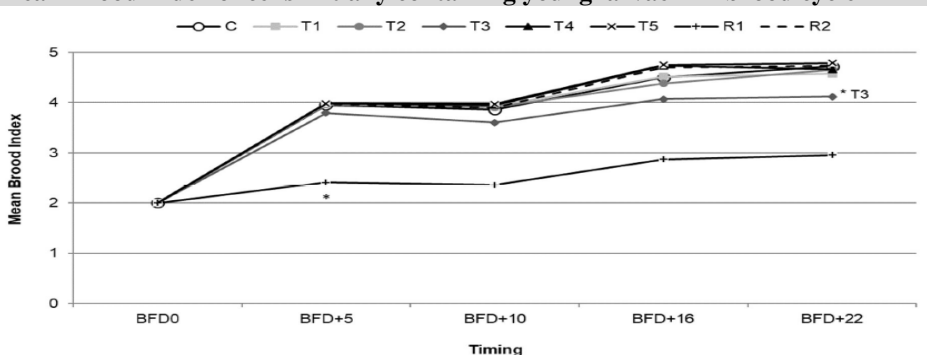


Figure 4. Development of cells initially containing young larvae: Brood index (1st brood cycle) BFD = Brood Area Fixing Day
*statistically significant difference to the Control (Bonferroni-Holms corrected U-Test or Mann-Whitney U-Test, left sided,

$p \leq 0.05$)

The mean compensation indices of cells initially containing young larvae were 2.00 in all treatment groups at the first assessment (BFD0) and they were 4.57 in C, 4.68 in T1, 4.48 in T2, 4.28 in T3, 4.80 in T4, 4.83 in T5, 2.90 in R1 and 4.77 in R2 at BFD+16 (maximum values, reached before emergence of the imagines). Differences of the compensation indices of young larvae compared to the control were statistically significant in T3 (BFD+22) and in R1 (BFD+5, BFD+22) (Dunnett's t-Test, Student's t-Test (pooled) or Mann-Whitney Exact, left-sided, $p \leq 0.05$).

Increased termination rates observed for T3 resulted from high values in replicate T3d. Values of the replicates a, b, c and e were within the range of the control and the other treatments. Therefore, no test item effect is assumed.

Mean Compensation index of cells initially containing young larvae - 1st brood cycle

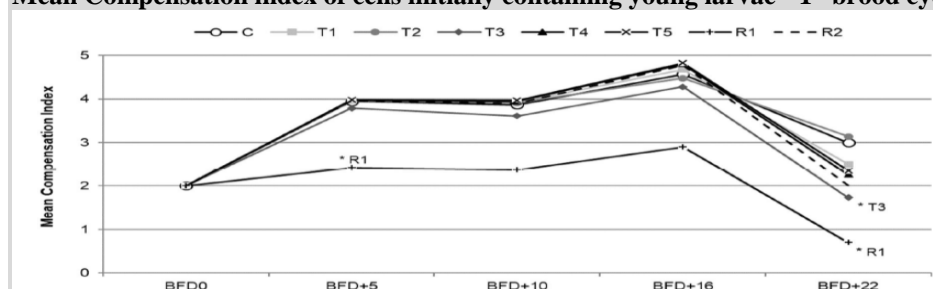


Figure 5. Development of cells initially containing young larvae: Compensation index (1st brood cycle)

BFD = Brood Area Fixing Day

* statistically significant difference to the Control (Dunnett's t-Test, Bonferroni U-Test, Student's t-Test (pooled) or Mann-Whitney Exact, left-sided, $p \leq 0.05$)

The mean termination rates of cells initially containing young larvae were 5.71% in C, 8.63% in T1, 7.02% in T2, 17.52% in T3, 6.90% in T4, 4.28% in T5, 40.94% in R1 and 5.18% in R2 at the end of the development cycle (BFD+22). Differences of the termination rates of young larvae compared to the control were statistically significant in T3 (all assessment days) and in R1 (BFD+5 and +10) (Dunnett's t-Test, Bonferroni-Holms, U-Test or Student's t-Test (pooled), right-sided, $p \leq 0.05$).

Mean Brood termination rate of cell containing young larvae - 1st brood cycle

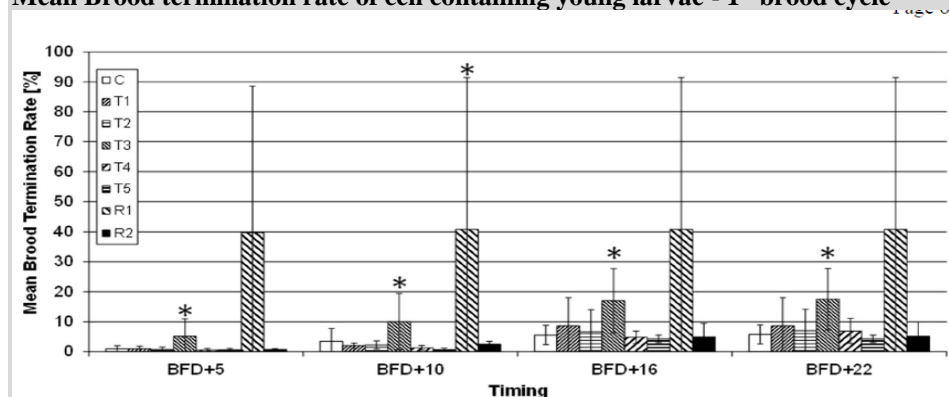


Figure 6. Termination rates of cells initially containing young larvae (1st brood cycle)

BFD = Brood Area Fixing Day

STD = Standard Deviation

* statistically significant difference to the Control (Dunnett's t-Test, Bonferroni-Holms U-Test or Student's t-Test (pooled), one-sided, $p \leq 0.05$)

Old Larvae

The mean brood indices of cells initially containing old larvae were 3.00 in all treatment groups at the first assessment (BFD0) and they were 4.85 in C, 4.87 in T1, 4.91 in T2, 4.73 in T3, 4.90 in T4, 4.97 in T5, 4.55 in R1 and 4.81 in R2 at the end of the development cycle (BFD+16). None of the differences of any test item treatment group or the reference item treatment groups R1 and R2 compared to the

control were statistically significant on any observation day.

Mean Brood Index of cells initially containing old larvae - 1st brood cycle

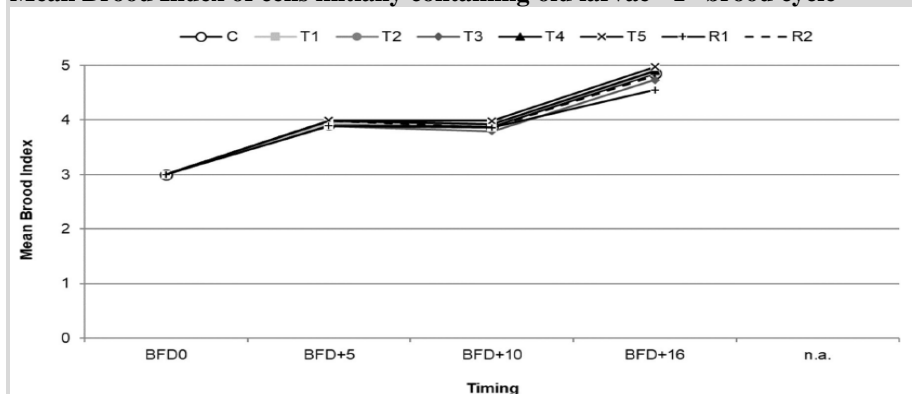


Figure 7. Development of cells initially containing old larvae: Brood index (1st brood cycle)
BFD = Brood Area Fixing Day, n.a.= not applicable (development of old larvae is expected to be complete by BFD+16), There were no statistically significant differences of the test item treatment groups or the reference item treatment group compared to the Control.

The mean compensation indices of cells initially containing old larvae were 3.00 in all treatment groups at the first assessment (BFD0) and they were 4.88 in C, 4.91 in T1, 4.94 in T2, 4.79 in T3, 4.92 in T4, 4.97 in T5, 4.78 in R1 and 4.86 in R2 at the end of the development cycle (BFD+16). None of the differences of any test item treatment group or the reference item treatment groups R1 and R2 compared to the control were statistically significant on any observation day.

Mean Compensation index of cells initially containing old larvae - 1st brood cycle

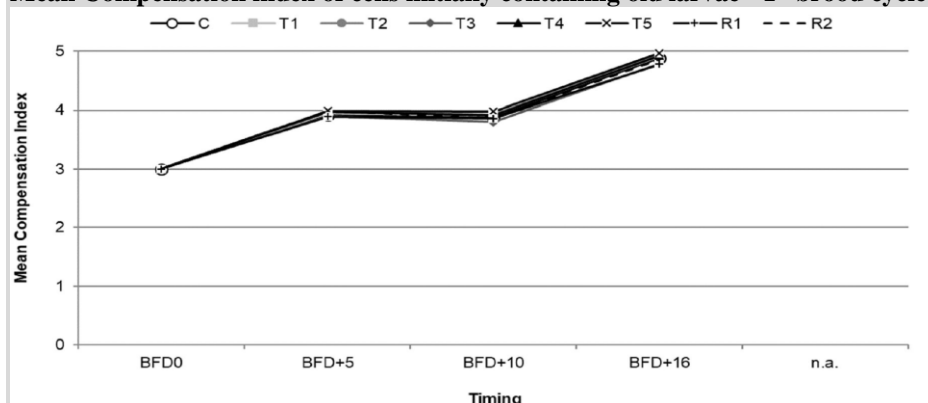


Figure 8. Development of cells initially containing old larvae: Compensation index (1st brood cycle)
BFD = Brood Area Fixing Day; n.a. = not applicable (development of old larvae is expected to be complete by BFD+16)
There were no statistically significant differences of the test item treatment groups or the reference item treatment group compared to the Control.

The mean termination rates of cells initially containing old larvae were 3.09% in C, 2.60% in T1, 1.76% in T2, 5.40% in T3, 2.04% in T4, 0.61% in T5, 9.06% in R1 and 3.84% in R2 at the end of the development cycle (BFD+16). None of the differences of any test item treatment group or the reference item treatment groups R1 and R2 compared to the control were statistically significant on any observation day.

Mean Brood Termination rate of cells initially containing old larvae - 1st brood cycle

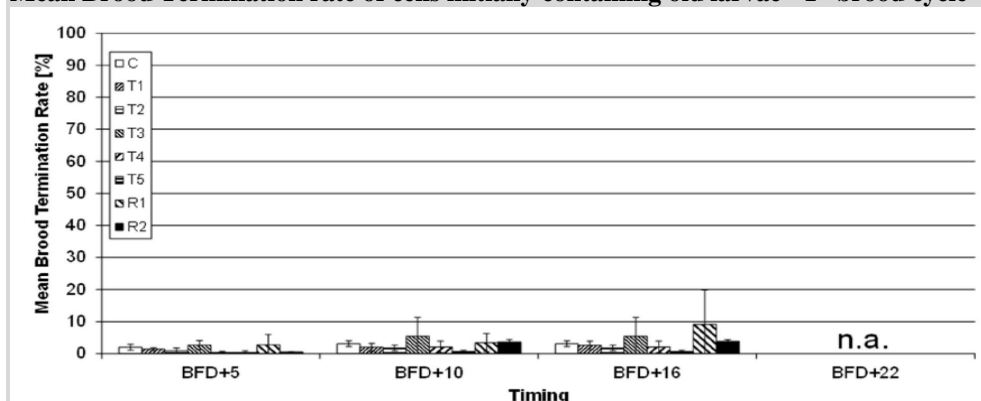


Figure 9. Termination rates of cells initially containing old larvae (1st brood cycle)

BFD = Brood Area Fixing Day

STD = Standard Deviation

n.a. = not applicable (development of old larvae is expected to be complete by BFD+16)

There were no statistically significant differences of the test item treatment groups or the reference item treatment group compared to the Control.

Overall, there was no effect of the test item treatments T1, T2, T3, T4 and T5 on the development of eggs, young larvae and old larvae in individual cells during the 1st brood cycle. Significant effects were observed in the reference item treatment R1 regarding the compensation and brood index and the termination rate for young larvae. Reference item R2 showed significant effects on brood and compensation indices as well as termination rates for eggs, confirming suitability of the test design and exposure of the bee colonies to the treated feeding solution.

2nd Brood Cycle

There was no effect of the test item treatments T1, T2, T3, T4, T5 and R1 on the brood indices, compensation indices or termination rates of eggs, young larvae or old larvae on any assessment day of the 2nd brood cycle (BFD0 to BFD+22 = 22DAF to 44DAF). Clear effects could be seen in the reference item group R2, where significant effects could be found in brood indices, compensation indices and termination rates of eggs, young and old larvae. No statistic evaluation could be done for R1 since only one replicate was left.

Eggs

The mean brood indices of cells initially containing eggs were 1.00 in all treatment groups at the first assessment (BFD0) and they were 3.96 in C, 4.45 in T1, 3.38 in T2, 3.71 in T3, 4.08 in T4, 4.50 in T5, 3.78 in R1 and 2.73 in R2 at the end of the development cycle (BFD+22). None of the differences of any test item treatment group compared to the control were statistically significant on any observation day. Statistically significant differences were observed only in the reference item group R2 on BFD+16 and BFD+22 (Student's t-Test (pooled), left-sided, $p \leq 0.05$).

Mean Brood Index of cell containing eggs – 2nd brood cycle

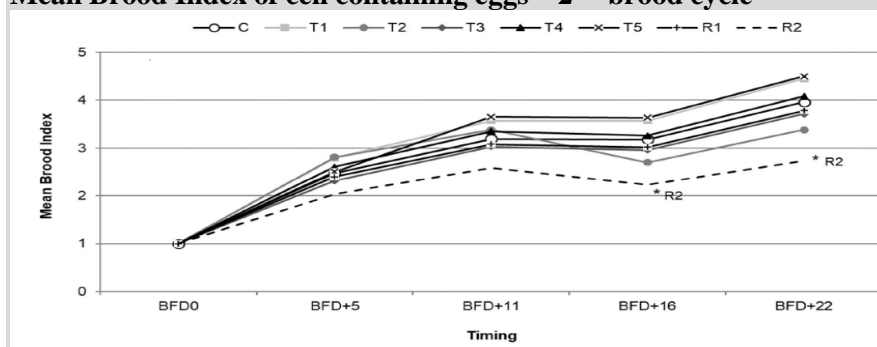


Figure 10. Development of cells initially containing eggs: Brood index (2nd brood cycle)
BFD = Brood Area Fixing Day

* statistically significant difference to the Control (Student's t-Test (pooled), left-sided, $p \leq 0.05$)

The mean compensation indices of cells initially containing eggs were 1.00 in all treatment groups at the first assessment (BFD0) and they were 4.38 in C, 4.67 in T1, 3.67 in T2, 4.10 in T3, 4.40 in T4, 4.68 in T5, 4.24 in R1 and 3.62 in R2 at the end of the development cycle (BFD+22). None of the differences of any test item treatment group compared to the control were statistically significant on any observation day. Statistically significant differences were observed only in the reference item group R2 on BFD+22 (Student's t-Test (pooled), left-sided, $p \leq 0.05$).

Mean Compensation Index cell containing eggs – 2nd brood cycle

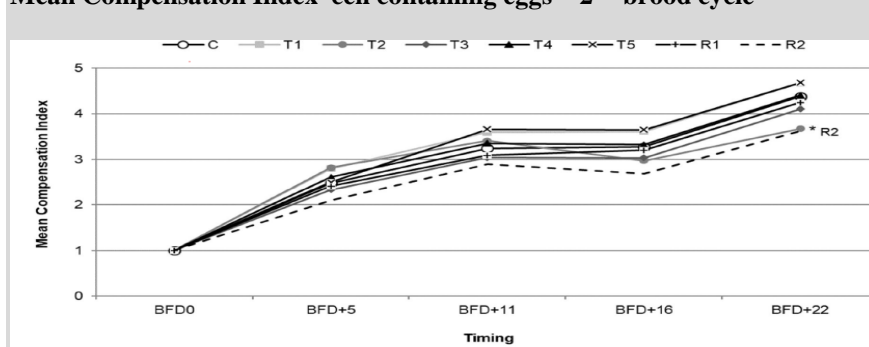


Figure 11. Development of cells initially containing eggs: Compensation index (2nd brood cycle)
No cells containing eggs marked in colony R1a.

BFD = Brood Area Fixing Day

* statistically significant difference to the Control (Student's t-Test (pooled), left-sided, $p \leq 0.05$)

The mean termination rates of cells initially containing eggs were 20.80% in C, 11.05% in T1, 32.41% in T2, 25.91% in T3, 18.44% in T4, 9.96% in T5, 24.42% in R1 and 45.49% in R2 at the end of the development cycle (BFD+22). None of the differences of any test item treatment group compared to the control were statistically significant on any observation day. Statistically significant differences were observed only in the reference item group R2 on BFD+16 and BFD+22 (Student's t-Test (pooled), right-sided, $p \leq 0.05$).

Mean Brood Termination rate of cell initially containing eggs – 2nd brood cycle

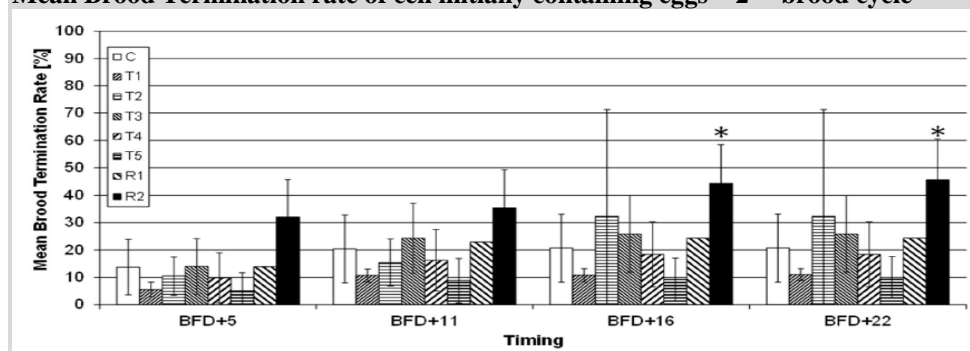


Figure 12. Mean termination rates and STD of cells initially containing eggs (2nd brood cycle)
BFD = Brood Area Fixing Day; STD = Standard Deviation; *Statistically significant difference to the Control (Student's t-Test (pooled), right sided, $p \leq 0.05$) No cells containing eggs marked in colony R1b.

Young Larvae

The mean brood indices of cells initially containing young larvae were 2.00 in all treatment groups at the first assessment (BFD0) and they were 4.44 in C, 4.65 in T1, 3.65 in T2, 4.28 in T3, 4.60 in T4, 4.26 in T5, 4.71 in R1 and 4.40 in R2 at the end of the development cycle (BFD+22). None of the differences of any test item treatment group or the reference item treatment groups R1 and R2 compared to the control were statistically significant on any observation day.

Mean brood Index of cell initially containing young larvae – 2nd brood cycle

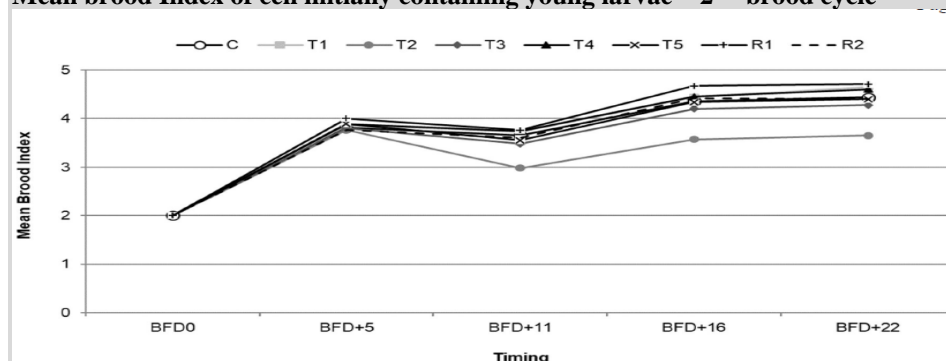


Figure 13. Development of cells initially containing young larvae: Brood index (2nd brood cycle)
No cells containing young larvae marked in the colony R1a
BFD = Brood Area Fixing Day;

There were no statistically significant differences of the test item treatment groups or the reference item treatment group compared to the Control. The mean compensation indices of cells initially containing young larvae were 2.00 in all treatment groups at the first assessment (BFD0) and they were 4.45 in C, 4.49 in T1, 4.05 in T2, 4.31 in T3, 4.50 in T4, 4.46 in T5, 4.74 in R1 and 4.48 in R2 at BFD+16 (maximum values, reached before emergence of the imagines). None of the differences of any test item treatment group or the reference item treatment groups R1 and R2 compared to the control were statistically significant on any observation day.

Mean Compensation Index of cell initially containing young larvae – 2nd brood cycle

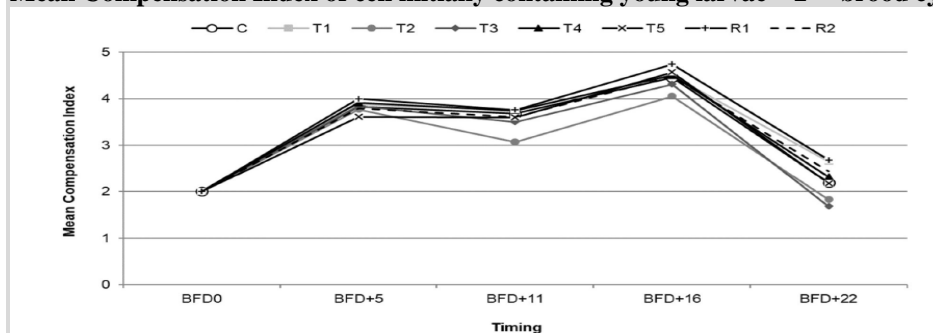


Figure 14. Development of cells initially containing young larvae: Compensation index (2nd brood cycle)
No cells containing young larvae marked in the colony R1a.

The mean termination rates of cells initially containing young larvae were 11.30% in C, 7.06% in T1, 26.98% in T2, 14.42% in T3, 8.07% in T4, 14.86% in T5, 5.88% in R1 and 12.05% in R2 at the end of the development cycle (BFD+22). None of the differences of any test item treatment group or the reference item treatment groups R1 and R2 compared to the control were statistically significant on any observation day.

Mean termination Index of cell initially containing young larvae – 2nd brood cycle

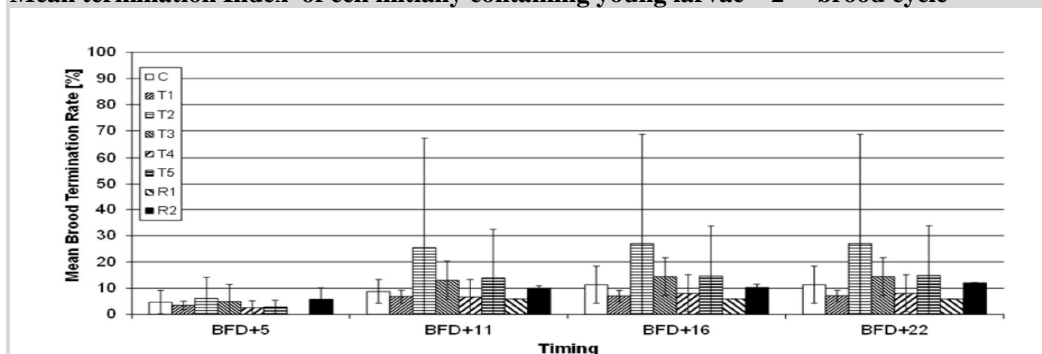


Figure 15. Termination rates of cells initially containing young larvae (2nd brood cycle)

BFD = Brood Area Fixing Day; STD = Standard Deviation

There were no statistically significant differences of the test item treatment groups or the reference item treatment group compared to the Control. No cells containing young larvae marked in the colonies R1a

Old Larvae

The mean brood indices of cells initially containing old larvae were 3.00 in all treatment groups at the first assessment (BFD0) and they were 4.79 in C, 4.77 in T1, 4.82 in T2, 4.66 in T3, 4.79 in T4, 4.82 in T5, 4.61 in R1 and 4.37 in R2 at the end of the development cycle (BFD+16). None of the differences of any test item treatment group compared to the control were statistically significant on any observation day. Statistically significant differences were observed only in the reference item group R2 on BFD+11 and BFD+16 (Student's t-Test (pooled) or Satterthwaite t-Test, left-sided, $p \leq 0.05$).

Mean Brood Index of cell initially containing old larvae – 2nd brood cycle

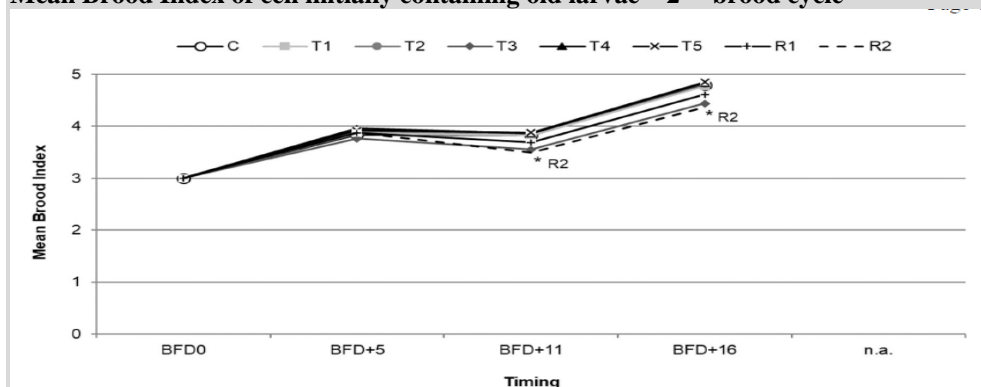


Figure 16. Development of cells initially containing old larvae: Brood index (2nd brood cycle)

No cells containing old larvae marked in the colony R1a.

BFD = Brood Area Fixing Day

n.a. = not applicable (development of old larvae is expected to be complete by BFD+16)

* statistically significant difference to the Control (Student's t-Test (pooled) or Satterthwaite t-Test, left-sided, $p \leq 0.05$)

The mean compensation indices of cells initially containing old larvae were 3.00 in all treatment groups at the first assessment (BFD0) and they were 4.83 in C, 4.80 in T1, 4.84 in T2, 4.70 in T3, 4.82 in T4, 4.84 in T5, 4.74 in R1 and 4.53 in R2 at the end of the development cycle (BFD+16). None of the differences of any test item treatment group compared to the control were statistically significant on any observation day. Statistically significant differences were observed only in the reference item group R2 on BFD+11 and BFD+16 (Student's t-Test (pooled), left-sided, $p \leq 0.05$).

Mean Compensation Index x of cell initially containing old larvae – 2nd brood cycle

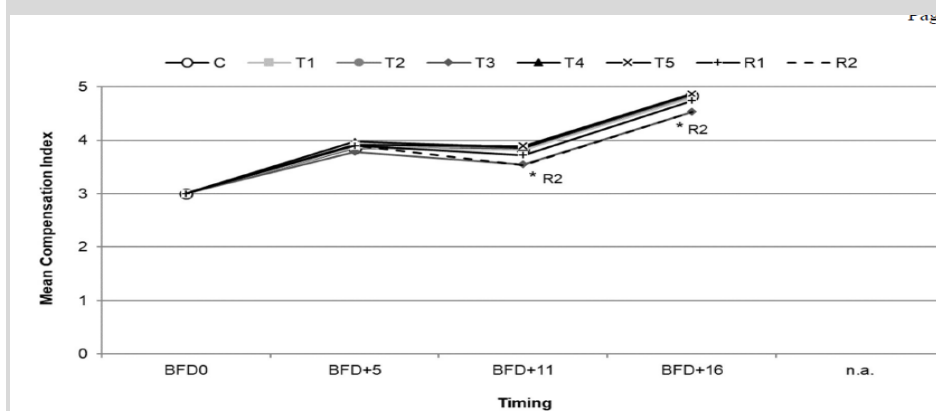


Figure 17. Development of cells initially containing old larvae: Compensation index (2nd brood cycle)

n.a. = not applicable (development of old larvae expected to be completed until BFD+16)

No cells containing old larvae marked in the colony R1a.

*Statistically significant difference to the Control (Student's t-Test (pooled), left sided, $p \leq 0.05$)

The mean termination rates of cells initially containing old larvae were 4.26% in C, 4.66% in T1, 3.52% in T2, 6.91% in T3, 4.20% in T4, 3.65% in T5, 7.76% in R1 and 12.74% in R2 at the end of the development cycle (BFD+16). None of the differences of any test item treatment group compared to the control were statistically significant on any observation day. Statistically significant differences were observed only in the reference item group R2 on BFD+11 and BFD+16 (Student's t-Test (pooled), right-sided, $p \leq 0.05$).

Mean Termination rates of cell initially containing old larvae – 2nd brood cycle

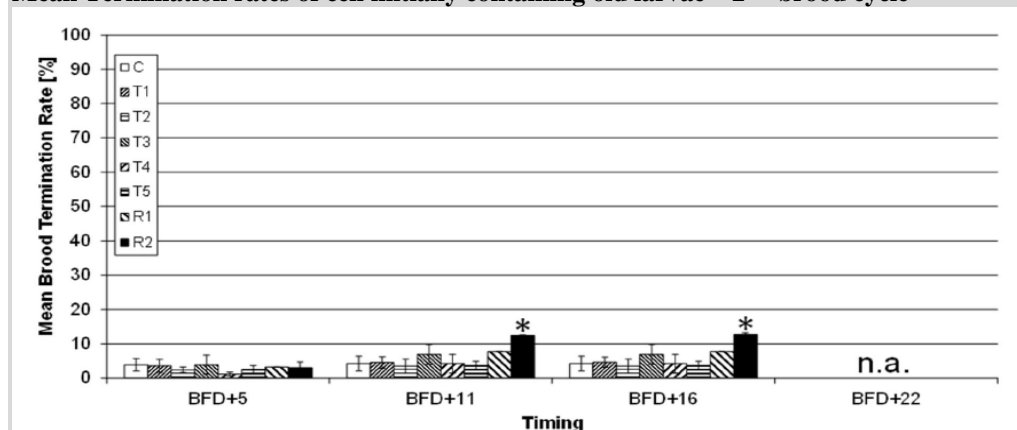


Figure 18. Termination rates of cells initially containing old larvae (2nd brood cycle)

No cells containing young larvae marked in the colonies R1a and R1b (lack of brood due to dead of queen in R1a); BFD= Brood Area Fixing Day; n.a.= not applicable (development of old larvae is expected to be complete by BFD+16); * Statistically significant difference to the Control (Student's t-Test (pooled), right-sided, ≤ 0.05)

Overall there was no effect of the test item treatments T1, T2, T3, T4 and T5 regarding the development of individual brood cells during the 2nd brood cycle. Significant effects were observed on brood index, compensation index and termination rate in the reference item treatments R2 for eggs and old larvae.

Condition of the Colonies

Colony Size

At start of the study (7/6DBF) the mean colony size (number of honey bees per colony) was on a similar level in all treatment groups with 12896 bees/colony in C, 12532 in T1, 12207 in T2, 12363 in T3, 12610 in T4, 11544 in T5, 11733 in R1 and 12740 bees/colony in R2.

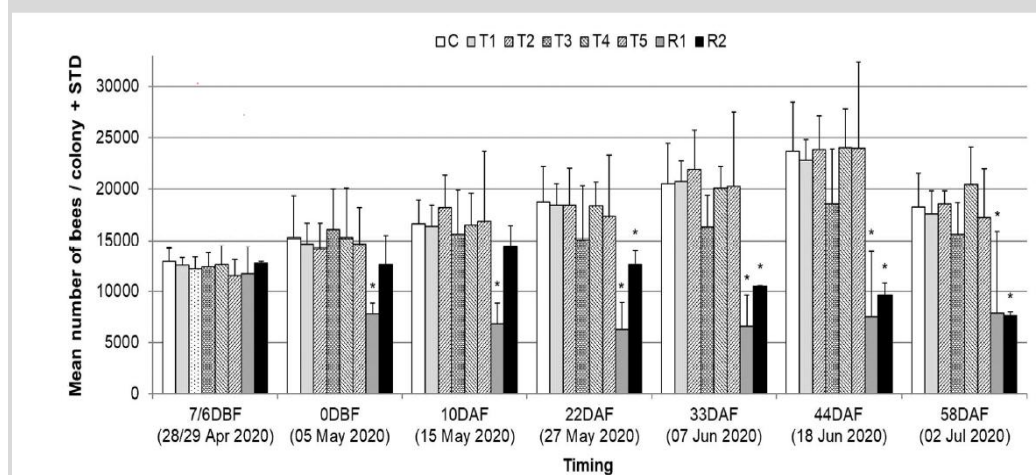


Figure 19. Mean colony size (numberof bees/hive) per treatment group (7/6 DBF to 58 DAF)

*statistically significant lower than control group (pooled t-test, left-side, $p \leq 0.05$)

The mean number of brood cells in all colonies of treatment C, T1, T2, T4 and T5 increased from 7/6DBF to 22DAF and remained constant with only slight variation until 58DAF (02 JUL 2020). Treatment group T3 forms an exception from this trend because of the aforementioned queen replacements in T3e which slowed average colony growth early in the season. The mean number of brood cells reached a similar level as that observed in the control group by 44DAF. After that date the mean number

of brood cells decreased in the control and treatment hives. The mean number of brood cells in the reference item treatment group R1 was significantly lower than that of the control group from 10DAF to 33DAF, after which date one replicate fully recovered while the other one died. The mean number of brood cells in reference item treatment group R2 was significantly lower than that of the control group from 22DAF to 58DAF and recovered to control levels afterwards and followed the decrease generally observed in the late season. After overwintering all brood stages were present in all colonies with exception of **T1e and T3e, which did not survive the winter**. The number of brood cells in treatment group R2 was significantly lower than that of the control. Overall, there was no obvious test item related effect of T1, T2, T3, T4 and T5. A clear effect on the total amount of brood or certain brood stages of on R1 and R2 were observed.

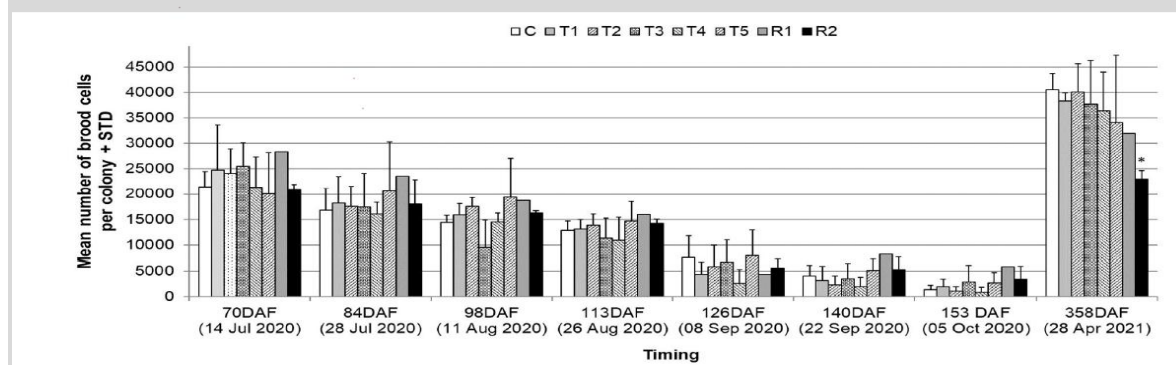


Figure 20. Mean number of brood cell (all brood stages) per treatment group (70 DAF to 358DAF)

Amount of Nectar and Pollen

Honey bees from the test colonies were free-flying, with access to natural nectar sources.

The mean number of nectar cells per colony was similar in all treatment groups at start of the study (7/6DBF): 32520 nectar cells/colony in C, 29520 in T1, 23040 in T2, 29320 in T3, 23440 in T4, 30440 in T5, 21000 in R1 and 21500 in R2. The mean number of pollen cells per colony was similar in all treatment groups at start of the study (7/6DBF): 3800 pollen cells/colony in C, 4080 in T1, 1720 in T2, 2720 in T3, 2920 in T4, 2520 in T5, 5400 in R1 and 3000 in R2. The mean number of nectar and pollen cells was similar for all treatment groups during the entire study period with the following exceptions: A significantly lower number of pollen cells than the control was observed in treatment group T2 on 0DBF (05 May 2020) and a significantly lower number of nectar cells was observed in treatment group T5 on 98DAF (11 Aug 2020). A significantly lower number of nectar cells was observed in the reference item treatment group R1 on 0DBF, 10DAF, 22DAF, 33DAF, 44DAF and 58DAF.

Afterwards, no statistical analysis was possible due to the death of replicate R1a. Significantly lower numbers of pollen cells were observed in treatment group R1 on 33DAF and 58DAF. A significantly lower number of nectar cells was observed in treatment group R2 on 0DBF, 10DAF, 58DAF, 84DAF, 98DAF, 113DAF, 126DAF, 140DAF and 153DAF.

**Overall, there was no obvious test item related effect of T1, T2, T3, T4 and T5.
A clear effect on the mean number of food cells of on R1 and R2 were observed.**

Weight and Assessment of Morphological Abnormalities of Pupae

1st Brood Cycle

No morphological malformations were observed in any treatment group. The mean weight of pupae collected from combs during the first brood cycle (17DAF) was 0.1419 g in the control group C, 0.1438 g in T1, 0.1386 g in T2, 0.1426 g in T3, 0.1354 g in T4, 0.1399 g in T5, 0.1229 g in R1 and 0.1529 g in R2. Pupal weight was significantly lower than the control in treatment groups T2, T4 and R1.

2nd Brood Cycle

No morphological malformations were observed in any treatment group. The mean weight of pupae collected from combs during the first brood cycle (38DAF) was 0.1395 g in the control group C, 0.1292 g in T1, 0.1470 g in T2, 0.1388 g in T3, 0.1338 g in T4, 0.1431 g in T5, 0.1269 g in R1 and 0.1428 in R2. Pupal weight was significantly lower than the control in the reference item treatment group R1.

Overall, there was a reduced mean weight of pupae collected from combs of treatment groups T2 and T4 as well as reference item treatment group R1 during the first brood cycle. As effects in the test item treatment groups were not dose-dependent, they are probably not test item-related. In the second brood cycle, a reduction in pupal weight was only observed in the reference item treatment group R1.

Determination of Colony Weight

All hive weight modifications made (e.g. adding or removal of frames or supers, additional feeding) were documented by direct weighing of the material added/removed, to determine the weight change. Hive weights were statistically analysed and are reported using one value per week (i.e. every 7th day) starting on 1DBF.

The mean colony weight per colony/day before start of feeding (1DBF) was 46.3 kg in C, 43.6 kg in T1, 41.5 kg in T2, 45.4 kg in T3, 42.4 kg in T4, 45.5 kg in T5, 40.1 kg in R1 and 40.5 kg in R2.

At the end of the 1st brood cycle (20DAF) mean colony weight measured was as follows: 50.5 kg in the control, 47.2 kg in T1, 45.9 kg in T2, 48.0 kg in T3, 47.6 kg in T4, 48.3 kg in T5, 35.6 kg in R1 and 43.1 kg in R2. There were no statistically significant differences in colony weight of the test item treatments T1, T2, T3, T4 and T5 on this date. Colony weights in the reference item treatment group R1 were significantly lower than those of the control group on every analysed date from 6DAF to 20DAF and in treatment group R2 on 20DAF.

At the end of the 2nd brood cycle (41DAF) mean colony weight measured was as follows: 51.3 kg in the control, 49.7 kg in T1, 47.3 kg in T2, 48.6 kg in T3, 49.0 kg in T4, 49.9 kg in T5, 36.3 kg in R1 and 43.9 kg in R2. There were no statistically significant differences in colony weight compared to the control group of the test item treatments T1, T2, T3, T4 and T5 in the second brood cycle. Statistically significant differences in colony weight during the 2nd brood cycle were found in treatment group R1 from 27DAF to 41DAF and in treatment group R2 on 27DAF.

The mean colony weight per colony/day until start of overwintering (1DBF to 153DAF, one value per week) was 58.0 kg in the control group C, 57.8 kg in T1, 54.8 kg in T2, 53.8 kg in T3, 56.0 kg in T4, 54.5 kg in T5, and 47.6 kg in R2. No statistically significant difference in hive weights over the period from 1DBF until 153DAF was observed for treatment groups T1-T5. The mean colony weight of test item treatment group T3 was significantly lower than that of the control group C on 83DAF which probably is a false positive and not test item-related due to its lack of any temporal correlation with the test item application. The mean colony weight in the reference item treatment group R2 was significantly lower than that of the control group for the period between 1DBF and 153DAF, and single-day measurements were also significantly lower for every analysed day between 48DAF and 153DAF.

The weight of R1a was recorded daily until its death on 69DAF. Therefore, no mean value or statistical analysis can be provided for the reference item treatment group R1 for the period from 1DBF until 153DAF. However, the mean colony weight of reference item treatment group R1 was significantly lower than that of the control on every analysed date from 48DAF until 69DAF. The mean colony weight/day in the period from 1DBF to 153DAF of the surviving replicate R1b was 46.5 kg.

According to local beekeeping practice the colonies were fed with sugar syrup on two further occasion until begin of overwintering: on 84DAF (5 kg/colony) and on 119DAF (12 kg/colony). After overwintering, mean values reduced to 43.4 kg for the control, 44.1 kg for T1, 42.2 kg for T2, 39.0 kg for T3, 41.9 for T4, 40.4 for T5 and 31.0 and 33.4 kg for R1 and R2.

The mean value of R2 is statistically significant different compared to control.

Table 3. Mean daily weight of colonies per treatment group

Treatment group (colony weight) mean \pm STD	C	T1	T2	T3	T4	T5	R1	R2
Mean (1DBF to 41DAF)	50.7 \pm 4.1	48.1 \pm 3.3	46.2 \pm 5.4	48.7 \pm 4.4	47.9 \pm 5.1	49.1 \pm 5.6	37.9* \pm 4.2	44.1* \pm 1.0
Mean (1DBF to 153DAF)	58.0 \pm 3.1	57.8 \pm 3.5	54.8 \pm 4.0	53.8 \pm 4.4	56.0 \pm 4.4	54.5 \pm 5.6	--	47.6 \pm 1.8
Mean (358DAF)	43.4 \pm 1.6	44.1 \pm 5.3	42.2 \pm 3.6	39.0 \pm 4.7	41.9 \pm 2.7	40.4 \pm 2.9	31.0 ¹⁾	33.4* \pm 3.6

DBF/DAF = Days before/after start of feeding, STD = standard deviation

* Statistically significantly lower compared to control $p \leq 0.05$: pooled t-test

1) one hive only (R1b), no statistics possible

Overall, there was no effect of the test item treatment in T1, T2, T3, T4 and T5 on colony weight throughout the whole observation period (1DBF to 153DAF). In the reference treatments R1 and R2 an effect on colony weight was observed throughout the study.

Counting of Varroa Mites Before Overwintering

The number of Varroa mites was counted before overwintering. For the colonies of the control between 0 – 2 mites were found. For T1: 0 – 1 mite, T2: 0-6 mites, T3: 0-4 mites, T5: 0-2 mites. For the reference item treatment, remaining replicate R1b 3 mites were counted and for R2 0 and 4 mites were counted. These numbers indicate a very low level of infestation with *Varroa* mites.

Overall, there was no effect of the test item treatment T1, T2, T3, T4 and T5 on the Varroa mite numbers.

Bee Disease

Bee Disease and AFB Analysis (Trial S20-02058-L1 (-02))

Nosema. sp. could be detected in ten hives. A low infection level was detected in one hive (Ce: colony 36). Mediate infection levels were detected in seven hives (R2s: colony 2, T2e: colony 3, colony 4 (not used), T2s: colony 13, Ca: colony 18, T2d: colony 32). A high infection level was detected in two hives (T2a: colony 21, T3a: colony 22). The infestation rate with Varroa mites in the honeybee samples taken from the colonies before start of exposure were 0.4 % in colony 2 (R2s), 0.3 % in colony 11 (T5d) and 0.7 % in colony 52 (T3b). In the honeybee samples taken from the control colonies before overwintering, Nosema sp. Spores were not detected. In the honeybee samples taken after overwintering, control colony Cb had a low infestation level and Ca had a high infestation level. In the honeybee samples taken from the control colonies before overwintering, the infestation rate with Varroa mite was 0.2 % in Ca, 0.4 % in Cb and 0.2 % in Cc. In the honeybee samples taken from the control colonies after overwintering, no Varroa mites were detected.

In the honeybee samples taken from the reference item treatment colonies before and after overwintering, neither Nosema sp. Spores, nor Varroa mites were detected. In the honeybee samples taken from the test item treatment colonies T1 before overwintering, infestation with Nosema sp. spores was on a medium level in the test item treatment colony T1b. In the honeybee samples taken from the test item treatment colonies T1 after overwintering, no infestation with Nosema sp. spores was detected. In the

honeybee samples taken from the test item treatment colonies T1 before overwintering, the infestation rate of Varroa mites was 0.6 % in T1b and 0.9 % in T1e. In the honeybee samples taken from the test item treatment colonies T1 after overwintering, the infestation rate of Varroa mites was 0.6 % in T1e. In the honeybee samples taken from the test item treatment colonies T2 before overwintering, no infestation with Nosema sp. spores was detected. In the honeybee samples taken from the test item treatment colonies T2 after overwintering, no infestation with Nosema sp. Spores was detected. In the honeybee samples taken from the test item treatment colonies T2 before overwintering, the infestation rate of Varroa mites was 0.4 % in T2b. In the honeybee samples taken from the test item treatment colonies T2 after overwintering, no Varroa mites were detected.

CBPV was detected in the samples taken from colonies T1a, T2e, and T5c of the test item treatment group at the time point ‘before start of exposure’ (sampling S1) in 2020 BQCV was detected in the samples taken from colonies T1c and T4d of the test item treatment group, and in the sample taken from colony R2b of reference item group at the time point “before start of exposure” (sampling S1).

Overall, no relevant differences in the bee health status in terms of virus infection between the colonies of the control group (Ca-Ce, Cs) and the corresponding test item treatment groups (T1a-T1e, T1s, T2a-e, T2s, T3a-e, T3s, T4a-e, T4s, T5a-e, T5s) were observed at any sampling date.

Residue analysis:

In the analytical phase S20-02058-L3 of this study, samples of pollen, nectar, honey, pupae, larvae, worker jelly and feeding solution were analysed for residues of fenpicoxamid, prothioconazole and prothioconazole-desthio with a limit of quantification of 0.001 mg/kg.

Additionally, samples of feeding solution were analysed for residues of dimethoate and fenoxycarb with a limit of quantification of 0.01 mg/kg.

Residues in Pollen:

No residues of fenpicoxamid were detected in any untreated pollen samples.

Residues of fenpicoxamid in treated pollen samples ranged from n.d. to 0.154 mg/kg.

Residues of prothioconazole in untreated pollen samples ranged from n.d. to 0.00280 mg/kg.

Residues of prothioconazole in treated pollen samples ranged from n.d. to 0.378 mg/kg.

Residues of prothioconazole-desthio in untreated pollen samples ranged from < n.d. to 0.00687 mg/kg.

Residues of prothioconazole-desthio in treated pollen samples ranged from n.d. to 0.157 mg/kg.

Residues in Nectar:

No residues of fenpicoxamid, prothioconazole and prothioconazole-desthio were detected in any untreated nectar samples.

Residues of fenpicoxamid in treated nectar samples ranged from n.d. to 3.24 mg/kg.

Residues of prothioconazole in treated nectar samples ranged from n.d. to 7.50 mg/kg.

Residues of prothioconazole-desthio in treated nectar samples ranged from n.d. to 0.519 mg/kg.

Residues in Honey:

No residues of fenpicoxamid and prothioconazole were detected in any untreated honey samples.

Residues of fenpicoxamid in treated honey samples ranged from n.d. to 0.942 mg/kg.

Residues of prothioconazole in treated honey samples ranged from n.d. to 5.49 mg/kg.

Residues of prothioconazole-desthio in untreated honey samples ranged from n.d. to < 0.001 mg/kg (0.000420 mg/kg).

Residues of prothioconazole-desthio in treated honey samples ranged from n.d. to 0.336 mg/kg.

Residues in Larvae:

No residues of fenpicoxamid, prothioconazole and prothioconazole-desthio were detected in any untreated larvae samples.

Residues of fenpicoxamid in treated larvae samples ranged from n.d. to 0.0174 mg/kg.
Residues of prothioconazole in treated larvae samples ranged from n.d. to 0.125 mg/kg.
Residues of prothioconazole-desthio in treated larvae samples ranged from n.d. to 0.0142 mg/kg.

Residues in Pupae:

No residues of fenpicoxamid, prothioconazole were detected in any untreated or treated pupae samples.
Residues of prothioconazole-desthio in untreated pupae samples ranged from n.d. to < 0.001 mg/kg (0.000414 mg/kg).
Residues of prothioconazole-desthio in treated pupae samples ranged from < n.d. to < 0.001 mg/kg (0.000393 mg/kg).

Residues in Worker Jelly:

No residues of fenpicoxamid, prothioconazole and prothioconazole-desthio were detected in any untreated worker jelly samples.
Residues of fenpicoxamid in treated worker jelly samples ranged from n.d. to 2.28 mg/kg.
Residues of prothioconazole in treated worker jelly samples ranged from n.d. to 2.80 mg/kg.
Residues of prothioconazole-desthio in treated worker jelly samples ranged from n.d. to 1.03 mg/kg.

Residues in Feeding Solution:

No residues of fenpicoxamid and prothioconazole were detected in any untreated feeding solution samples.
Residues of fenpicoxamid in treated feeding solution samples ranged from 0.182 mg/kg to 21.7 mg/kg.
Residues of prothioconazole in treated feeding solution samples ranged from 0.354 mg/kg to 54.6 mg/kg.
Residues of dimethoate in treated feeding solution samples ranged from n.d. to 9.46 mg/kg.
Residues of fenoxycarb in treated feeding solution samples ranged from n.d. to 72.3 mg/kg.

CONCLUSION

The test item, Fenpicoxamid and Prothioconazole (formulation GF-3307), were applied via feeding solution at rates of 4, 20, 50, 100, 500 mg product/L in treatment group T1, T2, T3, T4 and T5. The toxic reference item group R1 and R2 were applied at rates of 11.21 mg a.i./L and 84 mg a.i./L, respectively.

The test and reference items were provided via Apiinvert® sugar solution for 9 consecutive days, 500 mL per colony and day (Control pure Apiinvert®).

The test item treatments T1, T2, T3, T4 and T5 had no effect on the mortality of worker bees during the entire exposure and monitoring period. In the reference item group R1 significant effects could be seen starting at 2DAF and lasting until 18DAF.

Test item treatments T1, T2, T3, T4 and T5 had no significant effect on the mortality of worker pupae and larvae bees during the whole exposure period. During the first brood cycle some significant effects were observed in the reference item treatment group R1. In R2 significant effects of treatment were observed through the exposure time, confirming suitability of the test design.

Test item treatments T1, T2, T3, T4 and T5 had no effect on the behaviour of worker bees.

There was no effect of the test item treatments T1, T2, T3, T4 and T5 on the development of eggs, young larvae and old larvae in individual cells during the 1st brood cycle. Significant effects were observed in the reference item treatment R1 regarding the brood and compensation index and termination rate of young larvae. Reference item R2 showed significant effects on brood and compensation indices as well as termination rates for eggs, confirming suitability of the test design and exposure of the bee colonies to the treated feeding solution.

There was no effect of the test item treatments T1, T2, T3, T4 and T5 regarding the development of individual brood cells during the 2nd brood cycle. Significant effects were observed on brood index,

compensation index and termination rate in the reference item treatments R2 for eggs and old larvae.

No statistical effect on the colony size was observed for the test item treatment groups T1, T2, T3, T4 and T5. One replicate each of treatment groups T1 and T3 did not survive overwintering, but this effect is probably not test item-related. However, clear effects of the reference items were observed in R1 and R2. One hive of the reference item group R1 did not survive the treatment, and the mean colony size of R2 failed recover from a significant reduction in colony size within the monitoring period.

There was no obvious test item related effect of T1, T2, T3, T4 and T5 on the total amount of brood or certain brood stages. A clear effect of on R1 and R2 were observed.

There was no obvious test item related effect on the mean number of food cells of T1, T2, T3, T4 and T5. A clear effect of on R1 and R2 were observed.

The mean weights of pupae were determined during the 1st and the 2nd brood cycle and Significant effects only were noticed for reference treatment R1.

The number of Varroa mites was counted and no effect of the test item treatment T1, T2, T3, T4 and T5 on infestation of the colonies with *Varroa* mites was determined.

Regarding disease, AFB and virus determination, no test item related differences could be observed.

Residues in Pollen:

No residues of fenpicoxamid were detected in any untreated pollen samples.

Residues of fenpicoxamid in treated pollen samples ranged from n.d. to 0.154 mg/kg.

Residues of prothioconazole in untreated pollen samples ranged from n.d. to 0.00280 mg/kg.

Residues of prothioconazole in treated pollen samples ranged from n.d. to 0.378 mg/kg.

Residues of prothioconazole-desthio in untreated pollen samples ranged from < n.d. to 0.00687 mg/kg.

Residues of prothioconazole-desthio in treated pollen samples ranged from n.d. to 0.157 mg/kg.

Residues in Nectar:

No residues of fenpicoxamid, prothioconazole and prothioconazole-desthio were detected in any untreated nectar samples.

Residues of fenpicoxamid in treated nectar samples ranged from n.d. to 3.24 mg/kg.

Residues of prothioconazole in treated nectar samples ranged from n.d. to 7.50 mg/kg.

Residues of prothioconazole-desthio in treated nectar samples ranged from n.d. to 0.519 mg/kg.

Residues in Honey:

No residues of fenpicoxamid and prothioconazole were detected in any untreated honey samples.

Residues of fenpicoxamid in treated honey samples ranged from n.d. to 0.942 mg/kg.

Residues of prothioconazole in treated honey samples ranged from n.d. to 5.49 mg/kg.

Residues of prothioconazole-desthio in untreated honey samples ranged from n.d. to < 0.001 mg/kg (0.000420 mg/kg).

Residues of prothioconazole-desthio in treated honey samples ranged from n.d. to 0.336 mg/kg.

Residues in Larvae:

No residues of fenpicoxamid, prothioconazole and prothioconazole-desthio were detected in any untreated larvae samples.

Residues of fenpicoxamid in treated larvae samples ranged from n.d. to 0.0174 mg/kg.

Residues of prothioconazole in treated larvae samples ranged from n.d. to 0.125 mg/kg.

Residues of prothioconazole-desthio in treated larvae samples ranged from n.d. to 0.0142 mg/kg.

Residues in Pupae:

No residues of fenpicoxamid, prothioconazole were detected in any untreated or treated pupae samples.

Residues of prothioconazole-desthio in untreated pupae samples ranged from n.d. to < 0.001 mg/kg

(0.000414 mg/kg).

Residues of prothioconazole-desthio in treated pupae samples ranged from < n.d. to < 0.001 mg/kg (0.000393 mg/kg).

Residues in Worker Jelly:

No residues of fenpicoxamid, prothioconazole and prothioconazole-desthio were detected in any untreated worker jelly samples.

Residues of fenpicoxamid in treated worker jelly samples ranged from n.d. to 2.28 mg/kg.

Residues of prothioconazole in treated worker jelly samples ranged from n.d. to 2.80 mg/kg.

Residues of prothioconazole-desthio in treated worker jelly samples ranged from n.d. to 1.03 mg/kg.

Residues in Feeding Solution:

No residues of fenpicoxamid and prothioconazole were detected in any untreated feeding solution samples.

Residues of fenpicoxamid in treated feeding solution samples ranged from 0.182 mg/kg to 21.7 mg/kg.

Residues of prothioconazole in treated feeding solution samples ranged from 0.354 mg/kg to 54.6 mg/kg.

Residues of dimethoate in treated feeding solution samples ranged from n.d. to 9.46 mg/kg.

Residues of fenoxycarb in treated feeding solution samples ranged from n.d. to 72.3 mg/kg.

A 2.3.1.6.3 Study 2 - Guidance for Assessing Pesticide Risks to Bees

Comments of zRMS:	The GD for Bees (US EPA) 2019 was not considered by zRMS's evaluation.
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Reference:	KCP 10.3.1.6/3
Report:	United States Environmental Protection Agency (US EPA). 2019. Guidance for Assessing Pesticide Risks to Bees. Office of Pesticide Programs United States Environmental Protection Agency; Published
Guideline(s):	
Deviations:	
GLP:	
Acceptability:	NA
Duplication (if vertebrate study)	NA

A 2.3.1.6.4 Study 3 - Final Bee Risk Assessment to Support the Registration Review of Clothianidin and Thiamethoxam

Comments of zRMS:	The GD for Bees (US EPA) 2019 was not considered by zRMS in the current evaluation
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Reference:	KCP 10.3.1.6/34
Report:	US EPA. 2020. Final Bee Risk Assessment to Support the Registration Review of Clothianidin and Thiamethoxam. United States Environmental Protection Agency Office of Chemical Safety and Pollution Prevention. PC Codes: 044309, 060109, DP Barcode: 455645; Published
Guideline(s):	
Deviations:	
GLP:	

Acceptability:	NA
Duplication (if vertebrate study)	NA

A 2.3.1.6.5 Study 4 - Regulatory report on the occurrence of flowering weeds in agricultural fields

Comments of zRMS:	The summary of the report was not provided by the applicant. The Applicant is kindly reminded that detailed summaries of all studies/articles used in evaluation must be presented in dRR.
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Reference:	KCP 10.3.1.6/5
Report:	Last G, Lewis G, Pap G. 2019. Regulatory report on the occurrence of flowering weeds in agricultural fields. ERM report Nr. 0482579. ERM, Harrogate, United Kingdom
Guideline(s):	
Deviations:	
GLP:	
Acceptability:	NA
Duplication (if vertebrate study)	NA

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-3307: Effects on the Parasitoid *Aphidius rhopalosiphi* in the Laboratory (Tier I) - Dose Response Test

Comments of zRMS:	<p>The study was conducted in line with the respective guideline with minor deviations.</p> <p>It was noted that the wasps were fed with a 10 % fructose solution instead of the guideline recommended 1:3 v/v solution of honey and water. It was also noted that the guideline recommend a minimum of 15 female wasps per treatment to be evaluated for their fecundity. However, in the study in the reproduction phase 12 – 20 wasps were introduced, but some wasps were dead after 24 hrs; therefore, the number of aphid mummies obtained from 7 - 19 replicates per treatment group was used to calculate the mean aphid mummies produced per female. Since the validity criteria were met, in zRMS opinion these deviations are 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"> - Mortality in the control treatment should not exceed 13% (observed 0%), - Wasps in the control treatment should produce ≥ 5 mummies/female (observed 29.3 mummies/female), - In the control there should be no more than 2 wasps producing zero values (observed 1 wasp producing zero values), - Reference item mortality (control corrected) should be between 50% and 100% (observed 100.0%). <p>Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>LR₅₀ = 89.5 mL product/ha</p>
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Reference:	KCP 10.3.2.1/1
Report:	Moll, M.; 2014; GF-3307: Effects on the Parasitoid <i>Aphidius rhopalosiphi</i> in the Laboratory (Tier I) - Dose Response Test; Institut für Biologische Analytik und Consulting IBA-CON GmbH, 64380 Rossdorf, Germany; Lab Study No. 90541001; DAS Study No. 140224; 29 August 2014; Unpublished
Guideline(s):	Mead-Briggs <i>et al.</i> 2000 and Mead-Briggs <i>et al.</i> 2010
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable Yes
Duplication (if vertebrate study)	NA

COMPLIANCE

Guideline(s):	Mead-Briggs <i>et al.</i> 2000 and Mead-Briggs <i>et al.</i> 2010
US EPA Guideline(s):	Not applicable
Deviations:	None
Dates of work:	30 June 2014 to 05 August 2014
GLP status:	Yes

Number of pages in final re- 70
port:

MATERIALS AND METHODS

Test Item(s)

ISO Common name:	Not applicable
Test item (chemical/other name):	GF-3307
Purity:	XDE-777 4.8% w/w and prothioconazole 9.4% w/w
Description (physical state):	Brown liquid
Lot/batch no.:	F1281-135-1 (TSN307579)
CAS no.:	Not applicable

Test System

Organism (<i>Species</i>):	Parasitic wasp (<i>Aphidius rhopalosiphi</i>); 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 days after treatment. 4 replicates, each consisting of 10 wasps (7 females and 3 males) in one arena per test concentration for mortality phase. 7 - 19 females wasps for each treatment (control and test item) taken through to the fecundity phase (12 - 20 were introduced, but some wasps were dead after 24 hrs). No reproduction testing was performed with the reference item.
Test concentrations:	0 (control), 30.0, 53.0, 94.0, 167 and 250 mL product/ha. All treatments were applied in 200 L water/ha.
Environmental conditions:	Temperature: 20 to 22°C Relative humidity: 68 - 81% (acclimatisation, exposure period) 81 - 84% (post-exposure period; within the test units) Photoperiod: 590 - 2750 lux (acclimatisation, exposure, parasitisation period) 9180 - 12330 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 days before the numbers of aphid mummies that had developed were assessed.

RESULTS AND DISCUSSION

At 30.0 mL product/ha there was no test item related mortality compared to the control. At all higher dose rates, up to and including a rate of 250 mL product/ha, there was statistically significant mortality compared to the control.

Reproduction was tested at 30.0 and 53.0 mL product/ha. There was no statistically significant effect on the reproduction (mummies per female) of the parasitoids at 30.0 mL product/ha compared to the control. At 53.0 mL product/ha reproduction was statistically significantly affected and the effect on reproduction was above the trigger value of 50 % (was 81.0 %).

Table 19: Effects of GF-3307 on the survival of *Aphidius rhopalosiphi*

Test concentrations (mL GF-3307/ha)	% Mortality	Abbott corrected % mortality
Control	0.0	-
30.0	2.5	2.5
53.0	42.5	42.5 *
94.0	52.5	52.5 *
167	60.0	60.0 *
250	97.5	97.5 *
Toxic Reference	100.0	100.0 *

* Statistically different from the control

Table 20: Effects of GF-3307 on the parasitism rate of *Aphidius rhopalosiphi*

Test concentrations (mL GF-3307/ha)	Reproduction [Mean no. of mummies per female]	Effect on reproduction [% difference compared to control]
Control	29.3	-
30.0	28.8	1.6
53.0	5.6	81.0 *

* statistically different from the control

CONCLUSION

Under worst case laboratory conditions the 48-hour LR₅₀ of GF-3307 on *Aphidius rhopalosiphi* is 89.5 mL product/ha in 200 L water/ha (95 %-confidence limits: 8.9 - 534 mL product/ha).

Reproduction was tested at 30.0 and 53.0 mL product/ha. There was no statistically significant effect on the reproduction (mummies per female) of the parasitoids at 30.0 mL product/ha compared to the control. At 53.0 mL product/ha reproduction was statistically significantly affected and the effect on reproduction was above the trigger value of 50 % (was 81.0 %).

Common name	Species	Test item	Time-scale	End-point	Toxicity value	Units of test item
Parasitic wasp	<i>Aphidius rhopalosiphi</i>	GF-3307	48 hour	LR ₅₀	89.5	mL/ha

A 2.3.2.1.2 Study 2 - GF-3307: Effects on the Predatory Mite *Typhlodromus pyri* in the Laboratory (Tier I)- Dose Response Test

Comments of zRMS:	<p>The study was conducted in line with the respective guideline with no deviations.</p> <p>All the validity criteria were met:</p> <ul style="list-style-type: none"> - Control mean mortality rate should not exceed 20% on day 7 (observed 11.7%), - Control cumulative mean number of eggs per female at the end of the study should be ≥ 4 eggs/female (observed 6.8 eggs/female), - Reference item cumulative mean mortality (control corrected) on day 7 should be between 50% and 100% (observed 100.0%).
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	Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment: LR ₅₀ = 3.23 L product/ha
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Reference:	KCP 10.3.2.1/2
Report:	Moll, M; 2014; GF-3307: Effects on the Predatory Mite <i>Typhlodromus pyri</i> in the Laboratory (Tier I)- Dose Response Test; Institut für Biologische Analytik und Consulting IBA-CON GmbH, 64380 Rossdorf, Germany; Lab Study No. 90541063; DAS Study No. 140226; 28 August 2014; Unpublished
Guideline(s):	Blümel <i>et al.</i> 2000
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable Yes
Duplication (if vertebrate study)	NA

COMPLIANCE

Guideline(s):	Blümel <i>et al.</i> 2000
US EPA Guideline(s):	Not applicable
Deviations:	None
Dates of work:	30 June 2014 to 05 August 2014
GLP status:	Yes
Number of pages in final report:	69

MATERIALS AND METHODS

Test Item(s)

ISO Common name:	Not applicable
Test item (chemical/other name):	GF-3307
Purity:	XDE-777 4.8% w/w and prothioconazole 9.4% w/w
Description (physical state):	Brown liquid
Lot/batch no.:	F1281-135-1 (TSN307579)
CAS no.:	Not applicable

Test System

Organism (<i>Species</i>):	Predatory mite <i>Typhlodromus pyri</i> ; protonymphs less than 24 hours old
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:	Control, 0.407, 0.692, 1.18, 2.00 and 3.40 L product/ha and reference item. All treatments were applied in 200 L water/ha.
Environmental conditions:	Temperature: 25-27°C Relative humidity: 71-86% Photoperiod: 16 hours light : 8 hours dark light intensity: 330 lux - 730 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 less than 50% corrected mortality were sexed and the number of eggs per female was recorded on 3 assessment days within one week. No reproduction assessment was performed for the reference item.

RESULTS AND DISCUSSION

There was no treatment-related mortality at 0.407 and 1.18 L product/ha. At 0.692, 2.00 and 3.40 L product/ha mortality was statistically significantly higher compared to the control. Reproduction of *T. pyri* was assessed in the control and at 0.407, 0.692, 1.18 and 2.00 L product/ha. There were no effects on the reproduction (eggs produced per female) of the mites up to and including 2.00 L product/ha compared to the control.

Table 21: Effects of GF-3307 on the survival of *Typhlodromus pyri*

Test concentrations (L GF-3307/ha)	% Mortality	Abbott corrected % mortality
Control	11.7	-
0.407	15.0	3.8
0.692	30.0	20.8 *
1.18	21.7	11.3
2.00	30.0	20.8 *
3.40	68.3	64.2 *
Toxic Reference	100.0	100.0 *

(Negative values indicate better survivorship compared to control)

* Statistically different from the control

Table 22: Effects of GF-3307 on the fecundity of *Typhlodromus pyri*

Test concentrations (L GF-3307/ha)	Reproduction [Mean no. of eggs mummies per female]	Effect on reproduction [% Difference compared to control]
Control	6.8	-
0.407	6.5	4.4
0.692	7.3	-7.2

1.18	6.9	-0.7
2.00	5.0	26.8

(Negative values indicate better performance compared to control)

CONCLUSION

Under worst case laboratory conditions the 7-day LR₅₀ of GF-3307 on *Typhlodromus pyri* is 3.23 L product/ha in 200 L water/ha. LR₅₀ was calculated with Probit-Analysis; 95-% confidence limits could not be determined due to mathematical reasons.

Reproduction of *T. pyri* was assessed in the control and at 0.407, 0.692, 1.18 and 2.00 L product/ha. There were no effects on the reproduction (eggs produced per female) of the mites up to and including 2.00 L product/ha compared to the control.

Common name	Species	Test item	Time-scale	End-point	Toxicity value	Units of test item
Predatory mite	<i>Typhlodromus pyri</i>	GF-3307	7 day	LR ₅₀	3.23	L/ha

A 2.3.2.1.3 Study 3 - GF-3307: A laboratory Study of the Effects of Freshly Treated Substrate on the Rove Beetle, *Aleochara bilineata* (Coleoptera, Staphylinidae)

Comments of zRMS:	<p>The study was conducted in line with the respective guideline with minor deviations.</p> <p>It was noted that during the mortality assessment period of the bioassay the actual temperature range was 19.7-22.1°C while the intended range was 18-22°C. The temperature fell below 18.0°C on one occasion for <2h, hence the threshold value has been reported but it is not considered as a deviation. On one occasion, however, there were 2 consecutive hourly readings that slightly exceeded 22.0°C, reaching a maximum of 22.1°C. This deviation from the intended range of 18-22°C was due to a malfunction of the controlled environment room. However, in zRMS opinion this deviation is considered to have no impact on the outcome of the study since the validity criteria were met.</p> <p>Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>ER₅₀ > 4000 mL product/ha</p>
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Reference:	KCP 10.3.2.1/3
Report:	Tew, G; 2020; GF-3307: A 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-20-32; DAS Study No. 200609; 09 November 2020; Unpublished
Guideline(s):	Grimm <i>et al.</i> (2000)
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable Yes
Duplication (if vertebrate study)	NA

COMPLIANCE

Guideline(s):	Grimm <i>et al.</i> (2000).
US EPA Guideline(s):	Not applicable
Deviations:	On one occasion, there were 2 consecutive hourly readings that exceeded the intended temperature range of 18-22.0°C, reaching a maximum of 22.1°C.
Dates of work:	07 May 2020 to 17 July 2020
GLP status:	Yes
Number of pages in final report:	38

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-3307
Purity:	fenpicoxamid 4.7% (49 g/L), prothioconazole 9.7% (101 g/L)
Description (physical state):	Straw Coloured liquid
Lot/batch no.:	MAR19CE01Q (TSN400550)

Test System

Organism (<i>Species</i>):	Rove beetle <i>Aleochara Bilineata</i> approximately 6 days old
Study type:	Tier 1 laboratory study
Study design:	Assessments of mortality of beetles exposed for 28 days; emergence of beetles up to 71 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 mL GF-3307/ha.
Sand type:	Quartz Sand with a particle size in the range of 0.4 to 0.8 mm; moistened by adding purified water at a ratio, sand:water of 10:1 (vol/vol); the moisture content of the sand was re-adjusted to its starting weight every 1-3 days.
Application method of test item to sand:	Sprayer
Environmental conditions:	Temperature: 18.0-22.1 °C Relative humidity: 63 - 86% Lighting: 700-1000 lux 16 h photoperiod Feeding: Raw minced beef
Reference substance:	BAS 152 65 I (Perfekthion), containing nominally 400g/L dimethoate, applied at 1.1L/ha.

Methodology

GF-3307 was evaluated in a laboratory bioassay with a single application rate equivalent to 4000 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 1.1 L 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

quartz sand. 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 third 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 sand drying period the number of new adults (F1 progeny) that subsequently developed from the parasitised fly pupae was recorded over a further 36 -day period, until 71 DAT.

RESULTS AND DISCUSSION

At 28 DAT, there was 8.8% mortality in the control treatment, compared with 8.8% mortality in the 4000 mL product/ha treatment rates of GF-3307, respectively. When adjusted for the control treatment deaths, corrected mortality was 0.0% in the 4000 mL GF-3307/ha test-item treatment rate. Therefore, the LR₅₀ value was > 4000 mL GF-3307/ha. The test item treatment rate did not differ significantly from the control (Student-t test for homogeneous variances, one-sided, < control, $\alpha = 0.05$). No behavioural or physical abnormalities were observed. Therefore, the NOER value for beetle survival was 4000 mL GF-3307, the only highest rate tested.

The mean number of progeny produced per replicate was 926.0 in the control treatment, compared with a value of 857.5 in the 4000 mL product/ha treatment rate of GF-3307. A 7.4% decrease in parasitic success was therefore observed for the test-item treatment, when compared to the control treatment. Both the test item and toxic reference treatments differed significantly from the control, (Student-t test for homogeneous variances, one-sided, > control, $\alpha = 0.05$). In terms of the reproductive success of the beetles, the ER₅₀ value is >4000 mL GF-3307/ha and NOER was not determined.

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 926.0); 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 1: Effects of test item on the mortality and reproduction of *Aleochara bilineata*

Test item rates (mL/ha) GF-3307	% Mortality	Abbott corrected % mortality	Mean hatching rate (number of beetles/treatment)	% of Control
Control	8.8	-	926.0	-
4000	8.8	0	857.5*	7.4
Toxic Reference	100 *	100	0.0*	100.0

(Positive values indicate worse hatching rates compared to control)

* Statistically different from the control

CONCLUSION

In a laboratory test where adults of the rove beetle *Aleochara bilineata* were exposed to a sand substrate freshly treated with GF-3307, the ER₅₀ value was >4000 mL GF-3307/ha. The NOER value for reproduction was not determined.

Common name	Species	Test item	Time-scale	End-point	Toxicity value	Units of test item
Rove beetle	<i>Aleochara bilineata</i>	GF-3307	71 day	ER ₅₀	>4000	mL/ha
Rove beetle	<i>Aleochara bilineata</i>	GF-3307	71 day	NOER	Not Determined	mL/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-3307: Effects on mortality and reproduction to *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) under extended Laboratory Conditions

Comments of zRMS:	<p>The study was conducted in line with the respective guideline with minor deviations.</p> <p>It was noted that due to test units handling during the course of the study, the temperature decreased down to 22.7 °C and increased up to 27.2 °C. The relative humidity decreased down to 23.3 %. However, for both parameters, mean values were within the range recommended by the guideline; therefore, in zRMS opinion these deviations are considered to have no impact on the outcome of the study.</p> <p>It was also noted that due to a calculation mistake (i.e. including the density) the actual application rates were by 4 % higher than the target (nominal rates). This deviation from the target application rates is not considered to impact the results of the study and the nominal rates were used for the presentation of the results.</p> <p>All the validity criteria were met:</p> <ul style="list-style-type: none"> - In the control treatment the pre-imaginal mortality should be $\leq 30\%$ (observed 7.5%), - In the reference item treatment mortality should be $\geq 40\%$ (observed 100%), - In the control treatment the number of eggs laid should be > 2 fertile eggs per viable female per day (observed 12.8 laid eggs/female/day and 9.9 hatched eggs/female/day). <p>Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>10 days $LR_{50} = 1520$ mL product/ha Lower 42 days $ER_{50} = 209$ mL product/ha (fertility)</p>
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Reference:	KCP 10.3.2.2/1
Report:	Kimmel, S.; 2016; GF-3307: 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. 20150129; DAS Study No. 150923 ; 15 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:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable Yes
Duplication (if vertebrate study)	NA

COMPLIANCE

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.
US EPA Guideline(s):	None
Deviations:	Short-term deviations from the set range of 23-27 °C and 60-90% relative humidity are due to handling of the test units and do not impact the outcome of the study.
Dates of work:	25 August 2015 to 04 March 2016
GLP status:	Yes
Number of pages in final report:	82

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-3307
Purity:	XDE-777 (50 g/L, 4.8 wt%) and Prothioconazole (98 g/L, 9.4 wt%)
Description (physical state):	Brown liquid
Lot/batch no.:	ENBK- 147245-050 (TSN309552)

Test System

Organism (<i>Species</i>):	Ladybird beetle (<i>Coccinella septempunctata</i>), five days old larvae
Study type:	Tier II laboratory study, bean leaves for mortality and filter paper in reproduction cages for reproduction
Study design:	Assessment of the survival of larvae and pupae, the number of eggs laid per female (fecundity) and the larval hatching rate (fertility)
(No. of replicates, assessments made etc.):	40 replicates consisting of one <i>C. septempunctata</i> larva in each arena per test item rate for mortality phase Exposure phase: DAT 0 to DAT 10; 40 larvae per treatment (4 glass plates with 10 larvae each). Pre-reproduction phase: DAT 10 to DAT 26 (first egg laying in the control at DAT 20). Reproduction phase: DAT 26 to DAT 40 (fecundity assessment: DAT 26 to DAT 40, fertility assessment: DAT 31 to DAT 42). For the control and the test item treatment 3.2 mL product/ha, three reproduction units were set up, for all other test item treatments two reproduction units were set up. No reproduction was assessed for the reference item.
Test rates:	3.2, 10.1, 32.4, 103.8, 332 and 1062.5 mL product/ha
Plant substrate:	Whole leaves from potted French bean plants (<i>Phaseolus vulgaris</i>)

Environmental conditions: Temperature: mean 25.3 °C (range 22.7 – 27.2 °C, six times > 27 ° C for ≤ 1 hour)
Relative humidity: mean 67.1% (range 23.3 – 78.5%; for several occasions 55-60 % for 1-2 hours, at 12 occasions < 50 % for ≤ 1 hour)
Photoperiod: 16 h/8 h light/dark
Lighting: 2665 to 3130 Lux
Feeding: aphids of the species *Acyrtosiphon pisum*
Reference substance: Roxion (60 mL product/ha)

Methodology

The test consisted of two major phases: an exposure phase (mortality assessment: DAT 0 to DAT 10) and a reproduction phase (fecundity assessment: DAT 26 to DAT 40; fertility assessment: DAT 31 to DAT 42) which were separated by 10 to 26 days of a pre- reproduction phase. After application of the test rates of 3.2, 10.1, 32.4, 103.8, 332 and 1062.5 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 10 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 20 later 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 10 days with the most frequent duration of 6 days. The mean corrected mortality in the test item treatments ranged between 10.8 and 48.6%. The reproduction performance of females in the test item treatments ranged from 2.8 to 9.8 fertile eggs per female per day.

Table 23: Effects of GF-3307 on the survival of *Coccinella septempunctata* L.

Test concentrations (mL product/ha)	% Mortality	Abbott corrected % mortality
Control	7.5	-
3.2	17.5	10.8
10.1	22.5	16.2
32.4	22.5	16.2
103.8	22.5	16.2
332	22.5	16.2
1062.5	52.5 *	48.6 *
Toxic Reference	100	100

(Negative values indicate better survivorship compared to control)

* Statistically different from the control

Table 24: Effects of GF-3307 on the 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	12.8	76	9.9
3.2	9.6	84	8.2
10.1	13.0	75	9.8
32.4	11.2	76	8.8
103.8	5.4 *	74	4.6 *
332	3.8 *	71	2.8 *
1062.5	8.0 *	59	4.7 *

* Statistically different from the control

CONCLUSION

After 10 days of exposure of *Coccinella septempunctata* larvae to dried residues of GF-3307 on bean leaves, the LR₅₀ was 1520 mL product/ha (extrapolated), with 95% confidence limits of 612 and 3777 mL product/ha. At application rates up to and including 32.4 mL product/ha, GF-3307 had no adverse effect on the subsequent reproductive capacity of the adult ladybird beetles.

Common name	Species	Test item	Time-scale	End-point	Toxicity value	Units of test item
Lady bird beetle	<i>Coccinella septempunctata</i>	GF-3307	10 days	NOER	332	mL product/ha
Lady bird beetle	<i>Coccinella septempunctata</i>	GF-3307	10 days	LOER	1062.5	mL product/ha
Lady bird beetle	<i>Coccinella septempunctata</i>	GF-3307	10 days	LR ₁₀	42	mL product/ha
Lady bird beetle	<i>Coccinella septempunctata</i>	GF-3307	10 days	LR ₂₀	175	mL product/ha
Lady bird beetle	<i>Coccinella septempunctata</i>	GF-3307	10 days	LR ₅₀	1520	mL product/ha
Lady bird beetle	<i>Coccinella septempunctata</i>	GF-3307	42 days	NOER	32.4	mL product/ha
Lady bird beetle	<i>Coccinella septempunctata</i>	GF-3307	42 days	LOER	103.8	mL product/ha
Lady bird beetle	<i>Coccinella septempunctata</i>	GF-3307	42 days	ER ₁₀	1.9	mL product/ha
Lady bird beetle	<i>Coccinella septempunctata</i>	GF-3307	42 days	ER ₂₀	9.7	mL product/ha
Lady bird beetle	<i>Coccinella septempunctata</i>	GF-3307	42 days	ER ₅₀	209	mL product/ha

A 2.3.2.2.2 Study 2 - GF-3307: Toxicity to the Parasitoid Rove Beetle *Aleochara bilineata* (Coleoptera: Staphylinidae) under Extended Laboratory Conditions

Comments of zRMS:	<p>The study was conducted in line with the respective guideline with minor deviations.</p> <p>It was noted that due to Easter time instead of feeding every 1 to 3 days the feeding occurred once (between Day 20 and 25) after five days and the insertion of the host pupae took place on Day 20 instead of Day 21. This prolonged interval had no further impact on the results of the study as shown by the performance of the control treatment.</p> <p>It was also noted that due to technical error and New Year's Day during the reproduction phase of Main Test 1 the interval of recording the number of hatching beetles was prolonged twice. Instead of recording every 1 to 3 days the assessment occurred two times on the fourth day.</p> <p>Further, it was noted that the variations of temperature and relative humidity observed were either only occasional 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. Additionally, the deviations of the target temperature (20 ± 2 °C) and humidity (60-90%) range did not last more than two hours which is generally acceptable.</p> <p>In zRMS opinion these deviations are considered to have no impact on the outcome of the study since all the validity criteria were met.</p>
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	Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment: ER ₅₀ > 4000 mL product/ha
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Reference:	KCP 10.3.2.2/2
Report:	Kimmel, S.; 2016; GF-3307: Toxicity to the Parasitoid Rove Beetle <i>Aleochara bilineata</i> (Coleoptera: Staphylinidae) under Extended Laboratory Conditions; Innovative Environmental Services (IES) Ltd, Witterswil, Switzerland; Lab Study No. 20150131; DAS Study No. 150926 ; 03 August 2016; Unpublished
Guideline(s):	Grimm C., Reber B., Barth M., Candolfi M. P., Drexler A., Maus C., Moreth L., Ufer A., Waltersdorfer A. (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. In: Candolfi M.P., Blümel S., Forster R., Bakker F.M., Grimm C., Hassan S.A., Heimbach U., Mead-Briggs M.A., Reber B., Schmuck R., Vogt H. (eds) Guidelines to evaluate side-effects of plant protection products to non-target arthropods. pp 1-12, IOBC/WPRS, Gent.
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable Yes
Duplication (if vertebrate study)	NA

COMPLIANCE

Guideline(s):	Grimm C., Reber B., Barth M., Candolfi M. P., Drexler A., Maus C., Moreth L., Ufer A., Waltersdorfer A. (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. In: Candolfi M.P., Blümel S., Forster R., Bakker F.M., Grimm C., Hassan S.A., Heimbach U., Mead-Briggs M.A., Reber B., Schmuck R., Vogt H. (eds) Guidelines to evaluate side-effects of plant protection products to non-target arthropods. pp 1-12, IOBC/WPRS, Gent.
US EPA Guideline(s):	None
Deviations:	None
Dates of work:	13 October 2015 to 12 May 2016
GLP status:	Yes
Number of pages in final report:	61

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-3307
Purity:	XDE-777 (50 g/L, 4.8 wt%) and Prothioconazole (98 g/L, 9.4 wt%)
Description (physical state):	Brown liquid
Lot/batch no.:	ENBK- 147245-050 (TSN309552)

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 33 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:	100, 500, 1000, 2000 and 4000 mL product/ha; a control (i.e. ultrapure water) and a reference item (i.e. Roxion) were tested in parallel; spray volume of 400 mL/ha
Soil type:	Standard soil Lufa 2.1, the mean water content of the test substrate at test initiation was 11.7% which corresponds to 36.0 % of MWHC and was therefore in the target range of 35 ± 5 % of MWHC
Application method of test item to soil:	Spray
Environmental conditions:	Temperature: 16.3 – 25.1 °C, avg. = 20.2 °C Relative humidity: 44.2 – 86.9%, avg. = 66.0 % Photoperiod: 16 hours Light intensity: 1405 to 1844 lux, mean 1575 lux
Reference substance:	Feeding: defrosted mealworm larvae <i>Tenebrio molitor</i> Roxion (a.i. dimethoate, nominal 400 g/L)

Methodology

The test consisted of two major phases: an exposure phase (mortality assessment; 28 days) and a hatching phase (hatching assessment; 33 days) which were separated by 8 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 on it either alive or dead. 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 (33 days).

RESULTS AND DISCUSSION

The values of mean hatching rate in the test item treatments ranged from 550 to 756 beetles per replicate resulting percentages between 100 and 137 % relative to the control. As reproduction in the test item treatments was similar or higher than in the control, the corresponding ER₅₀ was determined (directly from the raw data) to be >4000 mL GF-3307/ha.

Table 25: Effects of GF-3307 on the mortality and reproduction of *Aleochara bilineata*

Test concentrations (mL/ha)	Mean mortality (%)	Mean hatching rate (# of beetles/treatment)	% of Control
Control	11 ± 10.3	553 ± 32*	-
100	0 ± 0	566 ± 43*	-2
500	9 ± 3	550 ± 31*	0
1000	9 ± 4.8	640 ± 99*	-16
2000	5 ± 4.1	730 ± 93*	-32
4000	6 ± 5	756 ± 36*	-37
Toxic Reference (8000)	100 ± 0.0	16 ± 12*	97

(Negative values indicate better hatching rates compared to control)

*:no statistical evaluation was conducted since the hatching rate value in all treatments was similar or higher than in the control treatment.

CONCLUSION

The study met all validity criteria since mean number of emerged F1-adults per replicate was > 400 (observed 553) and the reduction of reproductive capacity in the reference treatment was > 50 % relative to the control (observed 97%). In all test item treatments, reproduction was as high as in the control or even higher. The ER50 value was determined to be higher than the highest rate tested (> 4000mL GF-3307/ha) (obtained directly from the raw data).

Common name	Species	Test item	Time-scale	End-point	Toxicity value	Units of test item
Rove beetle	<i>Aleochara bilineata</i>	GF-3307	28 day	ER ₅₀	> 4000*	mL/ha
Rove beetle	<i>Aleochara bilineata</i>	GF-3307	28 day	NOER	4000*	mL/ha

*: obtained directly from the raw data.

A 2.3.2.2.3 Study 3 - GF-3307: Effects on the Lacewing *Chrysoperla carnea* under Extended Laboratory Conditions (Tier II)

Comments of zRMS:	<p>The study was conducted in line with the respective guideline with no deviations.</p> <p>All validity criteria were met:</p> <ul style="list-style-type: none"> - mortality in the control group was ≤ 20 % (observed 5 %), - mortality in the reference item group was ≥ 50 % (observed 100 %), - fecundity in the control was ≥ 15 eggs/female/day (observed 27.6 eggs/female/day), - fertility in the control (mean hatching rate) was ≥ 70 % (observed 92.1 %). <p>Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>LR₅₀ > 405 mL product/ha ER₅₀ > 405 mL product/ha</p>
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Reference:	KCP 10.3.2.2/3
Report:	Moll, M.; 2014; GF-3307: Effects on the Lacewing <i>Chrysoperla carnea</i> under Extended Laboratory Conditions (Tier II); Institut für Biologische Analytik und Consulting IBA-CON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany; Lab Study No. 90541047; DAS Study No. 140948; 16 December 2014; Unpublished
Guideline(s):	Vogt <i>et al.</i> 2000
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable Yes
Duplication (if vertebrate study)	NA

COMPLIANCE

Guideline(s): Vogt *et al.* 2000
US EPA Guideline(s): None
Deviations: None
Dates of work: 17 September 2014 to 24 November 2014
GLP status: Yes
Number of pages in final report: 54

MATERIALS AND METHODS

Test Item(s)

ISO Common name: Not applicable
Test item (chemical/other name): GF-3307
Purity: Prothioconazole: 9.4 % w/w
XDE-777: 4.8 % w/w
Description (physical state): Brown liquid
Lot/batch no.: F1281-135-1
CAS no.: Not applicable

Test System

Organism (*Species*): Lacewing (*Chrysoperla carnea*), larvae (2 - 3 days old)
Study type: Tier 2 extended laboratory study, leaf discs for mortality and fecundity and fertility
Study design: 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. Treatments where lacewings showed less than 50% mortality were continued through to the reproduction assessment.
Test concentrations: 0 (control), 405 and 3400 mL product/ha; treatments were applied in 200 L water/ha
Environmental conditions: Temperature: 23 - 26 °C
Relative humidity: 75 - 90 %
Photoperiod light intensity: 1010 - 1540 lux
The photoperiod is 16 h light : 8 h dark.
Feeding:
Larvae: UV-sterilised *Sitotroga cerealella* Oliv. eggs
Adults: artificial diet: 1 egg, 1 egg yolk, 15 mL condensed milk, 20 g fructose, 30 g honey, 30 g brewer's yeast, 50 g wheat germ and deionised water (approximately 45 mL) mixed homogeneously
Reference substance: 140 mL Perfekthion/ha (nominal: 400 g dimethoate/L)

Methodology

This study comprised 4 treatment groups (2 dose rates of the test item, control, reference item) with 40 replicates each containing 1 larva. The larvae were exposed to dried residues on treated leaf surfaces (bean leaves). Exposure time lasted until pupae were transferred to the reproduction units for development of adults. Mortality checks were carried out regularly until hatching of adult lacewings. In addition,

for the control and the test item treatment group where the corrected mortality was < 50 % the reproduction performance, *i.e.* egg deposition and larval hatching rate, was determined (2 checks/week, 24 hours period each assessment).

RESULTS AND DISCUSSION

There was a statistically significant treatment-related mortality at the two tested dose rates of 405 and 3400 mL product/ha compared to the control. The corrected mortality was below the trigger value of 50 % at 405 mL product/ha and therefore mortality was not affected at this rate. At 3400 mL product/ha the corrected mortality was above the trigger value of 50 %.

Reproduction was assessed at 405 mL product/ha. There were no effects on the reproduction (fecundity and fertility) of hatched lacewings at this dose rate.

Table 26: Effects of GF-3307 on the survival of *Chrysoperla carnea*

Test concentrations (mL GF-3307/ha)	% Mortality	Abbott corrected % mortality
Control	5.0	-
405	47.5	44.7 *
3400	72.5	71.1 *
Toxic Reference	100.0	100.0 *

* Statistically different from the control

Table 27: Effects of GF-3307 on the fecundity and fertility of *Chrysoperla carnea*

Test concentrations (mL GF-3307/ha)	Mean no. of eggs per female per day (fecundity)	Mean % larval hatching rate (fertility)
Control	27.6	92.1
405	23.6	89.6

CONCLUSION

Under extended laboratory conditions, mortality was statistically significantly affected at 405 mL product/ha compared to the control, but the corrected mortality was below the trigger value of 50 %. Reproduction was tested at 405 mL product/ha and the number of eggs per female per day was > 15 and the mean larval hatching rate was > 70 %. This indicates that GF-3307 had no adverse effects on either the survival of the larvae or the subsequent reproductive performance of *Chrysoperla carnea* at 405 mL product/ha.

At 3400 mL product/ha mortality was statistically significantly affected compared to the control and the corrected mortality was above the trigger value of 50 % under extended laboratory conditions. Reproduction was not assessed at this dose rate due to the high mortality.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Lacewing	<i>Chrysoperla carnea</i>	GF-3307	14 - 19 days	LR ₅₀	> 405	mL/ha
Lacewing	<i>Chrysoperla carnea</i>	GF-3307	14 - 19 days	ER ₅₀	> 405	mL/ha

A 2.3.2.2.4 Study 4 - GF-3307: Effects on the Parasitoid *Aphidius rhopalosiphii*, Extended Laboratory Study (Tier II) - Dose Response Test

Comments of zRMS:	<p>The study was conducted in line with the respective guideline with minor deviations.</p> <p>It was noted that due to the breakdown of the humidifier during the acclimatization and start of exposure period the relative humidity fell below the recommended 60 % down to 55% for approximately 6 hours. However, since the validity criteria for the control were met, in zRMS opinion this deviation is considered to have no 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"> - Mortality in the control treatment should be $\leq 10\%$ (observed 0%), - Corrected mortality in the reference item treatment should be $> 50\%$ (observed 100%), - In the control treatment there should be a minimum mean value of 5 mummies per female (observed 36.6 mummies per female), - In the control treatment no more than 2 of the surviving wasps should produce zero values (observed no wasps producing zero values). <p>Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>LR₅₀ = 769 mL product/ha</p>
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Reference:	KCP 10.3.2.2/4
Report:	Moll, M.; 2014; GF-3307: Effects on the Parasitoid <i>Aphidius rhopalosiphi</i> , Extended Laboratory Study (Tier II) - Dose Response Test -; Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany; Lab Study No. 90541002; DAS Study No. 140947; 04 December 2014; Unpublished
Guideline(s):	Mead-Briggs <i>et al.</i> , 2010
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable Yes
Duplication (if vertebrate study)	NA

COMPLIANCE

Guideline(s):	Mead-Briggs <i>et al.</i> , 2010
US EPA Guideline(s):	None
Deviations:	For approximately 6 hours during acclimatisation and start of exposure period, the relative humidity was $< 60\%$ (minimum 55 %). This is presumed to have no effect on the study as the deviation was slight and the test organisms were in good condition during exposure and reproduction.
Dates of work:	08 September 2014 to 21 October 2014
GLP status:	Yes
Number of pages in final report:	74

MATERIALS AND METHODS

Test Item(s)

ISO Common name:	Not applicable		
Test item (chemical/other name):	GF-3307		
Purity:	Prothioconazole:	9.4 % w/w	
	XDE-777:	4.8 % w/w	
Description (physical state):	Brown liquid		
Lot/batch no.:	F1281-135-1		
CAS no.:	Not applicable		

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 15 days after treatment. 6 replicates, each consisting of 5 wasps in one arena per test concentration for mortality phase; 20 replicates per treatment group were prepared with 1 female per replicate for parasitisation phase, 17-19 females were alive after the 24-hour parasitisation period
Test concentrations:	0 (control), 22.7, 79.3, 278, 971 and 3400 mL product/ha; all treatments were applied in 400 L water/ha
Environmental conditions:	Temperature: 19 - 22 °C Relative humidity: 55 - 78 % (acclimatisation, exposure) 75 - 86 % (post-exposure period, within the test units) Photoperiod: 750 - 1040 lux (acclimatisation, exposure) 1160 - 1330 lux (parasitisation period) 9400 - 13220 lux (post-parasitisation period) Feeding: A 10 %-fructose solution (acclimatisation and exposure)
Reference substance:	10.0 mL Perfekthion/ha (nominal: 400 g dimethoate/L); no fecundity assessment was performed for the reference item.

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

There was no treatment-related mortality at 22.7 and 79.3 mL product/ha compared to the control. At 278, 971 and 3400 mL product/ha mortality was statistically significantly affected compared to the control.

No repellent effect of the test item was observed compared to the control.
Reproduction was tested at 22.7, 79.3 and 278 mL product/ha. There was no statistically significant effect on reproduction (parasitisation efficiency) up to and including 278 mL product/ha (highest rate where reproduction was assessed) compared to the control.

Table 28: Effects of GF-3307 on the survival of *Aphidius rhopalosiphi*

Test concentrations (mL GF-3307/ha)	% Mortality	Abbott corrected % mortality
Control	0.0	-
22.7	0.0	0.0
79.3	0.0	0.0
278	20.0	20.0 *
971	60.0	60.0 *
3400	90.0	90.0 *
Toxic Reference	100.0	100.0 *

* Statistically different from the control

Table 29: Effects of GF-3307 on the parasitism rate of *Aphidius rhopalosiphi*

Test concentrations (mL GF-3307/ha)	Mean no. of mummies per female	% Difference compared to control
Control	36.6	-
22.7	38.1	-4.1
79.3	44.7	-22.0
278	29.8	18.5

(Negative values indicate better performance compared to control)

* Statistically different from the control

CONCLUSION

Under extended laboratory conditions the LR₅₀ of GF-3307 is 769 mL product/ha in 400 L water/ha (95%-confidence limits: 558 - 1078 mL product/ha).

No repellent effect of the test item was observed compared to the control.

Reproduction was tested at 22.7, 79.3 and 278 mL product/ha. There was no statistically significant effect on reproduction (parasitisation efficiency) up to and including 278 mL product/ha (highest rate where reproduction was assessed) compared to the control.

Common name	Species	Test item	Time-scale	End-point	Tox-icity value	Units of test item
Parasitic Wasp	<i>Aphidius rhopalosiphi</i>	GF-3307	15 days	LR ₅₀	769	mL/ha

A 2.3.2.2.5 Study 5 - GF-3307: Effects on the Wolf Spider *Pardosa spec.* in the Laboratory - Extended Laboratory Study (Tier II) -Dose Response Study

Comments of zRMS:	<p>The study was conducted in line with the respective guideline with minor deviations.</p> <p>It was noted that the body weight of the spiders recommended by the guideline should be within 12-25 mg/spider 3-4 days before application while in the study the body of one spider introduced to the reference item group was 26 mg. This occurred due to an inadvertent handling by one technician. However, in zRMS opinion this slight deviation in the body weight of one spider 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"> - Mortality in the control treatment should not exceed 8.8% at day 14 (3 out of 34 dead spiders are acceptable) (observed 2.9% at day 14; one spider died), - Mortality in the reference item treatment should be 65% ± 35% (control corrected) (observed 100%).
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	Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment: EC ₅₀ > 4000 mL product/ha
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Reference:	KCP 10.3.2.2/5
Report:	Schmitzer, S.; 2015; GF-3307: Effects on the Wolf Spider <i>Pardosa spec.</i> in the Laboratory - Extended Laboratory Study (Tier II) -Dose Response Study -; Ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany ; Lab Study No. 105531066; DAS Study No. 150927; 05 November 2015; Unpublished.
Guideline(s):	Heimbach <i>et al.</i> , 2000
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable Yes
Duplication (if vertebrate study)	NA

COMPLIANCE

Guideline(s):	Heimbach <i>et al.</i> , 2000
US EPA Guideline(s):	None
Deviations:	The body weight of one spider was 26 mg instead of 12 – 25 mg as indicated in the guideline
Dates of work:	24 August 2015 to 07 September 2015
GLP status:	Yes
Number of pages in final report:	75

MATERIALS AND METHODS

Test Item(s)

ISO Common name:	Not applicable
Test item (chemical/other name):	GF-3307
Purity:	prothioconazole: 9.59 wt%, DE-777: 4.80 wt%
Description (physical state):	Brown liquid
Lot/batch no.:	220/123-A, TSN309374
CAS no.:	Not applicable

Test System

Organism (<i>Species</i>):	Wolf Spider <i>Pardosa spec.</i> ; <i>Pardosa agrestis</i> (5.9 %), <i>Pardosa palustris</i> (2.5 %), <i>Pardosa agricola</i> (2.1 %) and subadults (89.5 %) (species determination at the end of the study); source: outdoor collected individuals near Roßdorf, South-Germany
Study type:	Tier 2 extended laboratory study
Study design:	Assessments of mortality, behaviour and feeding capacity measured up to 14 days after exposure to residues. Prolongation of Experiment: not necessary, because not more than 3 spiders died between day 7 and 14 after application and the feeding rate was not reduced by more than 50 % during the second week relative to the control.
No of replicates:	Each treatment group consisted of 34 replicates with a single spider per replicate (17 female and 17 male spiders).
Test concentrations:	0 (control), 100, 500, 1000, 2000 and 4000 mL GF-3307 per ha
Soil type:	LUFA 2.1. Soil was moistened at the beginning to about 55 % of its maximum water holding capacity (=WHK). The water holding capacity of this LUFA 2.1 Batch was 31.1 %, accordingly approx. 171 g deionised water in 1000 g dry soil was used.
Application method of test item to soil:	Laboratory-spraying equipment. Single application onto the soil of the trays and the spiders according to agricultural practice.
Environmental conditions:	Temperature: 18 - 22 °C Relative humidity: 61 - 86 % Photoperiod: 16 h light: 8 h dark light intensity: 820 lux - 960 lux Feeding: with deep frozen <i>Drosophila spec.</i> on days 0, 1, 2, 3, 7 and 10 after application at a rate of 5 flies per spider.
Reference substance:	1000 g a.i. dimethoate (nominal) in 400 L water/ha

Methodology

The spiders were exposed to direct application on natural soil (LUFA 2.1). After 2 hours and on day 1, 2, 3, 4, 7, 8, 10, 11 and 14 the number of dead or damaged individuals were assessed. The food consumption was assessed on day 1, 2, 3, 4, 8 and 11.

There were five treatments: the test item applied at rates of 100, 500, 1000, 2000 and 4000 mL GF-3307/ha, in 400 L water/ha, a water treated control and a reference item (dimethoate).

Each treatment group consisted of 34 replicates with a single spider per replicate (17 female and 17 male spiders).

RESULTS AND DISCUSSION

Mortality rates 14 days after treatment of the spiders with 100, 500, 1000, 2000 and 4000 mL GF-3307 per ha was: 3.1 % (100 mL/ha), 0.0 % (500, 1000 and 2000 mL/ha) and 6.1 % (4000 mL/ha) (corrected to the control). In the control group one spider died over the course of the study. This was not significantly different from the mortality rates of the control spiders.

Food consumption of the spiders which were exposed to 100, 500 and 1000 mL/ha GF-3307 was slightly reduced (max. - 8 % compared to the control values). In the 2000 and 4000 mL/ha GF-3307 treatment group a higher food consumption was noted compared to that of the control spiders. All these values were not statistically significant different to the control group.

One male spider was apathetic before dying in the 4000 mL/ha GF-3307 treatment group.

No further test item related behavioural abnormalities occurred.

Table 30: Effects of GF-3307 on the survival of *Pardosa spec.*

Test concentrations (mL GF-3307 per ha)	% Mortality	Abbott corrected % mortality
Control	2.9	-
100 mL	5.9	3.1
500 mL	2.9	0.0
1000 mL	0.0	0.0
2000 mL	2.9	0.0
4000 mL	8.8	6.1
Toxic Reference	100.0 *	100.0

* Statistically different from the control

Table 31: Effects of GF-3307 on the feeding capacity of *Pardosa spec.*

Test concentrations (mL GF-3307 per ha)	Flies consumed per spider per day	Difference in feeding capacity (%) compared to control
Control	2.4	-
100 mL	2.4	+2
500 mL	2.2	+8
1000 mL	2.3	+7
2000 mL	2.5	-2
4000 mL	2.5	-4
Toxic Reference	-	

(Negative values indicate better performance compared to control)

* Statistically different from the control

CONCLUSION

Mortality rates 14 days after treatment of the spiders with 100, 500, 1000, 2000 and 4000 mL GF-3307 was not significantly different from the mortality rates of the control spiders. 100.0 % mortality occurred after treatment with 1000 g a.i. dimethoate/ha.

Food consumption of the spiders were not statistically significant different to the control group.

Under extended laboratory conditions the 14-d EC₅₀ > 4000 mL/ha in 400 L water/ha.

Common name	Species	Test item	Time-scale	End-point	Tox-icity value	Units of test item
Wolf spider	<i>Pardosa spec.</i>	GF-3307	14 day	EC ₅₀	> 4000	mL/ha

A 2.3.2.2.6 Study 6 - GF-3307: A Rate-Response Extended Laboratory Study of the Effects of Freshly Treated Substrate on the Rove Beetle, *Aleochara bilineata* (Coleoptera, Staphylinidae)

Comments of zRMS:	<p>The study was conducted in line with the respective guideline with minor deviations.</p> <p>It was noted that while the beetles were held in warm storage, prior to use in the bioassay, the humidity fell below the specified range of 60-90% for four consecutive hourly readings, reaching a minimum of 28% relative humidity. This deviation from the intended range was due to a malfunction of the controlled environment room. Based on the performance of the <i>Aleochara</i> in the control treatment, it was considered that this deviation did not have any adverse effects on the test beetles used in the bioassay and the test passed all validity criteria. In zRMS opinion this deviation did not affect the integrity or outcome of the study.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment:</p>
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	ER ₅₀ > 4000 mL product/ha
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Reference:	KCP 10.3.2.2/6
Report:	Tew, G; 2020; GF-3307: 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-20-33; DAS Study No. 200610 ; 09 November 2020; Unpublished
Guideline(s):	Grimm et al. (2000)
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable Yes
Duplication (if vertebrate study)	NA

COMPLIANCE

Guideline(s):	Grimm et al. (2000).
US EPA Guideline(s):	Not applicable
Deviations:	During the culture phase of the bioassay, the humidity within the controlled environment room fell below the specified range of 60-90% RH for four consecutive hourly readings, reaching a minimum of 28% RH.
Dates of work:	01 April 2020 to 25 June 2020
GLP status:	Yes
Number of pages in final report:	42

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-3307
Purity:	fenpicoxamid 4.7% (49 g/L), prothioconazole 9.7% (101 g/L)
Description (physical state):	Straw Coloured liquid
Lot/batch no.:	MAR19CE01Q (TSN400550)

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 69 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 GF3307/ha. Individual treatments were applied at a volume rate equivalent to 400 L spray solution/ha.
Soil type:	LUFA 2.1, 35% ± 5% WHC

Application method of test item to soil:	Sprayer
Environmental conditions:	Temperature: 18.0-22.0 °C Relative humidity: 63-86% Lighting: 16 h, 800-1000 lux Feeding: Raw minced beef
Reference substance:	BAS 152 65 I (Perfekthion), containing nominally 400g/L dimethoate, applied at 3800 mL/ha.

Methodology

GF-3307 was evaluated in an 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 third 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 34 -day period, until 69 DAT.

RESULTS AND DISCUSSION

At 28 DAT, there was 11.3% mortality in the control treatment, compared with 15.0%, 13.8%, 18.8%, 15.0%, and 22.5% mortality in the 4000, 2000, 1000, 500 and 100 mL product/ha treatment rates of GF-3307, respectively. Therefore, the LR₅₀ value was > 4000 mL GF-3307/ha. None of the treatment rates differed significantly from the control (Multiple sequentially-rejective Fisher Test After Bonferroni-Holm, one-sided, > control, $\alpha = 0.05$). No behavioural or physical abnormalities amongst the adult beetles were observed. Therefore, the NOER value for beetle survival was 4000 mL GF-3307, the highest rate evaluated.

The mean number of progeny produced per replicate was 811.0 in the control treatment, compared with values of 953.8, 903.3, 915.8, 914.5 and 854.8 in the 4000, 2000, 1000, 500 and 100 mL product/ha treatment rates of GF-3307, respectively. The ER₅₀ value was > 4000 mL GF-3307/ha. None of the test item treatment rates differed significantly from the control, Williams multiple sequential t-test procedure (one sided, < control, $\alpha = 0.05$). The NOER was 4000 mL GF 3307/ha, the highest rate evaluated.

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 811.0); 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 32: Effects of test item on the mortality and reproduction of *Aleochara bilineata*

Test item rates (mL/ha) GF-3307	% Mortality	Abbott corrected % mortality	Mean hatching rate (number of bee- tles/treatment)	% of Control
Control	11.3	-	811.0	-
100	22.5	12.7	854.8	-5.4
500	15.0	4.2	914.5	-12.8
1000	18.8	8.5	915.0	-12.8
2000	13.8	2.8	903.3	-11.4
4000	15.0	4.2	953.8	-17.6
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-3307, the ER₅₀ value was >4000 mL GF-3307/ha. The NOER value for reproduction was 4000 mL GF-3307/ha, the highest rate tested.

Common name	Species	Test item	Time-scale	End- point	Tox- icity value	Units of test item
Rove beetle	<i>Aleochara bilineata</i>	GF-3307	69 day	ER ₅₀	>4000	mL/ha
Rove beetle	<i>Aleochara bilineata</i>	GF-3307	69 day	NOER	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-3307: An aged residue extended laboratory study on the parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera, Braconidae)

Comments of zRMS:	<p>The study was conducted in line with the respective guideline with no deviations.</p> <p>All the validity criteria were met:</p> <ul style="list-style-type: none"> - Mortality in the control should not exceed 13% at 48 h (observed 2.5 at 0 DAT, 7.5 at 14 DAT, 2.5 at 28 DAT), - Mortality in the toxic reference item treatment (0 DAT bioassay) should exceed 50% at 48 h (observed 100%), - In the control treatment the mean number of mummies should be > 5 per female and there should not be more than two wasps producing zero values (observed 24.6 at 14 DAT, 38.2 at 28 DAT; no wasps producing zero values). <p>Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>ER₅₀ > 2000 mL product/ha</p>
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Reference:	KCP 10.3.2.3/1
Report:	Stevens, J.; 2016; GF-3307: An aged residue extended laboratory study on the parasitic wasp <i>Aphidius rhopalosiphi</i> (Hymenoptera, Braconidae); Mambo-Tox Ltd., 2 Venture Road, University Science Park, Southampton SO16 7NP, UK; Lab Study No. DOW-16-37; DAS Study No. 150924; 26 September 2016; Unpublished
Guideline(s):	Mead-Briggs <i>et al.</i> (2000). A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, <i>Aphidius rhopalosiphi</i> (De Stephani-Perez) (Hymenoptera, Braconidae)
Deviations:	None

GLP:	Yes
Acceptability:	Acceptable Yes
Duplication (if vertebrate study)	NA

COMPLIANCE

Guideline(s):	Mead-Briggs <i>et al.</i> (2000). A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, <i>Aphidius rhopalosiphi</i> (De Stephani-Perez) (Hymenoptera, Braconidae)
US EPA Guideline(s):	Not applicable
Deviations:	None
Dates of work:	15 June 2016 to 09 August 2016
GLP status:	Yes
Number of pages in final report:	45

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-3307
Purity:	prothioconazole 9.59% w/w (100 g/L) DE-777 4.80% w/w (50 g/L)
Description (physical state):	amber to brown liquid (Emulsifiable Concentrate formulation)
Lot/batch no.:	220/123-A (TSN309374)

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 (with a minimum of five females) in one arena per test concentration for mortality phase. For the subsequent fecundity assessments, 15 female wasps per treatment were individually confined over pots of aphid-infested barley.
Test concentrations:	0 (Control), two applications of 2 L product/ha; application volume of 400 L/ha
Environmental conditions:	Temperature: 20.5-21.2°C Relative humidity: 68-75% Photoperiod: 16 h (1101-2019 lux during mortality phase, 5212-5332 lux during reproduction phase) Feeding: 10 % fructose solution applied to leaves prior to arena assembly.
Reference substance:	Dimethoate EC, nominally 400 g/L, applied one time at 60 mL product/ha.

Methodology

The aim was to evaluate the effects of both fresh dry and field-aged residues of the test item on the parasitic wasp *Aphidius rhopalosiphii* in a series of bioassays carried out under extended laboratory test conditions. GF-3307 was evaluated at a single application rate of 2 L product/ha, this being applied on two occasions (times T1 and T2) with a 14-day interval. A water-treated control (also applied at T1 and T2) was included in all of the bioassays, whilst a toxic reference treatment (applied at T2) was included in the initial bioassay only. Treatments were applied French bean plants (*Phaseolus vulgaris*) which were then maintained outdoors, but protected from any rainfall by storing them under a suspended sheet of UV light-permeable polythene.

Bioassays were initiated on the day of the T2 treatment application, i.e. 0 days after treatment (DAT), and also at 14 and 28 DAT. For each bioassay leaves were collected from the plants and returned to the laboratory. The leaves were prepared by applying a 10% w/v solution of fructose in water as a thin stripe across their exposed upper surface, to provide a food source and foraging stimulus for wasps. Once this had dried, the leaves were used to line the floor and ceiling of shallow test arenas, with their upper surfaces facing inwards. Ten adult wasps (including a minimum of five females), less than 48 h old, were placed into each replicate arena (n = 4 per treatment). Assessments of mortality were made over 48 h.

To determine any sub-lethal effects on the reproductive capacity of the surviving wasps, assessments were then carried out for the control and treatment rate of the test item. Fifteen female wasps from each treatment were confined individually for 24 h over untreated barley plants that had previously been infested with cereal aphids (*Metopolophium dirhodum* and *Rhopalosiphum padi*). The wasps were then removed and the plants left for a further 10 days before the number of ‘mummies’ (parasitised aphids containing wasp pupae) that had developed was recorded.

RESULTS AND DISCUSSION

In the bioassay initiated with fresh residues (0 DAT), there was 2.5% mortality in the control, compared with 100% mortality (100% corrected mortality) in both the GF-3307 treatment and the toxic reference. These results differed significantly (Fisher’s Exact Test, $\alpha = 0.05$).

In the bioassay initiated 14 DAT, there was 7.5% mortality in the control, compared with 30.0% mortality (24.3% corrected mortality) in the GF-3307 treatment. When compared statistically, these results differed significantly (Fisher’s Exact Test, $\alpha = 0.05$). The mean number of mummies produced per surviving female was 24.6 in the control, compared with 25.1 in the GF-3307 treatment. This was equivalent to an increase of 1.9% in the test-item treatment, a result which did not differ significantly from the control (t-test for independent samples, $\alpha = 0.05$).

In the bioassay initiated 28 DAT, there was 2.5% mortality in the control, compared with 10.0% mortality (7.7% corrected mortality) in the GF-3307 treatment. When compared statistically, these results did not differ significantly (Fisher’s Exact Test, $\alpha = 0.05$). The mean number of mummies produced per surviving female was 38.2 in the control, compared with 31.4 in the GF-3307 treatment. This was equivalent to a decrease of 17.7% in the test-item treatment, a result which did not differ significantly from the control (t-test for independent samples, $\alpha = 0.05$).

Since the GF-3307 treatment had resulted in < 50% reduction in both wasp survival and reproduction, relative to the control, in two consecutive bioassays, the testing programme was ended.

Table 33: Effects of GF-3307 on the survival of *Aphidius rhopalosiphi*

Bioassay initiated	Application rate (mL/ha)	% Mortality (at 48h)	Abbott corrected % mortality
0 DAT	Control	2.5	-
	2 x 2000	100 *	100
	Toxic Reference	100 *	100
14 DAT	Control	7.5	-
	2 x 2000	30.0 *	24.3
28 DAT	Control	2.5	-
	2 x 2000	10.0	7.7

* Statistically different from the control

Table 34: Effects of GF-3307 on the parasitism rate of *Aphidius rhopalosiphi*

Bioassay initiated	Test concentrations (mL/ha)	Mean no. of mummies per female	Effect on reproduction* % difference compared to control
14 DAT	Control	24.6	-
	2 x 2000	25.1	-1.9
28 DAT	Control	38.2	-
	2 x 2000	31.4	17.7

*A positive value indicates a decrease in reproduction, a negative value an increase.

CONCLUSION

In an aged-residue study to determine the effects of GF-3307 on the parasitic wasp, *Aphidius rhopalosiphi*, a series of extended laboratory tests were performed on both fresh and outdoor-aged foliar residues. Following two applications to French bean plants at a rate of 2000 mL product/ha, with a 14-day interval, both 14- and 28-day-old foliar residues of GF-3307 had no unacceptable effects on either wasp survival or reproduction (i.e. < 50% effects, relative to the control).

Bioassay initiated	Common name	Species	Test item	Endpoint	Value	Toxicity value	Units of test item
0 DAT	Parasitic wasp	<i>Aphidius rhopalosiphi</i>	GF-3307	48 h mortality	NOER	< 2000	mL product/ha (applied twice)
14 DAT	Parasitic wasp	<i>Aphidius rhopalosiphi</i>	GF-3307	48 h mortality	NOER	> 2000	mL product/ha (applied twice)
14 DAT	Parasitic wasp	<i>Aphidius rhopalosiphi</i>	GF-3307	Fecundity	NOER	> 2000	mL product/ha (applied twice)
28 DAT	Parasitic wasp	<i>Aphidius rhopalosiphi</i>	GF-3307	48 h mortality	NOER	> 2000	mL product/ha (applied twice)
28 DAT	Parasitic wasp	<i>Aphidius rhopalosiphi</i>	GF-3307	Fecundity	NOER	> 2000	mL product/ha (applied twice)

A 2.3.2.3.2 Study 2 - GF-3307: An aged-residue extended laboratory study with the green lacewing *Chrysoperla carnea* (Neuroptera, Chrysopidae)

Comments of zRMS:	<p>The study was conducted in line with the respective guideline with minor deviations.</p> <p>It was noted that during the bioassays the ambient relative humidity recorded within the test room was 56-87% (instead of the intended guideline range of 60-90%), but only 4 out of 869 hourly readings were outside the intended range. In zRMS opinion this deviation did not affect the outcome of the bioassay or the integrity of the study.</p> <p>It was also noted that there were two deviations to the study plan:</p> <ul style="list-style-type: none"> - The protocol indicated that a laboratory track-sprayer would be used to make treatment applications. However, for reasons of practicality (there being a large number of plants to spray), a hand-held boom sprayer (Azo Ltd., Ede, The Netherlands) powered by compressed air was used instead. This was fully calibrated
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	<p>to deliver the intended target output of 400 L spray solution/ha. Due to an oversight, an amendment was not raised at the time of the T1 treatment application, although one was raised prior to the T2 application. It was considered that this deviation did not affect the integrity of the study.</p> <ul style="list-style-type: none"> - Due to a malfunction of the data-logger being used to record the ambient temperatures alongside the treated plants, during their period of storage outdoors, no data were available from immediately after the second treatment application, i.e. from 0 DAT (full details provided in Appendix III of the study report). However, it was considered that this deviation did not affect the overall integrity of the study. <p>All the validity criteria were met:</p> <ul style="list-style-type: none"> - Pre-imaginal mortality in the control group should not exceed 20%. Actual mortality was 17.5% and 20.0% in the 0 and 14 DAT bioassays, respectively. - Mean egg production in the control should be ≥ 15 eggs per female per day. Actual egg production was 31.8 and 32.7 eggs per female in the 0 and 14 DAT bioassays, respectively. - Mean hatching rate of the eggs in the control should be $\geq 70\%$. Actual egg hatching was 90.6% and 90.8% in the 0 and 14 DAT bioassays, respectively. - Mortality in the toxic reference treatment should be $\geq 50\%$. Actual mortality was 90%. <p>Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>$ER_{50} > 2.0$ L product/ha</p>
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Reference:	KCP 10.3.2.3/2
Report:	Vaughan, R.; 2015; GF-3307: An aged-residue extended laboratory study with the green lacewing <i>Chrysoperla carnea</i> (Neuroptera, Chrysopidae); Mambo-Tox Ltd.; Lab Study No. DOW-15-37; DAS Study No. 150925; 21 December 2015; Unpublished.
Guideline(s):	Vogt <i>et al.</i> (2000). Laboratory method to test effects of plant protection products on larvae of <i>Chrysoperla carnea</i> (Neuroptera: Chrysopidae)
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable Yes
Duplication (if vertebrate study)	NA

COMPLIANCE

Guideline(s):	Vogt <i>et al.</i> (2000). Laboratory method to test effects of plant protection products on larvae of <i>Chrysoperla carnea</i> (Neuroptera: Chrysopidae)
US EPA Guideline(s):	Not applicable
Deviations:	During the bioassays the ambient relative humidity recorded within the test room was 56-87% (against guideline range of 60-90%), but only 4 out of 869 hourly readings were outside the guideline range. It was considered that this deviation did not affect the outcome of the bioassay or the integrity of the study.
Dates of work:	15 September 2015 to 25 November 2015
GLP status:	Yes
Number of pages in final report:	45

MATERIALS AND METHODS

Test Item(s)

ISO Common name:	Not applicable
Test item (chemical/other name):	GF-3307
Purity:	9.59% w/w (100 g/L) prothioconazole 4.80% w/w (50 g/L) DE-777
Description (physical state):	brown liquid (emulsifiable concentrate)
Lot/batch no.:	220/123-A (TSN309374)
CAS no.:	Not applicable

Test System

Organism (<i>Species</i>):	Green Lacewing (<i>Chrysoperla carnea</i>)
Study type:	Tier 2 extended laboratory tests to evaluate effects of both fresh (0-day-old) and aged (14-day-old) foliar residues.
Study design:	Treatments applied to French bean plants, from which leaves were collected for bioassays initiated 0 and 14 days after treatment (DAT). For each bioassay, 40 replicate lacewing larvae evaluated per treatment. Endpoints in each bioassay were assessment of pre-imaginal mortality and of the reproductive success of surviving insects (counts of eggs laid per female over two days and check on egg viability).
Test-item concentrations:	2.0 L GF-3307/ha, applied on two occasions with a 14-day interval; applied at a volume rate of 400 L spray solution/ha
Environmental conditions:	Temperature: 23.7-25.6°C Relative humidity: 56-78% Photoperiod: 16 h (2300-4400 lux) Feeding: lacewing larvae fed every 1-3 days with untreated UV light-killed eggs of the Angoumois grain moth, <i>Sitotroga cerealella</i> .
Reference substance:	BAS 152 11 I (Perfekthion), nominally 400 g/L dimethoate, applied at 200 mL product/ha on one occasion.

Methodology

GF-3307 was evaluated at a single application rate, equivalent to 2.0 L product/ha, this being applied on two separate occasions, with 14-day interval in-between. This treatment was compared to a control of water, also applied twice. A toxic reference treatment of BASF Perfekthion (nominally 400 g/L dimethoate) was applied on one occasion only, at a rate of 200 mL product/ha. All treatments were applied at a volume rate of 400 L spray solution/ha to potted French bean plants (*Phaseolus vulgaris* L.). The treated plants were stored outdoors (but protected from rainfall) both between applications and afterwards.

As a test substrate for bioassays, leaves were collected from the plants either immediately once residues had dried (i.e. 0 days after final treatment (DAT)), or after 14 days ageing of the residues outdoors (i.e. 14 DAT). For each bioassay, larvae of *C. carnea* (2-3 days old, n = 40 per treatment) were confined individually on the leaves and were fed every 1-3 days with untreated eggs of the Angoumois grain moth, *Sitotroga cerealella*. 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 evaluated for the control and test-item treatments. 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

In the bioassay initiated 0 DAT, corrected pre-imaginal mortality was 24.2% in the GF-3307 treatment and 87.9% in the toxic reference treatment. In the bioassay initiated 14 DAT, corrected pre-imaginal mortality was 0.0% in the GF-3307 treatment (Table 1).

In both bioassays, the reproductive performance of the surviving lacewings in the control and test-item treatments exceeded the thresholds of ≥ 15 eggs/female/day and $\geq 70\%$ hatching rate that are currently viewed as being indicative of there being no harmful treatment effects on reproduction (Table 2). All validity criteria imposed for the study were met.

Table 35: Effects of GF-3307 on the survival of *Chrysoperla carnea*.

Bioassay initiated	Test concentrations (L GF-3307/ha)	% Mortality	Abbott-corrected % mortality
0 DAT	Control	17.5	-
	2 x 2.0	37.5	24.2
	Toxic Reference	90.0 *	87.9
14 DAT	Control	20.0	-
	2 x 2.0	20.0	0

* Statistically different to the control ($\alpha = 0.05$).

Table 36: Effects of GF-3307 on the fecundity and fertility of *Chrysoperla carnea*.

Bioassay initiated	Test concentrations (L GF-3307/ha)	Mean no. of eggs per female per day (fecundity)	Mean % larval hatching rate (fer- tility)	Mean viable eggs/female/day	Effects on repro- duction* [%]
0 DAT	Control	31.8	90.6	28.8	-
	2 x 2.0	32.7	90.5	29.6	-2.8
	Toxic Reference	~	~	~	~
14 DAT	Control	32.7	90.8	29.7	-
	2 x 2.0	28.3	88.9	25.2	15.2

~ not assessed.

*Percentage change in the calculated mean number of viable eggs per female, relative to the control. A negative value indicates an increase, a positive value indicates a decrease.

CONCLUSION

The effects of both fresh and field-aged residues of GF-3307 on the green lacewing *Chrysoperla carnea* were evaluated under extended laboratory test conditions. Following application at a rate of 2.0 L product/ha on two separate occasions, with a 14-day interval in-between, both fresh (0-day-old) and field-aged (14-day-old) residues of GF-3307 had no adverse effect on either the survival of larvae or the subsequent reproductive capacity of the adult lacewings.

Common name	Species	Test item	Endpoint	Toxicity value	Units of test item
Lacewing	<i>Chrysoperla carnea</i>	GF-3307	NOER, Pre-imaginal mortality	2 x 2.0	L/ha
Lacewing	<i>Chrysoperla carnea</i>	GF-3307	NOER, Fecundity	2 x 2.0	L/ha

A 2.3.2.3.3 Study 3 - GF-3307: Aged-residue extended laboratory tests to determine effects on the ladybird beetle, *Coccinella septempunctata* (Coleoptera, Coccinellidae) ~~An aged-residue extended laboratory study with the green lacewing *Chrysoperla carnea* (Neuroptera, Chrysopidae)~~

Comments of zRMS:	<p>The study was conducted in line with the respective guideline with no deviations.</p> <p>All the validity criteria were met:</p> <ul style="list-style-type: none"> - Pre-imaginal mortality in the control group should not exceed 30% (observed 25% at 0 DAT, 0% at 14 DAT and 2.5% at 28 DAT). - Mean egg production in the control should be > 2 viable eggs per female per day in the control treatment (observed 20.9 at 0 DAT, 11.8 at 14 DAT and 4.7 at 28 DAT) - Mortality in the toxic reference treatment should be ≥ 50% (0 DAT Bioassay only) (observed 97.5%, corrected 96.7%). <p>Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>ER₅₀ > 2.0 L product/ha</p>
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Reference:	KCP 10.3.2.3/3
Report:	Vaughan R.; 2017; GF-3307: 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-17-6; DAS Study No. 170778; 16 January 2018; Unpublished
Guideline(s):	Schmuck <i>et al.</i> (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 Yes
Duplication (if vertebrate study)	NA

COMPLIANCE

Guideline(s):	Schmuck <i>et al.</i> (2000). A laboratory test system for assessing effects of plant protection products on the plant-dwelling insect <i>Coccinella septempunctata</i> L. (Coleoptera: Coccinellidae).
US EPA Guideline(s):	Not applicable
Deviations:	None
Dates of work:	18 September 2017 to 26 December 2017
GLP status:	Yes
Number of pages in final report:	55

MATERIALS AND METHODS

Test Item(s)

Test item (chemical/other name):	GF-3307
Purity:	48 g/L fenpicoxamid 96 g/L prothioconazole
Description (physical state):	Pale brown liquid (emulsifiable concentrate formulation)
Lot/batch no.:	ENBK-169144-026 (TSN314279)

Test System

Organism (<i>Species</i>):	Seven-spotted ladybird (<i>Coccinella septempunctata</i> 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 (<i>Phaseolus vulgaris</i> 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. All treatments were applied at a volume rate of 400 L spray solution/ha using a calibrated sprayer.
Environmental conditions:	Temperature: 23.6-25.5°C Relative humidity: 50-81% Photoperiod: 16 h (2700-4400 lux) Feeding: insects provided daily with untreated pea aphids (<i>Acyrtosiphon pisum</i> (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, 14 and 28 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 every 1-3 days 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%, 0.0% and 2.5% in the bioassays initiated 0, 14 and 28 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-3307 treatment, pre-imaginal mortality was 57.5%, 5.0% and 22.5% for the 0, 14 and 28 DAT bioassays, respectively. Corrected pre-imaginal mortality was therefore 43.3%, 5.0% and 20.5% for the 0, 14 and 28 DAT bioassays, respectively.

Reproduction assessments were carried out for the control and test-item treatment only. In each of the bioassays, the reproductive performance in the control and test-item treatments exceeded the threshold of > 2 viable eggs/female/day, currently viewed as being indicative of no harmful treatment effects (Schmuck et al., 2000). The validity criteria imposed for the control treatment was therefore met.

Table 37: Effects of GF-3307 on the survival of *Coccinella septempunctata*.

Bioassay initiated (days after 2 nd treatment application)	Test concentrations (L product/ha, applied on two occasions)	% Mortality	Abbott-corrected % mortality
0	Control	25.0	-
	2.0	57.5 *	43.3
	Toxic Reference	97.5 *	96.7
14	Control	0.0	-
	2.0	5.0	5.0
28	Control	2.5	-
	2.0	22.5 *	20.5

* Statistically different from the control ($\alpha = 0.05$).

Table 2: Effects of GF-3307 on the reproductive capacity of *Coccinella septempunctata*.

Bioassay initiated (days after 2 nd treatment applica- tion)	Test concentrations (L product/ha, applied on two occa- sions)	Mean number eggs/fe- male/day	Mean percent- age egg viabil- ity	Mean viable eggs/fe- male/day	Effects on repro- duction*[%]
0	Control	30.0	69.7	20.9	-
	2.0	28.2	50.6	14.3	31.6
14	Control	16.7	70.6	11.8	-
	2.0	14.5	73.9	10.7	9.1
28	Control	7.2	65.5	4.7	-
	2.0	11.0	66.2	7.3	-54.0

*Percentage change in mean number of viable eggs per female, relative to control. A positive value indicates a decrease; a negative value indicates an increase, compared to the control.

CONCLUSION

The effects of both freshly-dried and field-aged residues of GF-3307 on the ladybird beetle, *Coccinella septempunctata*, were evaluated under extended laboratory test conditions. Following two applications of GF-3307 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 14 and 28 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-3307	Pre-imaginal mortality	Time to pre-imaginal mortality being < 50%	14 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-3307: Effects on Reproduction and Growth of Earthworms *Eisenia fetida* in Artificial Soil with 10% Peat

Comments of zRMS:	<p>The study was conducted in line with OECD 222 with no deviations.</p> <p>The test design was relevant to derive only the NOEC value (5 concentrations, 8 replicates for control, 4 replicates per treatment group). Observed effects were not statistically significantly different compared to the control.</p> <p>All the validity criteria were met:</p> <ul style="list-style-type: none"> - Adult mortality in the control treatment should not exceed 10% over the initial 4 weeks of the test (observed 0%). - In the control treatment the number of juveniles per replicate should be ≥ 30 by the end of the test (observed 253 to 349). - The coefficient of variation of reproduction in control treatment should be $\leq 30\%$ (observed 11%). <p>Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>56d NOEC_{reproduction} = 120 mg product/kg soil dw</p>
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Reference:	KCP 10.4.1.1/1
Report:	Ganßmann, M.; 2014; GF-3307: 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, Arheilger Weg 17, 64380 Rossdorf, Germany; Lab Study No. 90541022; DAS Study No. 140234; 06 November 2014; Unpublished
Guideline(s):	OECD 222
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable Yes
Duplication (if vertebrate study)	NA

COMPLIANCE

Guideline(s):	OECD 222, 2004 and ISO 11268-2, 2012
US EPA Guideline(s):	None
Deviations:	None
Dates of work:	05 August 2014 to 06 October 2014
GLP status:	Yes

MATERIALS AND METHODS

Test Item(s)

ISO Common name:	Not applicable
Test item (chemical/other name):	GF-3307
Content:	XDE-777: 4.8% w/w (nominal), 4.8 % w/w (analysed) Prothioconazole: 9.6 % w/w (nominal), 9.4 % w/w (analysed)
Description (physical state):	Brown liquid
Batch no.:	TSN307579
Lot No.:	F1281-135-1
CAS no.:	Not applicable

Test System

Organism (<i>Species</i>):	Earthworm (<i>Eisenia fetida</i>), adult worms (with clitellum and weight range 300 to 580 mg), approximately 6 months old, source: from an in-house culture.
Study type:	56 day earthworm acute 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, 7.5, 15, 30, 60 and 120 mg GF-3307/kg soil dry weight
Soil parameters:	Artificial soil according to OECD 222. pH at initiation: 5.7 to 6.0 pH at termination: 5.7 to 5.8 Water content at initiation: 32.1% to 32.7% (54.3% to 55.4% of the maximum water holding capacity) Water content at termination: 34.4% to 37.0% (58.3% to 62.8% of the maximum water holding capacity)
Environmental conditions:	Temperature: within the range of 18°C to 22°C 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.

Reference substance:

Carbendazim (499 g/kg nominal). In the most recent test with the reference item Luxan Carbendazim 500 FC (performed under IBACON Study Number 46646022 from August 2013 to October 2013), there were statistically significant effects on reproduction at a concentration of 1.30 mg carbendazim/kg soil and higher; the EC50 for reproduction was calculated as 1.32 mg carbendazim/kg soil. The results are shown in Appendix 2 indicating that the sensitivity of the worms was consistent with the level proposed by the OECD 222 guideline (significant effects between 1 and 5 mg carbendazim/kg soil).

Methodology

56-day test in treated artificial soil according to OECD 222; different concentrations of the test item were incorporated into the soil; 6 treatment groups (5 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

A slight mortality of 2.5% was found at the concentration of 120 mg test item/kg soil which was not statistically significantly different compared to the control, where none of the worms died (Fisher's Exact Test, one-sided greater, $\alpha = 0.05$).

Body weight of the earthworms exposed to GF-3307 were not statistically significantly different compared to the control up to and including the highest test concentration of 120 mg GF-3307/kg soil, (Williams t-test, two-sided $\alpha = 0.05$).

No statistically significant effects on reproduction were observed up to and including the highest test item concentration of 120 mg test item/kg soil compared to the control group (Williams t-test, one-sided smaller, $\alpha = 0.05$, 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 38: Effects of GF-3307 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	0.0	46.8	292	-
7.5	0.0	54.2	288	98.6
15	0.0	41.4	326	111.5
30	0.0	37.2	307	104.9
60	0.0	35.4	258	88.2
120	2.5	43.0	294	100.6

CONCLUSION

In an earthworm reproduction and growth study with GF-3307 the LC₅₀ was determined to be greater than 120 mg test item/kg soil. The No Observed Effect Concentration (NOEC) for mortality, growth and reproduction of the earthworm *Eisenia fetida* was determined to be 120 mg test item/kg soil, *i.e.* the highest concentration tested. The Lowest Observed Effect Concentration (LOEC) for mortality, growth and reproduction of the earthworm *Eisenia fetida* was estimated to be greater than 120 mg test item/kg soil.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Earthworm	<i>Eisenia fetida</i>	GF-3307	56 day	NOEC	120	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-3307: Effects on Reproduction of the Collembola *Folsomia candida* in Artificial Soil with 5% Peat

Comments of zRMS:	<p>The study was conducted 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) even though the spacing factor was ≥ 2 instead of the recommended maximum of 1.8.</p> <p>The reliability of the EC₁₀ value was evaluated in line with recommendations of EFSA Supporting publication 2019:EN-1673:</p> <ul style="list-style-type: none"> NW (normalised width) of 0.21 was calculated, which results in rating “good” in line with Table E9 in EFSA Supporting publication 2019:EN-1673, median EC₁₀ is lower than EC_{20,low}, the dose-response curve is steep with steepness of 0.73 (i.e. >0.66). <p>Based on the above indications the calculated EC₁₀ may be considered reliable.</p> <p>All the validity criteria were met:</p> <ul style="list-style-type: none"> In the control the mean mortality should be $\leq 20\%$ (observed 15%). In the control the mean number of juveniles per replicate should be ≥ 100 (observed 323 to 472). In the control the coefficient of variation of reproduction should be $< 30\%$ (observed 12%). <p>Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>28d NOEC (mortality, reproduction) = 38 mg product/kg soil dw 28d EC₁₀ (reproduction) = 57.5 mg product/kg soil dw 28d EC₂₀ (reproduction) = 64.1 mg product/kg soil dw 28d EC₅₀ (reproduction) = 79.1 mg product/kg soil dw</p>
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Reference:	KCP 10.4.2.1/1
Report:	Ganßmann, M.; 2014; GF-3307: Effects on Reproduction of the Collembola <i>Folsomia candida</i> in Artificial Soil with 5% Peat; Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany; Lab Study No. 90541016; DAS Study No. 140227; 08 January 2015; Unpublished
Guideline(s):	OECD 232
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable Yes
Duplication (if vertebrate study)	NA

COMPLIANCE

Guideline(s):	OECD 232, 2009 and ISO 11267, 1999
US EPA Guideline(s):	None
Deviations:	None
Dates of work:	31 October 2014 to 01 December 2014
GLP status:	Yes
Number of pages in final report:	46

MATERIALS AND METHODS

Test Item(s)

ISO Common name:	Not applicable
Test item (chemical/other name):	GF-3307
Purity:	XDE-777: 4.8 % w/w (analysed) Prothioconazole: 9.4 % w/w (analysed)
Description (physical state):	Brown Liquid
Lot No.:	F1281-135-1
CAS no.:	Not applicable

Test System

Organism (<i>Species</i>):	Collembola (<i>Folsomia candida</i>), 10-12 days old, from cultures held at the laboratory
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, 1, 2, 5, 9, 19, 38, 75 and 150 mg GF-3307/kg soil dry weight
Soil parameters:	Soil type: Artificial soil according to OECD 232 pH at initiation: 6.4 pH at termination: 6.1 to 6.2 Water content at initiation: 24.1% to 24.6% (52.4% to 53.4% of the maximum water holding capacity) Water content at termination: 23.2% to 24.4% (50.5% to 53.0% of the maximum water holding capacity) WHCmax: 46%
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: With <i>ca.</i> 2 mg dry yeast for each test vessel at the beginning of the test.
Reference substance:	Boric acid (conducted and reported as a separate study). In a separate study (study code 61405016) the reference item Boric acid showed statistically significant effects on reproduction at concentrations of ≥ 30.5 mg/kg soil; the EC ₅₀ for reproduction was calculated to be 160 mg/kg soil.

Methodology

28-d 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. Assessment of adult mortality, behavioral effects and reproduction after 28 d.

RESULTS AND DISCUSSION

After 28 days of exposure, GF-3307 caused no statistical significant effects on mortality or reproduction of *Folsomia candida* up to and including the concentration of 38 mg test item/kg soil. At the concentration of 75 and 150 mg test item/kg soil the mortality and reproduction were statistically significantly increased (Fisher's Exact Test, $\alpha = 0.05$, one-sided greater for mortality and Williams t-test, $\alpha = 0.05$, one-sided smaller for reproduction). No behavioural abnormalities were observed in any of the treatment groups.

Therefore, the overall No Observed Effect Concentration (NOEC) was determined to be 38 mg test item/kg soil. The overall Lowest Observed Effect Concentration (LOEC) was determined to be 75 mg test item/kg soil. The LC₅₀ was estimated to be 89.9 mg test item/kg soil. The EC₁₀, EC₂₀ and EC₅₀ values were determined to be 57.5, 64.1, and 79.1 mg test item/kg soil, respectively (Probit Analysis).

Table 39: Effects of GF-3307 on *Folsomia candida* survival and reproduction on day 28

Test concentrations (mg/kg)	Mean mortality of adults (%)	Mean no. of juveniles	% Change in no. of juveniles compared to control
Control	15	382	-
1	10	374	98
2	10	364	95
5	18	364	95
9	13	395	103
19	18	384	100
38	10	376	98
75	43*	244*	59*
150	100*	0*	0*

* Statistically different from the control

CONCLUSION

In a 28-day Collembola reproduction study with GF-3307, the overall NOEC was equivalent to 38 mg test item/kg soil dry weight. The LC₅₀ was estimated to be 89.9 mg test item/kg soil. The EC₁₀, EC₂₀ and EC₅₀ values were determined to be 57.5, 64.1, and 79.1 mg test item/kg soil, respectively.

Common name	Species	Test item	Time-scale	End-point	Toxicity value	Units of test item
Collembola	<i>Folsomia candida</i>	GF-3307	28 day	NOEC	38	mg/kg
Collembola	<i>Folsomia candida</i>	GF-3307	28 day	LOEC	75	mg/kg
Collembola	<i>Folsomia candida</i>	GF-3307	28 day	EC ₁₀	57.5	mg/kg
Collembola	<i>Folsomia candida</i>	GF-3307	28 day	EC ₂₀	64.1	mg/kg
Collembola	<i>Folsomia candida</i>	GF-3307	28 day	EC ₅₀	79.1	mg/kg
Collembola	<i>Folsomia candida</i>	GF-3307	28 day	LC ₅₀	89.9	mg/kg

A 2.4.2.2 Study 2 – GF-3307: Effects on Reproduction of the Predatory Mite *Hypoaspis aculeifer* in Artificial Soil with 5% Peat

Comments of zRMS:	<p>The study was conducted in line with OECD 226 with no deviations.</p> <p>The study design (6 concentrations, 8 replicates for control, 4 replicates per treatment group) was relevant to derive only the NOEC values and not the EC_x values. Observed effects were not statistically significantly different compared to the control.</p> <p>All the validity criteria were met:</p> <ul style="list-style-type: none"> - In the control treatment the mortality should be ≤ 20 % (observed 9%). - The mean number of juveniles per replicate in the control treatment should be ≥ 50 at the end of the test (observed 131 to 250 juveniles per replicate). - The coefficient of variation of reproduction in the control should be ≤ 30 % (observed 23.1%). <p>Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>14d NOEC (reproduction) > 150 mg product/kg soil dw</p>
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Reference:	KCP 10.4.2.1/2
Report:	Ganßmann, M.; 2014; GF-3307: 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, Arheilger Weg 17, 64380 Rossdorf, Germany; Lab Study No. 90541089; DAS Study No. 140230; 04 December 2014; Unpublished
Guideline(s):	OECD 226
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable Yes
Duplication (if vertebrate study)	NA

COMPLIANCE

Guideline(s):	OECD 226, 2008
US EPA Guideline(s):	None
Deviations:	None
Dates of work:	October 08, 2014 to October 24, 2014
GLP status:	Yes
Number of pages in final report:	42

MATERIALS AND METHODS

Test Item(s)

ISO Common name:	Not applicable
Test item (chemical/other name):	GF-3307
Purity:	XDE-777: 4.8 % w/w (analysed) Prothioconazole: 9.4 % w/w (analysed)
Description (physical state):	Brown liquid
Lot/batch no.:	F1281-135-1
CAS no.:	Not applicable

Test System

Organism (<i>Species</i>):	Predatory soil mite (<i>Hypoaspis aculeifer</i>), adult females, approximately 12 days after reaching the adult stage (33 days after placing adult females in clean rearing vessels), cultured by the laboratory
Study type:	14-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, 8, 14, 26, 46, 83 and 150 mg GF-3307/kg soil dry weight
Soil parameters:	Soil type: Artificial soil according to OECD 226. pH at initiation: 6.2 to 6.3 pH at termination: 5.9 to 6.0 Water content at initiation: 23.4% to 23.8% (50.9% to 51.8% of the maximum water holding capacity) Water content at termination: 22.8% to 24.0% (49.6% to 52.1% of the maximum water holding capacity) WHCmax: 46%
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 (<i>Tyrophagus putrescentiae</i> cultured by IBACON), two spatulas after the introduction of the test organisms and on day 2 and day 5, one spatula on day 7 and 9, and half a spatula on day 12.
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; 6 concentrations; 4 replicates/concentration and 8 replicates for the control, with 10 female predatory mites each. Feeding of the mites with cheese mites. Assessment of adult mortality and reproduction after 14 d (counted after extraction on day 16 after application).

RESULTS AND DISCUSSION

A slight mortality of up to 15% was observed in the test item treated groups, which was not statistically significantly different compared to the control, where 9% of the adult mites died (Fisher's Exact Test, $\alpha = 0.05$, one-sided greater).

The reproduction of the predatory mites exposed to GF-3307 was not statistically significantly different compared to the control up to and including the highest test concentration of 150 mg/kg soil (Williams ttest, $\alpha = 0.05$, one-sided smaller).

No differences in morphology or any abnormalities were observed in any of the treatment groups.

The reference item dimethoate showed statistically significant effects on reproduction at a concentration of 4.5 mg dimethoate/kg soil and above. The EC₅₀ for reproduction was 5.5 mg dimethoate/kg soil.

Table 40: Effects of GF-3307 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	9	169	-
8	15	182	108
14	5	185	110
26	15	159	94
46	5	151	90
83	13	164	97
150	10	189	112

* Statistically different from the control

CONCLUSION

GF-3307 caused no significant effects on mortality or reproduction of *Hypoaspis aculeifer* up to and including the concentration of 150 mg test item/kg soil.

Therefore, the overall No Observed Effect Concentration (NOEC) was determined to be 150 mg test item/kg soil. The overall Lowest Observed Effect Concentration (LOEC) as well as the EC₅₀ for reproduction were estimated to be greater than 150 mg test item/kg soil.

Common name	Species	Test item	Time-scale	End-point	Toxicity value	Units of test item
Predatory soil mite	<i>Hypoaspis aculeifer</i>	GF-3307	14 day	EC ₅₀	>150	mg/kg
Predatory soil mite	<i>Hypoaspis aculeifer</i>	GF-3307	14 day	NOEC	150	mg/kg

A 2.4.2.3 KCP 10.4.2.1 Species level testing

A 2.4.2.4 KCP 10.4.2.2 Higher tier testing

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

A 2.5.1 Study 1 - GF-3307: Effects on the Activity of the Soil Microflora in the Laboratory

Comments of zRMS:	<p>The study was conducted 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 is struck through.</p> <p>All validity criteria were met:</p> <ul style="list-style-type: none"> - the variation between replicate control samples was ≤ 15 % (observed max. 2.03 %). <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 < 25 % at the end of the study period (28 days) up to 13.29 mg product/kg soil dw.</p>
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Reference:	KCP 10.5/1
Report:	Hammesfahr, U.; 2014; GF-3307: Effects on the Activity of the Soil Microflora in the Laboratory; Institut für Biologische Analytik, und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany; Lab Study No. 90541080; DAS Study No. 140237; November 04, 2014; Unpublished
Guideline(s):	OECD Guideline 216 – Soil Microorganisms – Nitrogen Transformation Test
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable Yes
Duplication (if vertebrate study)	NA

COMPLIANCE

Guideline(s):	OECD Guideline 216 – Soil Microorganisms – Nitrogen Transformation Test OECD Guideline 217 – Soil Microorganisms – Carbon Transformation Test
US EPA Guideline(s):	Not Applicable
Deviations:	No Deviations
Dates of work:	14 August 2014 to 12 September 2014
GLP status:	Yes
Number of pages in final report:	73

MATERIALS AND METHODS

Test Item(s)

ISO Common name:	Not applicable
Test item (chemical/other name):	GF-3307
Purity:	Prothioconazole: 9.4 % w/w XDE-777: 4.8 % w/w
Description (physical state):	Liquid Brown
Lot/batch no.:	F1281-135-1/ TSN307579
CAS no.:	Not applicable

Test System

Organism (<i>Species</i>):	Soil micro-organisms
Study type:	Laboratory study with OECD guideline natural soil, assessed for: <ul style="list-style-type: none">• Nitrate formation• Microbial respiration
Study duration:	28 days
Parameters measured:	Nitrogen transformation: analysis of nitrate, nitrite and ammonium in extracted soil samples, via continuous flow analysis; limits of quantification: NO ₃ -N: 0.281 mg/kg soil dry weight NO ₂ -N: 0.184 mg/kg soil dry weight NH ₄ -N: 0.073 mg/kg soil dry weight <ul style="list-style-type: none">• soil water content 50% to 52%• pH 6.9 to 7.0 Microbial respiration: <ul style="list-style-type: none">• soil respiration rates after addition of glucose 2 g/kg• soil water content 47% to 51%• pH 6.8 to 6.9
Observation intervals:	0, 7, 14 and 28 days
Test concentrations:	Dose 1: 2.78 mg GF-3307/kg soil dry weight Dose 2: 13.92 mg GF-3307/kg soil dry weight
Toxic reference:	Sodium chloride The inhibition of soil respiration 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.
Method of test item application:	Incorporation into the soil
Environmental conditions:	Conducted in the dark. Temperature: 20 ± 2°C pH: 6.8– 7.0
Soil properties	Soil source: The soil batch used in this study was according to the Guidelines and was taken from fallow grassland: District authority: Darmstadt-Dieburg Municipality: 64380 Rossdorf, Germany Geographical position: longitude 8° 44' 38.70" E latitude 49° 51' 59.59" N Moisture content of soil at start: 48% - 51% of WHC _{max} Moisture content of soil at end: 47% - 52% of WHC _{max} Clay (%): 8.7 Silt (%): 33.3 Sand (%): 58.0 Organic Carbon (%): 0.96 Textural classification: Loamy sand

Methodology

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 CO₂-production over a period of up to 24 hours at different sampling intervals. 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. NH₄⁺-, NO₂⁻- and NO₃⁻-nitrogen formed from the nitrification process were determined by means of a Continuous Flow Analyser (AA3, XY-2 / XY-3Sampler).

RESULTS AND DISCUSSION

No adverse effects of GF-3307 on nitrogen transformation in soil could be observed in both test item concentrations (2.78 mg/kg dry soil, 13.92 mg/kg dry soil) after 28 days. Deviations from the control of -1.50% (application rate 2.78 mg/kg dry soil) and -1.87% (application rate 13.92 mg/kg dry soil) were measured at the end of the 28-day incubation period. There were no statistically significant differences on day 28 between control and the test item treated groups (Student t-test, $\alpha = 0.05$).

The soil nitrate formation rates were calculated on an incremental basis (i.e. between successive sampling dates). In the last interval between days 14 and 28, the deviations from control were -0.53% and -11.23% for the low and high test rate of GF-3307. Thus, the difference in the soil nitrate formation rates between the control and both test item treatments was clearly below the OECD guideline 216 trigger value of 25% at the 14 to 28 day interval. The deviation was statistically significant different from the control for the high test rate (Student t-test, $\alpha = 0.05$).

Table 41: Effects of GF-3307 on the nitrate formation rate

Interval sampling days	Control	2.78 mg GF-3307/kg soil dw				13.92 mg GF-3307/kg soil dw		
	[mg/kg/day ¹]	[mg/kg/day ¹]	[% ²]	[sig ³]		[mg/kg/d ¹]	[% ²]	[sig ³]
0-7	-1.26	-1.18	-6.35	n.s.		-0.78	-38.10	*
7-14	2.53	2.42	-4.35	*		2.54	0.40	n.s.
14-28	1.87	1.86	-0.53	n.s.		1.66	-11.23	*

¹ mean mg NO₃-N/kg soil dry weight per day

² deviation from control

³ statistical significance; *statistically significant from control (Student t-test; $\alpha = 0.05$)

Table 42: Effects of GF-3307 on the respiration rate

Sampling days	Control	2.78 mg GF-3307/kg soil dw				13.92 mg GF-3307/kg soil dw		
	Respiration Rate ¹	Respiration Rate ¹	[% ²]	[sig ³]		Respiration Rate ¹	[% ²]	[sig ³]
0	11.171	11.529	3.20	n.s.		11.776	5.42	n.s.
7	8.967	9.262	3.29	n.s.		10.115	12.80	n.s.
14	10.698	11.395	6.52	*		11.479	7.30	*
28	9.614	9.967	3.67	n.s.		10.102	5.08	n.s.

¹: respiration, mean values of 3 replicates

²: deviation from control

³: statistical significance

*: significant

n.s.: not significant

CONCLUSION

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 3.67% and 5.08% from the control for the low and high test rate, respectively.

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 -0.53% and -11.23% for the low and high test rate of GF-3307.

Based on the results of this study, it is concluded that GF-3307 had no significant impact on soil microorganisms (~~carbon and~~ nitrogen transformation) when applied at test item concentrations up to 13.92 mg/kg soil dry weight.

It can be concluded that GF-3307 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-3307	28 day – nitrogen transformation	NOEC	13.92	mg/kg soil
Soil micro organisms	N/A	GF-3307	28 day – carbon respiration	NOEC	13.92	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-3307 (XDE-777 + prothioconazole 50 + 100 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 2014

Comments of zRMS:	<p>The study was conducted in line with OECD 227 with minor deviations.</p> <p>It was noted that the temperature on occasions exceeded the recommended maximum of 32°C for cucumber and tomato reaching 35°C and the relative humidity reached the extremes of 29% and 100%. Moreover, the light intensity was not measured during the study. Additionally, even though OECD 227 does not specify the size of the pot nor the number of plants per pot, recommendations of OECD 208 could be adopted. According to OECD 208, the maximum plant density would be around 3-10 seeds per 100 cm² depending on the size of the seeds. As an example, one to two corn, soybean, tomato, cucumber, or sugar beet plants per 15cm container; three rape or pea plants per 15 cm container; and 5 to 10 onion, wheat, or other small seeds per 15 cm container are recommended. In the present study 5-6 pots with 4-7 plants per pot were used which resulted in 24-35 plants per species per treatment. However, since all the control plants survived and did not exhibit any phytotoxic symptoms, and the validity criteria were met, the approach to the number of plants per pot and the number of pots employed in the study is considered acceptable.</p> <p>In summary, in zRMS opinion all the deviations are considered to have no impact on the integrity or the outcome of the study.</p> <p>It should be noted that GF-3307 contains two active substances and in line with the requirements of the Central Zone the test concentrations of all active substances should be verified in the respective chemical analyses or at a minimum the least stable active substance should be analysed. However, in the present study only the concentration of prothioconazole was measured and the analysis for fenpicoxamid was not carried out. No explanation or justification for the active substance selected for chemical analysis was provided in the study report. Based on the information from the area of environmental fate and behavior of both active substances it can be concluded that fenpicoxamid is the least stable substance, not prothioconazole. Furthermore, in line with the recommendations in Appendix J EFSA Supporting publication 2019:EN-1673 when only one active substance is measured it has to also be confirmed that this substance is the driver of the toxicity (contribution of > 90 %) of the product to non-target plants.</p> <p>If it is confirmed that prothioconazole is the driver of the toxicity to non-target plants, then, since the measured concentrations of prothioconazole in the present study were within 80-120% of nominal, the zRMS is of the opinion that the endpoints can be based on nominal concentrations as provided in the study report.</p> <p>For this reason the Applicant is requested to provide justification for selection of prothioconazole for chemical analyses, especially fenpicoxamid is also rapidly degraded in water and it thus expected to dissipate rapidly in the spray solutions. Please note that in case of bee chronic and larvae studies fenpicoxamid was selected for chemical analyses, so it is not clear why in case of studies with NTTPs the concentrations of prothioconazole was analysed, although reason for selection of fenpicoxamid in bee studies is also unknown and requires clarification</p> <p>The following justification were provided by the Applicant.</p> <p>Both fenpicoxamid and prothioconazole are unstable in water with a DT₅₀ of 0.7 days (EFSA, 2018) and 0.8 – 1.0 days (EFSA, 2007), respectively. In non-target plant studies (DAS study No. 160372 and 160373) with a similar formulation (GF-3308; 50g fenpicoxamid/L EC), fenpicoxamid recoveries of 98 – 100% were reported. Therefore, it can be assumed that also in the non-target plant studies with GF-3307 fenpicoxamid was present</p>
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	<p>close to its nominal concentration in the freshly prepared and applied spray solution. Furthermore, no effects on seedling emergence, plant development, survival and biomass were observed at an application rate of 4 L GF-3307/ha, which is more than two-fold of the max. application rate. Lastly, the study was done at a GLP certified facility according to GLP and the OECD Guidelines 208 and 227, which say that the concentrations must be confirmed by an appropriate analytical verification but do not specify details. Therefore, it should be acceptable that prothioconazole only was determined in the spray solution of GF-3307.</p> <p>The zRMS agrees with the Applicant approach.</p> <p>It is also noted that endpoints based on phytotoxic effects were not calculated although they are required in line with the agreements taken during the Central Zone harmonisation meetings. Very slight or slight effects were observed in some species in the highest application rate but these effects are considered to be not significant enough to provide any meaningful information relevant for the risk assessment. Therefore, the Applicant was not requested to provide any additional calculations on phytotoxic effects.</p> <p>The study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>ER₅₀ (mortality and fresh shoot weight) > 4 L product/ha</p>
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Reference:	KCP 10.6.2/1
Report:	Brockmann, A.; 2014; GF-3307 (XDE-777 + prothioconazole 50 + 100 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 2014; agro-check, Dr.Teresiak & Erdmann GbR; Lab Study No. AC/DOW/14/04; DAS Study No. 140555; 10 December 2014; Unpublished
Guideline(s):	OECD 227
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	NA

COMPLIANCE

Guideline(s):	Good Laboratory Practice Regulations: Appendix 1 to § 19a, Section 1, of the German Chemikaliengesetz (Chemicals Act). OECD Principles of Good Laboratory Practice ENV/MC/CHEM(98)17, Paris 1998 OECD Consensus Document “The application of the OECD Principles of GLP to the Organisation and Management of Multi-Site Studies” No 13 (2002) ENV/JM/MONO(2002)9 OECD Guideline for the Testing of Chemicals; Test No. 227 Terrestrial Plant Test: Vegetative Vigour Test, 19 July 2006
US EPA Guideline(s):	Not Applicable
Deviations:	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 ER ₅₀ was > 4 L GF-3307/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	7	5
Onion	5	5
OSR	5	5
Soybean	5	5
Carrot	5	5
cucumber	4	6
Sugar beet	5	5
Sunflower	5	5
tomato	5	5

Dates of work:	25 September 2014 to 16 October 2014
GLP status:	Yes
Number of pages in final report:	142

MATERIALS AND METHODS

Test Item(s)

ISO Common name: Not applicable
Test item (chemical/other name): GF-3307
Purity: XDE-777:4.8% w/w
Prothioconazole: 9.6% w/w
Description (physical state): emulsifiable concentrate (EC)
Lot/batch no.: F1281-135-1/TSN307579
CAS no.: Not applicable

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 dead plants: 7, 14 and 21 days after application
Foliar fresh weight: 21 days after application

Phytotoxicity rating:

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		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		Long lasting crop injury, no recovery, stunting
4		Long 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
1		Only occasional live crop plants left, almost dead
0	Complete effects	Complete crop destruction, all dead

Growth conditions: Temperature (range): 18.8°C - 25.7°C with extremes of 35°C, temperatures higher than 32°C for cucumber and tomato for short time only
Photoperiod: 16/8
Light intensity (range): not measured but adding artificial light if outdoor light intensity was lower than 10 klux.
Relative humidity: 54.1% to 83.2% with extremes of 29% and 100%
Water regime and schedules: as needed
Water source/type: rain water
Pest control method /fertilisation, if used:
Pest control none, Fertilizer was added to each pot with a NPK-fertilizer as a 0.2% solution (Hakaphos blau). P- and K-fertilizer (Gabi Plus P and Gabi Plus K) were added as needed. All pots of one plant species and treatment rate obtained the same level of fertilizer during the trial.

Growth medium:	Soil type: Light silty sand Details of nutrient medium, if used: Hakaphos blau, Gabi Plus K, Gabi Plus P pH: 7.23; C _{org} : 0.64%, Texture: 1.4% of < 2 µm, 23.3% of 2-63 µm, 75.3% of >63 µm
Test concentrations:	Nominal: 0 (control), 0.250, 0.500, 1.000, 2.000, 3.000, 4.000 L test item /ha Mean calculated concentrations: 4.000 L test item /ha
Analytical verification:	94% (based on the concentration of prothiconazole only)
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 plants per replicate pot: 4-7 Growth stage at application: 12-14
Number of control replicates:	5-6
Number of test concentration replicates:	5-6

Methodology

Greenhouse trial, dose response design; GF-3307 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. Shoot fresh weight was determined at study termination 21 DAT.

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 ≥ 16 hour day length. 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.

The influence of GF-3307 was tested for survival, phytotoxicity and biomass production at rates up to 4.000 L GF-3307/ha.

No phytotoxic damages were observed for the monocotyledonous species oat, ryegrass and onion and for the dicotyledonous species carrot 21 DAT. Very slight to slight phytotoxic effects on leaves, which occurred at application time, were seen in the tested species oilseed rape, soybean, cucumber and tomato. Sugar beet and sunflower as the most influenced species showed slight phytotoxic effects also in the new growth after application of the highest tested rate of 4.000 L GF-3307/ha.

No negative impact of GF-3307 was found for survival and biomass production for all tested species belonging to the plant families Poaceae, Liliaceae, Brassicaceae, Fabaceae, Apiaceae, Cucurbitaceae, Chenopodiaceae, Asteraceae and Solanaceae.

Table 43: Observations of plant mortality, foliar fresh weight, and visual phytotoxicity ratings: Monocotyledonous species at 21DAT

Treatment [L/ha]	Oat				Ryegrass				Onion			
	Mortality	Fresh weight [g/pot]	%Survival	Phytotox Rating	Mortality	Fresh weight [g/pot]	%Survival	Phytotox Rating	Mortality	Fresh weight [g/pot]	%Survival	Phytotox Rating
Control	0	108,262	100	10	0	41,038	100	10	0	54,439	100	10
0.250 0.188	0	108,123	100	10	0	42,673	100	10	0	58,466	100	10
0.500 0.375	0	114,610	100	10	0	42,845	100	10	0	65,945	100	10
1.000 0.750	0	112,847	100	10	0	42,243	100	10	0	62,205	100	10
2.000 1.500	0	111,620	100	10	0	41,776	100	10	0	74,304	100	10
4.000 3.000	0	115,382	100	10	0	41,362	100	10	0	66,076	100	10

Table 44: Observations of plant mortality, foliar fresh weight, and visual phytotoxicity ratings: Dicotyledonous species at 21DAT

Treatment [L/ha]	Treatment	Oilseed rape				Soybean				Carrot			
		Mortality	Fresh weight [g/pot]	%Survival	Phytotox Rating	Mortality	Fresh weight [g/pot]	%Survival	Phytotox Rating	Mortality	Fresh weight [g/pot]	%Survival	Phytotox Rating
Control	Control	0	131,064	100	10	0	92,832	100	10	0	64,860	100	10
0.250 0.188	0.188	0	131,169	100	10	0	96,393	100	10	0	65,282	100	10
0.500 0.375	0.375	0	128,920	100	10	0	96,710	100	10	0	64,783	100	10
1.000 0.750	0.750	0	131,716	100	10	0	97,656	100	10	0	67,589	100	10
2.000 1.500	1.500	0	128,117	100	9	0	98,488	100	9	0	64,387	100	10
4.000 3.000	3.000	0	132,343	100	9	0	101,141	100	8	0	68,035	100	10

Treatment [L/ha]	Treatment	Cucumber				Sugar beet				Sunflower			
		Mortality	Fresh weight [g/pot]	%Survival	Phytotox Rating	Mortality	Fresh weight [g/pot]	%Survival	Phytotox Rating	Mortality	Fresh weight [g/pot]	%Survival	Phytotox Rating
Control	Control	0	214,541	100	10	0	117,745	100	10	0	238,228	100	10
0.250 0.188	0.188	0	223,122	100	10	0	125,489	100	10	0	242,900	100	10
0.500 0.375	0.375	0	221,529	100	10	0	121,856	100	9	0	238,568	100	10
1.000 0.750	0.750	0	220,513	100	9	0	117,536	100	9	0	240,246	100	9
2.000 1.500	1.500	0	224,925	100	9	0	116,655	100	9	0	235,695	100	9
4.000 3.000	3.000	0	211,727	100	8	0	111,830	100	7	0	233,969	100	7

Treatment [L/ha]	Treatment	Tomato			
		Mortality	Fresh weight [g/pot]	%Survival	Phytotox Rating
Control	Control	0	180,989	100	10
0.250 0.188	0.188	0	181,324	100	9

Treatment [L/ha]	Treatment	Tomato			
		Mortality	Fresh weight [g/pot]	% Survival	Phytotox Rating
0.500 0.375	0.375	0	185,119	100	9
1.000 0.750	0.750	0	178,042	100	9
2.000 1.500	1.500	0	181,033	100	9
4.000 3.000	3.000	0	186,545	100	9

Table 45: Reported ER₅₀ values based on foliar fresh weight and survival

Species	ER ₅₀ (L/ha)	Regression R-Square
Monocotyledonous		
Oat	> 4.000	NA
Ryegrass	> 4.000	NA
Onion	> 4.000	NA
Dicotyledonous		
Oilseed rape	> 4.000	NA
Soybean	> 4.000	NA
Carrot	> 4.000	NA
Cucumber	> 4.000	NA
Sugar beet	> 4.000	NA
Sunflower	> 4.000	NA
Tomato	> 4.000	NA

CONCLUSION

Based on the results of this study, conducted under greenhouse conditions it can be concluded that the fungicide GF-3307 applied at BBCH 12-14 with rates up to 4.000 L GF-3307/ha did not result in adverse effects on survival or fresh weight of any of the plant species tested.

The NOER for plant mortality and plant weight appeared to be higher than or equal 4.000 L GF-3307/ha. ER₅₀ values could not be calculated for any of the tested species.

Very slight to slight phytotoxic effects were seen in oilseed rape, soybean, cucumber and tomato. Slight effects were found in sugar beet and sunflower, also in few of the new developed leaves of the plants. But no influence on plant development and biomass could be not found.

No negative impact of GF-3307 was found for the tested plant species oat, ryegrass, onion and carrot 21 DAT.

A 2.6.2.2 Study 2 - GF-3307 (XDE-777 + prothioconazole 50 + 100 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 2014

Comments of zRMS:	<p>The study was conducted in line with OECD 208 with minor deviations.</p> <p>It was noted that the temperature on occasions exceeded the recommended maximum of 32°C reaching 38°C and the relative humidity reached the extremes of 26% and 80%. Moreover, the light intensity was not measured during the study. Additionally, according to OECD 208, the maximum plant density would be around 3-10 seeds per 100 cm² depending on the size of the seeds. As an example, one to two corn, soybean, tomato, cucumber, or sugar beet plants per 15cm container; three rape or pea plants per 15 cm container; and 5 to 10 onion, wheat, or other small seeds per 15 cm container are recommended. In the present study pots of 9-12 cm in diameter were used. There were 5-6 pots with 4-10 seeds per pot which resulted in 24-50 plants per species per treatment. However, since all the control plants survived and did not exhibit any phytotoxic symptoms, and the validity criteria were met, the approach to the number of seeds per pot and the number of pots employed in the study is considered acceptable.</p> <p>In summary, all the deviations are considered to have no impact on the integrity or the outcome of the study.</p> <p>According to OECD 208, the maximum plant density would be around 3-10 seeds per 100 cm² depending on the size of the seeds. As an example, one to two corn, soybean, tomato, cucumber, or sugar beet plants per 15cm container; three rape or pea plants per 15 cm container; and 5 to 10 onion, wheat, or other small seeds per 15 cm container are recommended.</p>
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	<p>It should be noted that GF-3307 contains two active substances and in line with the requirements of the Central Zone the test concentrations of all active substances should be verified in the respective chemical analyses or at a minimum the least stable active substance should be analysed. However, in the present study only the concentration of prothioconazole was measured and the analysis for fenpicoxamid was not carried out. No explanation or justification for the active substance selected for chemical analysis was provided in the study report. Based on the information from the area of environmental fate and behavior of both active substances it can be concluded that fenpicoxamid is the least stable substance, not prothioconazole. Furthermore, in line with the recommendations in Appendix J EFSA Supporting publication 2019:EN-1673 when only one active substance is measured it has to also be confirmed that this substance is the driver of the toxicity (contribution of > 90 %) of the product to non-target plants.</p> <p>If it is confirmed that prothioconazole is the driver of the toxicity to non-target plants, then, since the measured concentrations of prothioconazole in the present study were within 80-120% of nominal, the zRMS is of the opinion that the endpoints can be based on nominal concentrations as provided in the study report.</p> <p>For this reason the Applicant is requested to provide justification for selection of prothioconazole for chemical analyses, especially fenpicoxamid is also rapidly degraded in water and it thus expected to dissipate rapidly in the spray solutions. Please note that in case of bee chronic and larvae studies fenpicoxamid was selected for chemical analyses, so it is not clear why in case of studies with NTTPs the concentrations of prothioconazole was analysed, although reason for selection of fenpicoxamid in bee studies is also unknown and requires clarification</p> <p>The following justification were provided by the Applicant.</p> <p>Both fenpicoxamid and prothioconazole are unstable in water with a DT50 of 0.7 days (EFSA, 2018) and 0.8 – 1.0 days (EFSA, 2007), respectively. In non-target plant studies (DAS study No. 160372 and 160373) with a similar formulation (GF-3308; 50g fenpicoxamid/L EC), fenpicoxamid recoveries of 98 – 100% were re-reported. Therefore, it can be assumed that also in the non-target plant studies with GF-3307 fenpicoxamid was present close to its nominal concentration in the fresh-ly prepared and applied spray solution. Furthermore, no effects on seedling emergence, plant development, survival and biomass were observed at an application rate of 4 L GF-3307/ha, which is more than two-fold of the max. application rate. Lastly, the study was done at a GLP certified facility according to GLP and the OECD Guidelines 208 and 227, which say that the concentrations must be confirmed by an appropriate analytical verification but do not specify details. Therefore, it should be acceptable that prothioconazole only was determined in the spray solution of GF-3307.</p> <p>The zRMS agrees with the Applicant approach.</p> <p>It is also noted that endpoints based on phytotoxic effects were not calculated although they are required in line with the agreements taken during the Central Zone harmonisation meetings. Slight discolourations and deformations were observed only in ryegrass at application rates of ≥ 1.000 L GF-3307/ha. However, these effects are considered to be not significant enough to provide any meaningful information relevant for the risk assessment. Therefore, the Applicant was not requested to provide any additional calculations on phytotoxic effects.</p> <p>The study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>ER₅₀ (survival and fresh weight) > 4.000 L product/ha</p>
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Reference:	KCP 10.6.2/2
Report:	Brockmann, A.; 2014; GF-3307 (XDE-777 + prothioconazole 50 + 100 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 2014; agro-check, Dr.Teresiak & Erdmann GbR; Lab Study No. AC/DOW/14/03; DAS Study No. 140707; 10 December 2014; Unpublished
Guideline(s):	OECD 208
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable Yes
Duplication (if vertebrate study)	NA

COMPLIANCE

Guideline(s):	Good Laboratory Practice Regulations: Appendix 1 to § 19a, Section 1, of the German Chemikaliengesetz (Chemicals Act). OECD Principles of Good Laboratory Practice ENV/MC/CHEM(98)17, Paris 1998 OECD Consensus Document “The application of the OECD Principles of GLP to the Organisation and Management of Multi-Site Studies” No 13 (2002) ENV/JM/MONO(2002) OECD Guideline for the Testing of Chemicals; Test No. 208 Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test, 19 July 2006
US EPA Guideline(s):	Not Applicable
Deviations:	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 ₅₀ was > 4 L GF-3307/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

Dates of work:	07 August 2014 to 07 September 2014
GLP status:	Yes
Number of pages in final report:	134

MATERIALS AND METHODS

Test Item(s)

ISO Common name:	Not applicable
Test item (chemical/other name):	GF-3307
Purity:	XDE-777:4.8% w/w Prothioconazole: 9.6% w/w
Description (physical state):	emulsifiable concentrate (EC)
Lot/batch no.:	F1281-135-1/ TSN307579
CAS no.:	Not applicable

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 counts: 7, 14, 21 days after seeding; if 7 days after application less than 50% of the plants in the untreated control emerged, assessments will start at 14 days after application and assessments will be extended to 28 days Number of dead plants: 7, 14, 21 days after seeding; if 7 days after application less than 50% of the plants in the untreated control emerged, assessments will start at 14 days after application and assessments will be extended to 28 days Shoot fresh weight: 21 days after seeding or 28 days after seeding if 7 days after application less than 50% of the plants in the untreated control emerged
Phytotoxicity rating:	Description of main categories

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:	<p>Temperature (range): 21.3°C to 26.8°C (extremes ranged from 15°C to 38°C, temperatures > 32°C for short time only)</p> <p>Photoperiod: 16/8</p> <p>Light intensity (range): not measured but adding artificial light if outdoor light intensity was lower than 10 klux.</p> <p>Relative humidity: 47.8% to 68.1% (extremes 26% and 88% relative humidity)</p> <p>Water regime and schedules: as needed</p> <p>Water source/type: rain water</p> <p>Pest control method /fertilisation, if used: Pest control none, Fertilizer was added to each pot with a NPK-fertilizer as a 0.2% solution (Hakaphos blau). K-fertilizer (Gabi Plus K) was added as needed. All pots of one plant species and treatment rate obtained the same level of fertilizer during the trial.</p>
Pots:	Pots used were new plastic containers with 9 cm (0.35 L), 10.5 cm (0.49 L), 11 cm (0.54 L) or 12 cm (0.68 L) in diameter (species dependent).
Growth medium:	<p>Soil type: Light silty sand</p> <p>Details of nutrient medium, if used: Hakaphos blau, Gabi Plus K, Gabi Plus P</p> <p>pH: 7.23, C_{org}: 0.64%, texture: 1.4% of <2 µm, 23.3% of 2-63 µm, 75.3% of >63 µm</p>
Test concentrations:	<p>Nominal: 0 (control), 0.250, 0.500, 1.000, 2.000, 3.000, 4.000 L test item /ha</p> <p>Mean calculated concentrations: 4.000 L test item /ha</p>
Analytical verification:	96% (based on the concentration of prothiconazole only)
Test material application:	<p>Method: track sprayer</p> <p>Application interval: 1 timing</p> <p>Reference chemical (if used): non/ plain tap water</p>
Seeds:	<p>Source: breeders</p> <p>Method of seeding: by hand</p> <p>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.</p> <p>Number of seeds per replicate pot: 4-10</p> <p>Growth stage at application: 00</p>
Number of control replicates:	5-6
Number of test concentration replicates:	5-6

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). 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 ≥16 hour day length. 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.

All plant species had reached the 50 % emergence rate after 7 days except carrot and onion (14 days). None of the tested plant species was affected concerning seedling emergence by the application of GF-3307 up to the highest tested rate of 4.000 L/ha. No reduction of plant stand 21 days after application (carrot and onion 28 DAT) of GF-3307 was found for all tested plant species. No ER_{50} values could be calculated for all tested plant species after application of GF-3307 up to a rate of 4.000 L/ha. None of the tested plants died, so there was found a survival of 100 %.

None of the tested plant species showed phytotoxic symptoms after use of GF-3307 pre emergence except ryegrass. In ryegrass were found slight discolourations and deformations at application rates of ≥ 1.000 L GF-3307/ha.

The plant biomass (shoot fresh weight) was determined 21 DAT (28 DAT for carrot and onion). No influence of GF-3307 on plant fresh weight was observed for all tested plant species after preemergence application up to 4.000 L/ha. No dose response could be found for all tested plant species. The ER_{50} was higher than the highest tested rate of 4.000 L GF-3307/ha.

The checked parameters emergence, survival and fresh biomass production were not negatively influenced after pre-emergence application of GF-3307 up to the highest tested rate of 4.000 L/ha.

Table 46: Observations of plant mortality, emergence, foliar fresh weight, and visual phytotoxicity ratings: Monocotyledonous species

Treatment [L/ha]	Oat				ryegrass				onion			
	Mortality	Fresh weight [g/pot]	% Emergence	Phytotox Rating	Mortality	Fresh weight [g/pot]	% Emergence	Phytotox Rating	Mortality	Fresh weight [g/pot]	% Emergence	Phytotox Rating
Control	0	13.607	88	10	0	6.296	96	10	0	8.135	92	10
0.250	0	15.020	96	10	0	5.918	88	10	0	8.032	90	10
0.500	0	15.797	100	10	0	6.602	96	10	0	7.957	94	10
1.000	0	15.058	96	10	0	6.208	88	10	0	7.817	94	10
2.000	0	14.435	92	10	0	5.624	88	9	0	8.876 8.450	94	10
4.000	0	13.866	96	10	0	6.082 5.695	92	8	0	8.089 8.044	94	10

Table 47: Observations of plant mortality, emergence, foliar fresh weight, and visual phytotoxicity ratings: Dicotyledonous species

Treatment [L/ha]	Oilseed rape				Soybean				Carrot			
	Mortality	Fresh weight [g/pot]	% Emergence	Phytotox Rating	Mortality	Fresh weight [g/pot]	% Emergence	Phytotox Rating	Mortality	Fresh weight [g/pot]	% Emergence	Phytotox Rating
Control	0	21.946	92	10	0	29.002	100	10	0	12.917 +11.546	78	10
0.250	0	21.320	92	10	0	26.994	92	10	0	13.677 +13.064	85	10
0.500	0	20.774	96	10	0	26.466	96	10	0	13.888	90	10
1.000	0	20.126	96	10	0	26.530	92	10	0	12.996	88	10
2.000	0	22.398	96	10	0	24.648	88	10	0	12.664	83	10
4.000	0	20.637	92	10	0	27.330 +26.757	92	10	0	12.438	78	10

Treatment [L/ha]	Cucumber				Sugar beet				Sunflower			
	Mortality	Fresh weight [g/pot]	% Emergence	Phytotox Rating	Mortality	Fresh weight [g/pot]	% Emergence	Phytotox Rating	Mortality	Fresh weight [g/pot]	% Emergence	Phytotox Rating
Control	0	33.301 +32.553	100	10	0	18.621	100	10	0	63.604	96	10
0.250	0	33.106	100	10	0	19.256	100	10	0	65.156	100	10
0.500	0	32.059	96	10	0	19.543	100	10	0	62.744	96	10
1.000	0	32.268	100	10	0	19.525	100	10	0	64.344	100	10
2.000	0	31.784	100	10	0	18.230	100	10	0	63.363	100	10
4.000	0	31.290	100	10	0	18.913	100	10	0	63.073	100	10

Treatment [L/ha]	Tomato			
	Mortality	Fresh weight [g/pot]	% Emergence	Phytotox Rating
Control	0	23.635	92	10
0.250	0	24.851	100	10
0.500	0	24.292	100	10
1.000	0	25.669 24.783	96	10
2.000	0	25.106 24.079	100	10
4.000	0	24.279	88	10

Table 48: Reported ER₅₀ values based on foliar fresh weight, seedling emergence and plant survival

Species	ER ₅₀ (L/ha)	Regression R-Square
Monocotyledonous		
Oat	> 4.000	NA
Ryegrass	> 4.000	NA
Onion*	> 4.000	NA
Dicotyledonous		
Oilseed rape	> 4.000	NA
Soybean	> 4.000	NA
Carrot*	> 4.000	NA
Cucumber	> 4.000	NA
Sugar beet	> 4.000	NA
Sunflower	> 4.000	
Tomato	> 4.000	

*Carrot and onion 28 DAT; other species 21 DAT

CONCLUSION

Based on the results of this study, conducted under greenhouse conditions, it can be concluded that the fungicide GF-3307 did not cause adverse effects to the seedling emergence, plant survival and fresh biomass production of all tested plant species. Ryegrass (Poaceae) showed slight phytotoxic effects after application of ≥ 1.000 L GF-3307/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.

Appendix 3 Aquatic Mixtures Assessment using the AGD MixTox Tool

Aquatic mixture toxicity has been assessed with the AGD_Aquamix_v122.xlsm Excel Aquatic MixTox tool, which can be found at <https://zenodo.org/records/7788826>. The input parameters/endpoints are shown below as well as the outcome and from the AGD MixTox tool.

The acute mixture assessment is presented for Fenpicoxamid + Prothioconazole.

The chronic mixture assessment is presented for Fenpicoxamid + Prothioconazole and alternatively for Fenpicoxamid + JAU 6476-desthio, since the metabolite JAU 6476-desthio is more toxic to fish (chronic) than the parent Prothioconazole.

For simplicity, only the input data and the final mixture assessment at Step 8b are presented.

Upon request of the zRMS, the NOEC = 0.1 µg/L im; AF=3; RAC = 0.033 µg/L for a.s. fenpicoxamid from the mesocosm study was considered in the aquatic invertebrate mixture toxicity risk assessment. Additionally, the fenpicoxamid fish acute geomean was used for the mixture assessment.

Acute aquatic organism mixture toxicity Fenpicoxamid + Prothioconazole

Input Tox tab

Table A3-1: Product acute Tier 1 data for GF-3307

Product data	
Product name	GF-3307
Density of product [g/cm ³]	1.044
LC ₅₀ fish [mg prod./L]	0.072
LC ₅₀ fish a.s. based [mg sum of a.s./L]	0.0103
EC ₅₀ invertebrates [mg prod./L]	0.015
LC ₅₀ invertebrates a.s. based [mg sum of a.s./L]	0.0022
EC ₅₀ algae [mg prod./L]	8
EC ₅₀ algae a.s. based [mg sum of a.s./L]	1.1494

Table A3-2: Active substance acute Tier 1 data for fenpicoxamid (FPN) and prothioconazole (PTZ)

Active Substance (a.s.) standard data (Tier 1 EP)				
Active substance names		FPN		PTZ
Concentration in product [g a.s./L or g a.s./kg]	FPN	50		100
p(X) (fraction in product)	Species	0.33	Species	0.67
LC ₅₀ fish [mg a.s./L]	O. mykiss (trout)	0.0022	O. mykiss	1.83
LC ₅₀ invertebrates [mg a.s./L]	D. magna	0.00093	D. magna	1.3
EC ₅₀ algae [mg a.s./L]	P. subcapitata	0.522	P. subcapitata	2.18
EC ₅₀ macrophytes [mg a.s./L]				
AF for RAC				
Fish	O. mykiss (trout)	10	O. mykiss	10
Invertebrates	D. magna	10	D. magna	10

Algae	P. subcapitata	10	P. subcapitata	10
RAC				
Fish	O. mykiss (trout)	0.00022	O. mykiss	0.183
Invertebrates	D. magna	0.000093	D. magna	0.13
Algae	P. subcapitata	0.0522	P. subcapitata	0.218

Input PEC tab

Table A3-3: Active substance FOCUS Steps 1-3 maximum PEC_{sw} values for fenpicoxamid and prothioconazole_sugar beet

Used FOCUS Scenarios	FNP		PTZ	
	PECs [µg/L]	Max PEC _{sw}	PECs [µg/L]	Max PEC _{sw}
Step 1	Step 1	7.1257	Step 1	32.5801
Step 2	Step 2		Step 2	
N-Europe	N-Europe	0.6898	N-Europe	1.3795
S-Europe	S-Europe	0.6898	S-Europe	1.3795
Step 3	Step 3		Step 3	
D1 Ditch	D1 Ditch		D1 Ditch	
D1 Stream	D1 Stream		D1 Stream	
D2 Ditch	D2 Ditch		D2 Ditch	
D2 Stream	D2 Stream		D2 Stream	
D3 Ditch	D3 Ditch	0.3863	D3 Ditch	0.7849
D4 Pond	D4 Pond	0.016	D4 Pond	0.0317
D4 Stream	D4 Stream	0.3211	D4 Stream	0.6529
D5 Pond	D5 Pond		D5 Pond	
D5 Stream	D5 Stream		D5 Stream	
D6 Ditch	D6 Ditch		D6 Ditch	
R1 Pond	R1 Pond	0.016	R1 Pond	0.032
R1 Stream	R1 Stream	0.265	R1 Stream	0.540
R2 Stream	R2 Stream		R2 Stream	
R3 Stream	R3 Stream	0.3775	R3 Stream	0.7672
R4 Stream	R4 Stream		R4 Stream	

Table A3-4: Fenpicoxamid and Prothioconazole FOCUS Step 4 maximum PEC_{sw} values at worst case 1 - 2 x 75 g a.s./ha with various mitigations_sugar beet

Active substance 1: FNP			Active substance 2: PTZ		
PEC _{sw} [µg/L]	Scenario	FOCUS Step 4	PEC _{sw} [µg/L]	Scenario	FOCUS Step 4

Nozzle	Vegetative strip [m]	10	10
reduction	No spray buffer [m]	10	30
None	D3 Ditch	0.06670	0.02340
50%			
75%		0.01660	
90%			
None	D4 Pond	0.01010	0.00500
50%			
75%		0.00250	
90%			
None	D4 Stream	0.07130	0.02500
50%			
75%		0.01770	
90%			
None	R1 Pond	0.00980	0.00500
50%			
75%		0.00250	
90%			
None	R1 Stream	0.05890	0.02060
50%			
75%		0.01460	
90%			
None	R3 Stream	0.08380	0.02940
50%			
75%		0.02080	
90%			

Nozzle	Vegetative strip [m]	10	10
reduction	No spray buffer [m]	10	30
None	D3 Ditch	0.13640	0.04800
50%			
75%		0.03410	
90%			
None	D4 Pond	0.02030	0.01030
50%			
75%		0.00510	
90%			
None	D4 Stream	0.14570	0.05130
50%			
75%		0.03640	
90%			
None	R1 Pond	0.02030	0.01030
50%			
75%		0.00510	
90%			
None	R1 Stream	0.12050	0.05470
50%			
75%		0.05470	
90%			
None	R3 Stream	0.17	0.06
50%			
75%		0.04	
90%			

Output: Mixture assessment, Step 8b, RQmix

Table A3-5: FOCUS Step 1, 2 and 3 Mixture assessment, Step 8b, RQmix _fenpicoxamid + prothioconazole

FOCUS Step 1, Step 2 and Step 3			
Fish		Invertebrates	
RQmix		RQmix	
Step 1	32.57	Step 1	76.87
Step 2		Step 2	
N-Europe	3.14	N-Europe	7.43
S-Europe	3.14	S-Europe	7.43
Step 3		Step 3	
D1 Ditch		D1 Ditch	
D1 Stream		D1 Stream	
D2 Ditch		D2 Ditch	
D2 Stream		D2 Stream	

Algae	
RQmix	
Step 1	0.29
Step 2	
N-Europe	0.02
S-Europe	0.02
Step 3	
D1 Ditch	
D1 Stream	
D2 Ditch	
D2 Stream	

D3 Ditch	1.76	D3 Ditch	4.16	D3 Ditch	0.01
D4 Pond	0.07	D4 Pond	0.17	D4 Pond	0.00
D4 Stream	1.46	D4 Stream	3.46	D4 Stream	0.01
D5 Pond		D5 Pond		D5 Pond	
D5 Stream		D5 Stream		D5 Stream	
D6 Ditch		D6 Ditch		D6 Ditch	
R1 Pond	0.07	R1 Pond	0.17	R1 Pond	0.00
R1 Stream	1.21	R1 Stream	2.86	R1 Stream	0.01
R2 Stream		R2 Stream		R2 Stream	
R3 Stream	1.72	R3 Stream	4.07	R3 Stream	0.01
R4 Stream		R4 Stream		R4 Stream	

Table A3-6: Results FOCUS Step 4 Mixture assessment, Step 8b, RQmix_fenpicoxamid + prothioconazole

FOCUS Step 4					
Fish				Invertebrates	
FOCUS Step 4	Scenario	RQmix		FOCUS Step 4	RQmix
	Vegetative strip [m]	10	10		
Nozzle re-duction	No spray buffer [m]	10	30	Nozzle re-duction	No spray buffer [m]
None	D3 Ditch	0.30	0.11	None	D3 Ditch
50%				50%	
75%		0.08		75%	0.18
90%				90%	
None	D4 Pond	0.05	0.02	None	D4 Pond
50%				50%	
75%		0.01		75%	0.03
90%				90%	
None	D4 Stream	0.32	0.11	None	D4
50%				50%	
75%		0.08		75%	0.19
90%				90%	
None	R1 Pond	0.04	0.02	None	R1 Pond
50%				50%	
75%		0.01		75%	0.03
90%				90%	
None	R1 Stream	0.27	0.09	None	R1
50%				50%	
75%		0.07		75%	0.16
90%				90%	
None	R3 Stream	0.38	0.13	None	R3
50%				50%	

75%		0.09		75%		0.22	
90%				90%			

Algae			
FOCUS Step 4	Scenario	RQmix	
	Vegetative strip [m]	10	10
Nozzle reduction	No spray buffer [m]	10	30
None	D3 Ditch	0.00	0.00
50%			
75%		0.00	
90%			
None	D4 Pond	0.00	0.00
50%			
75%		0.00	
90%			
None	D4 Stream	0.00	0.00
50%			
75%		0.00	
90%			
None	R1 Pond	0.00	0.00
50%			
75%		0.00	
90%			
None	R1 Stream	0.00	0.00
50%			
75%		0.00	
90%			
None	R3 Stream	0.00	0.00
50%			
75%		0.00	
90%			

Acceptable acute risk is demonstrated for the mixture of fenpicoxamid + prothioconazole in sugar beet at 1 to 2 applications of 1.5 L GF-3307/ha (1 to 2 x 75 g fenpicoxamid/ha + 150 g prothioconazole/ha) with a:

- 10 m NSZ + 10 m NSZ and
- 30 m NSZ + 10 m NSZ and
- 10 m NSZ + 10 m VFS + 75% DRN

zRMS comments:

Acute mixture toxicity the mixture of fenpicoxamid + prothioconazole

Based on the calculations based on Tier 1 data performed in sugar beet at 1 to 2 applications of 1.5 L GF-3307/ha (1 to 2 x 75 g fenpicoxamid/ha + 150 g prothioconazole/ha) the following risk mitigation measures are required

- 10 m NSZ + 10 m NSZ or
- 30 m NSZ + 10 m NSZ or

▲ 10 m NSZ + 10 m VFS + 75% DRN

The details of the calculation are presented in the exe.file.



AGD_AquaMix_v1.22_
1to2x_75gFPN+150gP

Chronic aquatic organism mixture toxicity Fenpicoxamid + Prothioconazole

Input Tox tab

Table A3-7: Active substance chronic Tier 1 data for fenpicoxamid (FPN) and prothioconazole (PTZ)

Active Substance (a.s.) standard data (Tier 1 EP)				
Active substance names		FPN		PTZ
Concentration in product [g a.s./L or g a.s./kg]	FPN	50		100
p(X) (fraction in product)	Species	0.33	Species	0.67
LC ₅₀ fish [mg a.s./L]	NOEC, ELS P. promelas	0.00091	NOEC, ELS O. mykiss	0.308
LC ₅₀ invertebrates [mg a.s./L]	NOEC, daph chronic	0.00053	NOEC, daph chronic	0.56
AF for RAC				
Fish	NOEC, ELS P. promelas	10	NOEC, ELS O. mykiss	10
Invertebrates	NOEC, daph chronic	10	NOEC, daph chronic	10
Algae		10		10
Macrophytes		10		10
RAC				
Fish	NOEC, ELS P. promelas	0.000091	NOEC, ELS O. mykiss	0.0308
Invertebrates	NOEC, daph chronic	0.000053	NOEC, daph chronic	0.056

Input PEC tab

Table A3-8: Active substance FOCUS Steps 1-3 maximum PEC_{sw} values for fenpicoxamid and prothioconazole_sugar beet

Used FOCUS Scenarios	FPN		PTZ	
	PECs [µg/L]	Max PEC _{sw}	PECs [µg/L]	Max PEC _{sw}
Step 1	Step 1	7.1257	Step 1	32.5801
Step 2	Step 2		Step 2	
N-Europe	N-Europe	0.6898	N-Europe	1.3795
S-Europe	S-Europe	0.6898	S-Europe	1.3795
Step 3	Step 3		Step 3	

D1 Ditch	D1 Ditch		D1 Ditch	
D1 Stream	D1 Stream		D1 Stream	
D2 Ditch	D2 Ditch		D2 Ditch	
D2 Stream	D2 Stream		D2 Stream	
D3 Ditch	D3 Ditch	0.3863	D3 Ditch	0.7849
D4 Pond	D4 Pond	0.016	D4 Pond	0.0317
D4 Stream	D4 Stream	0.3211	D4 Stream	0.6529
D5 Pond	D5 Pond		D5 Pond	
D5 Stream	D5 Stream		D5 Stream	
D6 Ditch	D6 Ditch		D6 Ditch	
R1 Pond	R1 Pond	0.016	R1 Pond	0.032
R1 Stream	R1 Stream	0.265	R1 Stream	0.540
R2 Stream	R2 Stream		R2 Stream	
R3 Stream	R3 Stream	0.3775	R3 Stream	0.7672
R4 Stream	R4 Stream		R4 Stream	

Table A3-9: Fenpicoxamid and Prothioconazole FOCUS Step 4 maximum PEC_{sw} values at worst case 1 - 2 x 75 g a.s./ha with various mitigations_sugar beet

Active substance 1: FNP				Active substance 2: PTZ			
PEC _{sw} [µg/L]	Scenario	FOCUS Step 4		PEC _{sw} [µg/L]	Scenario	FOCUS Step 4	
Nozzle reduction	Vegetative strip [m]	10	10	Nozzle reduction	Vegetative strip [m]	10	10
	No spray buffer [m]	10	30		No spray buffer [m]	10	30
None	D3 Ditch	0.06670	0.02340	None	D3 Ditch	0.13640	0.04800
50%				50%			
75%		0.01660		75%		0.03410	
90%				90%			
None	D4 Pond	0.01010	0.00500	None	D4 Pond	0.02030	0.01030
50%				50%			
75%		0.00250		75%		0.00510	
90%				90%			
None	D4 Stream	0.07130	0.02500	None	D4 Stream	0.14570	0.05130
50%				50%			
75%		0.01770		75%		0.03640	
90%				90%			
None	R1 Pond	0.00980	0.00500	None	R1 Pond	0.02030	0.01030
50%				50%			
75%		0.00250		75%		0.00510	
90%				90%			
None	R1 Stream	0.05890	0.02060	None	R1 Stream	0.12050	0.05470
50%				50%			
75%		0.01460		75%		0.05470	

90%					90%				
None	R3 Stream	0.08380	0.02940		None	R3 Stream	0.17	0.06	
50%					50%				
75%		0.02080			75%		0.04		
90%					90%				

Output: Mixture assessment, Step 8b, RQmix

Table A3-10: FOCUS Step 1, 2 and 3 Mixture assessment, Step 8b, RQmix _Fenpicoxamid + Prothioconazole

FOCUS Step 1, Step 2 and Step 3			
Fish		Invertebrates	
RQmix		RQmix	
Step 1	79.36	Step 1	135.03
Step 2		Step 2	
N-Europe	7.63	N-Europe	13.04
S-Europe	7.63	S-Europe	13.04
Step 3		Step 3	
D1 Ditch		D1 Ditch	
D1 Stream		D1 Stream	
D2 Ditch		D2 Ditch	
D2 Stream		D2 Stream	
D3 Ditch	4.27	D3 Ditch	7.30
D4 Pond	0.18	D4 Pond	0.30
D4 Stream	3.55	D4 Stream	6.07
D5 Pond		D5 Pond	
D5 Stream		D5 Stream	
D6 Ditch		D6 Ditch	
R1 Pond	0.17	R1 Pond	0.29
R1 Stream	2.93	R1 Stream	5.02
R2 Stream		R2 Stream	
R3 Stream	4.17	R3 Stream	7.14
R4 Stream		R4 Stream	

Table A3-11: Results FOCUS Step 4 Mixture assessment, Step 8b, RQmix _Fenpicoxamid + Prothioconazole

FOCUS Step 4				
Fish				
FOCUS Step 4	Scenario	RQmix		
	Vegetative strip [m]	10	10	
Nozzle reduction	No spray buffer [m]	10	30	
None	D3 Ditch	0.74	0.26	
50%				
75%		0.18		
90%				
None	D4 Pond	0.11	0.06	
50%				
75%		0.03		
90%				
None	D4 Stream	0.79	0.28	
50%				
75%		0.20		
90%				
None	R1 Pond	0.11	0.06	
50%				
75%		0.03		
90%				
None	R1 Stream	0.65	0.23	
50%				
75%		0.16		
90%				
None	R3 Stream	0.93	0.33	
50%				
75%		0.23		
90%				

Invertebrates			
FOCUS Step 4	Scenario	RQmix	
	Vegetative strip [m]	10	10
Nozzle reduction	No spray buffer [m]	10	30
None	D3 Ditch	1.26	0.44
50%			
75%		0.31	
90%			
None	D4 Pond	0.19	0.09
50%			
75%		0.05	
90%			
None	D4 Stream	1.35	0.47
50%			
75%		0.33	
90%			
None	R1 Pond	0.19	0.09
50%			
75%		0.05	
90%			
None	R1 Stream	1.11	0.39
50%			
75%		0.28	
90%			
None	R3 Stream	1.58	0.56
50%			
75%		0.39	
90%			

Acceptable chronic risk is demonstrated for the mixture of fenpicoxamid + prothioconazole in sugar beet at 1 to 2 applications of 1.5 L GF-3307/ha (1 to 2 x 75 g fenpicoxamid/ha + 150 g prothioconazole/ha) with a:

- 30 m NSZ + 10 m NSZ and
- 10 m NSZ + 10 m VFS + 75% DRN

Chronic aquatic organism mixture toxicity Fenpicoxamid + JAU 6476-desthio

Input Tox tab

Table A3-12: Active substance chronic Tier 1 data for fenpicoxamid (FPN) and JAU 6476-desthio (PTZ-desthio)

Active Substance (a.s.) standard data (Tier 1 EP)				
Active substance names		FPN		PTZ-desthio
Concentration in product [g a.s./L or g a.s./kg]	FPN	50		100
p(X) (fraction in product)	Species	0.33	Species	0.67

LC ₅₀ fish [mg a.s./L]	NOEC, ELS	0.00091	NOEC, ELS	0.00334
LC ₅₀ invertebrates [mg a.s./L]	NOEC, daph chronic	0.00053	NOEC, daph chronic	0.1
AF for RAC				
Fish	NOEC, ELS	10	NOEC, ELS	10
Invertebrates	NOEC, daph chronic	10	NOEC, daph chronic	10
RAC				
Fish	NOEC, ELS	0.000091	NOEC, ELS	0.000334
Invertebrates	NOEC, daph chronic	0.000053	NOEC, daph chronic	0.01

Input PEC tab

Table A3-13: Active substance FOCUS Steps 1-3 maximum PEC_{sw} values for fenpicoxamid and JAU 6476-desthio_sugar beet

FOCUS Step 1, Step 2 and Step 3				
Used FOCUS Scenarios	FNP		PTZ-desthio	
	PECs [µg/L]	Max PEC _{sw}	PECs [µg/L]	Max PEC _{sw}
Step 1	Step 1	7.1257	Step 1	58.5727
Step 2	Step 2		Step 2	
N-Europe	N-Europe	0.6898	N-Europe	2.745
S-Europe	S-Europe	0.6898	S-Europe	5.0409
Step 3	Step 3		Step 3	
D1 Ditch	D1 Ditch		D1 Ditch	
D1 Stream	D1 Stream		D1 Stream	
D2 Ditch	D2 Ditch		D2 Ditch	
D2 Stream	D2 Stream		D2 Stream	
D3 Ditch	D3 Ditch	0.3863	D3 Ditch	0.0798
D4 Pond	D4 Pond	0.016	D4 Pond	0.026
D4 Stream	D4 Stream	0.3211	D4 Stream	0.0424
D5 Pond	D5 Pond		D5 Pond	
D5 Stream	D5 Stream		D5 Stream	
D6 Ditch	D6 Ditch		D6 Ditch	
R1 Pond	R1 Pond	0.016	R1 Pond	0.053
R1 Stream	R1 Stream	0.265	R1 Stream	0.223
R2 Stream	R2 Stream		R2 Stream	
R3 Stream	R3 Stream	0.3775	R3 Stream	0.3583
R4 Stream	R4 Stream		R4 Stream	

Table A3-14: Fenpicoxamid and JAU 6476-desthio FOCUS Step 4 maximum PEC_{sw} values at worst case 1 - 2 x 75 g a.s./ha with various mitigations_sugar beet

Active substance 1: FNP				Active substance 2: PTZ-desthio			
PEC _{sw} [µg/L]	Scenario	FOCUS Step 4		PEC _{sw} [µg/L]	Scenario	FOCUS Step 4	
Nozzle	Vegetative strip [m]	10	10	Nozzle	Vegetative strip [m]	10	10
reduction	No spray buffer [m]	10	30	reduction	No spray buffer [m]	10	30
None	D3 Ditch	0.06670	0.02340	None	D3 Ditch	0.01380	0.00490
50%				50%			
75%		0.01660		75%		0.00350	
90%				90%			
None	D4 Pond	0.01010	0.00500	None	D4 Pond	0.01620	0.00950
50%				50%			
75%		0.00250		75%		0.00740	
90%				90%			
None	D4 Stream	0.07130	0.02500	None	D4 Stream	0.03570	0.03570
50%				50%			
75%		0.01770		75%		0.03570	
90%				90%			
None	R1 Pond	0.00980	0.00500	None	R1 Pond	0.02450	0.02110
50%				50%			
75%		0.00250		75%		0.01950	
90%				90%			
None	R1 Stream	0.05890	0.02060	None	R1 Stream	0.10100	0.10100
50%				50%			
75%		0.01460		75%		0.10100	
90%				90%			
None	R3 Stream	0.08380	0.02940	None	R3 Stream	0.16	0.16
50%				50%			
75%		0.02080		75%		0.16	
90%				90%			

Output: Mixture assessment, Step 8b, RQmix

Table A3-15: FOCUS Step 1, 2 and 3 Mixture assessment, Step 8b, RQmix_Fenpicoxamid + JAU 6476-desthio

FOCUS Step 1, Step 2 and Step 3			
Fish		Invertebrates	
RQmix		RQmix	
Step 1	253.67	Step 1	140.30
Step 2		Step 2	
N-Europe	15.80	N-Europe	13.29
S-Europe	22.67	S-Europe	13.52
Step 3		Step 3	

D1 Ditch		D1 Ditch	
D1 Stream		D1 Stream	
D2 Ditch		D2 Ditch	
D2 Stream		D2 Stream	
D3 Ditch	4.48	D3 Ditch	7.30
D4 Pond	0.25	D4 Pond	0.30
D4 Stream	3.66	D4 Stream	6.06
D5 Pond		D5 Pond	
D5 Stream		D5 Stream	
D6 Ditch		D6 Ditch	
R1 Pond	0.33	R1 Pond	0.30
R1 Stream	3.58	R1 Stream	5.03
R2 Stream		R2 Stream	
R3 Stream	5.22	R3 Stream	7.16
R4 Stream		R4 Stream	

Table A3-16: Results FOCUS Step 4 Mixture assessment, Step 8b, RQmix_Fenpicoxamid + JAU 6476-desthio

FOCUS Step 4							
Fish				Invertebrates			
FOCUS Step 4	Scenario	RQmix		FOCUS Step 4	Scenario	RQmix	
	Vegetative strip [m]	10	10		Vegetative strip [m]	10	10
Nozzle reduction	No spray buffer [m]	10	30	Nozzle reduction	No spray buffer [m]	10	30
None	D3 Ditch	0.77	0.27	None	D3 Ditch	1.26	0.44
50%				50%			
75%		0.19		75%		0.31	
90%				90%			
None	D4 Pond	0.16	0.08	None	D4 Pond	0.19	0.10
50%				50%			
75%		0.05		75%		0.05	
90%				90%			
None	D4 Stream	0.89	0.38	None	D4 Stream	1.35	0.48
50%				50%			
75%		0.30		75%		0.34	
90%				90%			
None	R1 Pond	0.18	0.12	None	R1 Pond	0.19	0.10
50%				50%			
75%		0.09		75%		0.05	
90%				90%			
None	R1 Stream	0.95	0.53	None	R1 Stream	1.12	0.40

50%				50%			
75%		0.46		75%		0.29	
90%				90%			
None	R3 Stream	1.41	0.81	None	R3 Stream	1.60	0.57
50%				50%			
75%		0.72		75%		0.41	
90%				90%			

Acceptable chronic risk is demonstrated for the mixture of fenpicoxamid + JAU 6476-desthio in sugar beet at 1 to 2 applications of 1.5 L GF-3307/ha (1 to 2 x 75 g fenpicoxamid/ha + 150 g prothioconazole/ha) with a:

- 30 m NSZ + 10 m NSZ and
- 10 m NSZ + 10 m VFS + 75% DRN

zRMS comments:

Acceptable chronic risk is demonstrated for the mixture of fenpicoxamid + JAU 6476-desthio in sugar beet at 1 to 2 applications of 1.5 L GF-3307/ha (1 to 2 x 75 g fenpicoxamid/ha + 150 g prothioconazole/ha) with the following risk mitigation measures:

- 30 m NSZ + 10 m NSZ and
- 10 m NSZ + 10 m VFS + 75% DRN

It should be noted that the Applicant used the chronic studies for *Daphnia magna* for fenpicoxamid with NOEC=0.0053 mg a.s./L, invalidated at EU level and for this reason this endpoint should be not used in Tier 1 risk assessment.

Therefore, the chronic mixture toxicity have been calculated with consideration NOEC value from mesocosm study.

COMMENTING PERIOD PROCESS:

Aquatic mixture toxicity has been assessed with the AGD_Aquamix_v122.xlsm Excel Aquatic MixTox tool, which can be found at <https://zenodo.org/records/7788826>. The input parameters/endpoints are shown below as well as the outcome and from the AGD MixTox tool.

The acute mixture assessment is presented for Fenpicoxamid + Prothioconazole.

The chronic mixture assessment is presented for Fenpicoxamid + Prothioconazole and alternatively for Fenpicoxamid + JAU 6476-desthio, since the metabolite JAU 6476-desthio is more toxic to fish (chronic) than the parent Prothioconazole.

For simplicity, only the input data and the final mixture assessment at Step 8b are presented.

Upon request of the zRMS (considering comments given by some of MSs), the NOEC = 0.1 µg/L im; AF=3; RAC = 0.033 µg/L for a.s. fenpicoxamid from the mesocosm study was considered in the aquatic invertebrate mixture toxicity risk assessment.

Additionally, the fenpicoxamid fish acute geomean was used by the Applicant for the mixture assessment.

Acute aquatic organism mixture toxicity Fenpicoxamid + Prothioconazole

Input Tox tab

Table A3-1: Product acute Tier 1 data for GF-3307

Product data	
Product name	GF-3307
Density of product [g/cm ³]	1.044
LC ₅₀ fish [mg prod./L]	0.072
LC ₅₀ fish a.s. based [mg sum of a.s./L]	0.0103
EC ₅₀ invertebrates [mg prod./L]	0.015
LC ₅₀ invertebrates a.s. based [mg sum of a.s./L]	0.0022
EC ₅₀ algae [mg prod./L]	8
EC ₅₀ algae a.s. based [mg sum of a.s./L]	1.1494

Table A3-2: Active substance acute Tier 1 data for fenpicoxamid (FPN) and prothioconazole (PTZ)

Active Substance (a.s.) standard data (Tier 1 EP)				
Active substance names		FPN		PTZ
Concentration in product [g a.s./L or g a.s./kg]	FPN	50		100
p(X) (fraction in product)	Species	0.33	Species	0.67
LC ₅₀ fish [mg a.s./L]	4 species geomean	0.0041	O. mykiss	1.83
LC ₅₀ invertebrates [mg a.s./L]	Inv. Mesocosm NOEC	0.0001	D. magna	1.3
EC ₅₀ algae [mg a.s./L]	P. subcapitata	0.522	P. subcapitata	2.18
EC ₅₀ macrophytes [mg a.s./L]				
AF for RAC				
Fish	4 species geomean	100	O. mykiss	100
Invertebrates	Inv. Mesocosm	3	D. magna	100
Algae	P. subcapitata	10	P. subcapitata	10
RAC				
Fish	4 species geomean	0.000041	O. mykiss	0.0183
Invertebrates	Inv. Mesocosm	0.000033333	D. magna	0.013
Algae	P. subcapitata	0.0522	P. subcapitata	0.218

Input PEC tab

Table A3-3: Active substance FOCUS Steps 1-3 maximum PECsw values for fenpicoxamid and prothioconazole_sugar beet

Used FO- CUS Scenarios	FNP		PTZ	
	PECs [µg/L]	Max PECsw	PECs [µg/L]	Max PECsw
Step 1	Step 1	7.1257	Step 1	32.5801
Step 2	Step 2		Step 2	
N-Europe	N-Europe	0.6898	N-Europe	1.3795
S-Europe	S-Europe	0.6898	S-Europe	1.3795
Step 3	Step 3		Step 3	
D1 Ditch	D1 Ditch		D1 Ditch	
D1 Stream	D1 Stream		D1 Stream	
D2 Ditch	D2 Ditch		D2 Ditch	
D2 Stream	D2 Stream		D2 Stream	
D3 Ditch	D3 Ditch	0.3863	D3 Ditch	0.7849
D4 Pond	D4 Pond	0.016	D4 Pond	0.0317
D4 Stream	D4 Stream	0.3211	D4 Stream	0.6529
D5 Pond	D5 Pond		D5 Pond	
D5 Stream	D5 Stream		D5 Stream	
D6 Ditch	D6 Ditch		D6 Ditch	
R1 Pond	R1 Pond	0.016	R1 Pond	0.032
R1 Stream	R1 Stream	0.265	R1 Stream	0.540
R2 Stream	R2 Stream		R2 Stream	
R3 Stream	R3 Stream	0.3775	R3 Stream	0.7672
R4 Stream	R4 Stream		R4 Stream	

Table A3-4: Fenpicoxamid and Prothioconazole FOCUS Step 4 maximum PECsw values at worst case 1 - 2 x 75 g a.s./ha with various mitigations_sugar beet

Active substance 1:		FNP			Active substance 2:		PTZ		
PECsw [µg/L]	Sce-nario	FOCUS Step 4			PECsw [µg/L]	Sce-nario	FOCUS Step 4		
Nozzle reduction	Vegetative strip [m]	10	10	10	Nozzle reduction	Vegetative strip [m]	10	10	10
	No spray buffer [m]	10	20	30		No spray buffer [m]	10	20	30
None	D3	0.06670		0.02340	None	D3	0.13640		0.04800
50%					50%				
75%		0.01660	0.0000		75%		0.03410	0.0177	
90%					90%				
None	D4 Pond	0.01010		0.00500	None	D4	0.02030		0.01030

50%					50%				
75%		0.00250	0.0003		75%		0.00510	0.0034	
90%					90%				
None	D4	0.07130		0.02500	None	D4	0.14570		0.05130
50%					50%				
75%		0.01770	0.0020		75%		0.03640	0.0189	
90%					90%				
None	R1 Pond	0.00980		0.00500	None	R1 Pond	0.02030		0.01030
50%					50%				
75%		0.00250	0.0054		75%		0.00510	0.0034	
90%					90%				
None	R1	0.05890		0.02060	None	R1	0.12050		0.05470
50%					50%				
75%		0.01460	0.0134		75%		0.05470	0.0547	
90%					90%				
None	R3	0.08380		0.02940	None	R3	0.17		0.06
50%					50%				
75%		0.02080	0.0204		75%		0.04	0.0222	
90%					90%				

Output: Mixture assessment, Step 8b, RQmix

Table A3-5: FOCUS Step 1, 2 and 3 Mixture assessment, Step 8b, RQmix _fenpicoxamid + prothioconazole

FOCUS Step 1, Step 2 and Step 3					
Fish		Invertebrates		Algae	
RQmix		RQmix		RQmix	
Step 1	175.58	Step 1	216.28	Step 1	0.29
Step 2		Step 2		Step 2	
N-Europe	16.90	N-Europe	20.80	N-Europe	0.02
S-Europe	16.90	S-Europe	20.80	S-Europe	0.02
Step 3		Step 3		Step 3	
D1 Ditch		D1 Ditch		D1 Ditch	
D1 Stream		D1 Stream		D1 Stream	
D2 Ditch		D2 Ditch		D2 Ditch	
D2 Stream		D2 Stream		D2 Stream	
D3 Ditch	9.46	D3 Ditch	11.65	D3 Ditch	0.01
D4 Pond	0.39	D4 Pond	0.48	D4 Pond	0.00
D4 Stream	7.87	D4 Stream	9.68	D4 Stream	0.01
D5 Pond		D5 Pond		D5 Pond	
D5 Stream		D5 Stream		D5 Stream	
D6 Ditch		D6 Ditch		D6 Ditch	
R1 Pond	0.38	R1 Pond	0.47	R1 Pond	0.00
R1 Stream	6.50	R1 Stream	8.00	R1 Stream	0.01
R2 Stream		R2 Stream		R2 Stream	

R3 Stream	9.25	R3 Stream	11.38	R3 Stream	0.01
R4 Stream		R4 Stream		R4 Stream	

Table A3-6: Results FOCUS Step 4 Mixture assessment, Step 8b, RQmix_fenpicoxamid + prothioconazole

FOCUS Step 4									
Fish					Invertebrates				
FOCUS Step 4	Scenario	RQmix			FOCUS Step 4	Scenario	RQmix		
	Vegetative strip [m]	10	10	10		Vegetative strip [m]	10	10	10
Nozzle reduction	No spray buffer [m]	10	20	30	Nozzle reduction	No spray buffer [m]	10	20	30
None	D3 Ditch	1.63		0.57	None	D3 Ditch	2.01		0.71
50%					50%				
75%		0.41	0.00		75%		0.50	0.00	
90%					90%				
None	D4 Pond	0.25		0.12	None	D4 Pond	0.30		0.15
50%					50%				
75%		0.06	0.01		75%		0.08	0.01	
90%					90%				
None	D4 Stream	1.75		0.61	None	D4	2.15		0.75
50%					50%				
75%		0.43	0.05		75%		0.53	0.06	
90%					90%				
None	R1 Pond	0.24		0.12	None	R1 Pond	0.30		0.15
50%					50%				
75%		0.06	0.13		75%		0.08	0.16	
90%					90%				
None	R1 Stream	1.44		0.51	None	R1	1.78		0.62
50%					50%				
75%		0.36	0.33		75%		0.44	0.41	
90%					90%				
None	R3 Stream	2.05		0.72	None	R3	2.53		0.89
50%					50%				
75%		0.51	0.50		75%		0.63	0.61	
90%					90%				

Algae				
FOCUS Step 4	Scenario	RQmix		
	Vegetative strip [m]	10	10	10
Nozzle reduction	No spray buffer [m]	10	20	30
None	D3 Ditch	0.00		0.00

50%				
75%		0.00	0.00	
90%				
None	D4 Pond	0.00		0.00
50%				
75%		0.00	0.00	
90%				
None	D4 Stream	0.00		0.00
50%				
75%		0.00	0.00	
90%				
None	R1 Pond	0.00		0.00
50%				
75%		0.00	0.00	
90%				
None	R1 Stream	0.00		0.00
50%				
75%		0.00	0.00	
90%				
None	R3 Stream	0.00		0.00
50%				
75%		0.00	0.00	
90%				

Acceptable acute risk is demonstrated for the mixture of fenpicoxamid + prothioconazole in sugar beet at 1 to 2 applications of 1.5 L GF-3307/ha (1 to 2 x 75 g fenpicoxamid/ha + 150 g prothioconazole/ha) with a:

- 30 m NSZ + 10 m NSZ and
- 20 m NSZ + 10 m VFS + 75% DRN
- 10 m NSZ + 10 m VFS + 75% DRN

zRMS comments:

Acute mixture toxicity assessment: Fenpicoxamid + prothioconazole



AGD_AquaMix_v1.22_
1to2x_75gFPN+150gP

Additional, **acute risk calculation considering higher Tier data** (mesocosm study with NOEC=0.1 microgram/L and geomean for 4 fish species demonstrated for the mixture of fenpicoxamid + prothioconazole in sugar beet at 1 to 2 applications of 1.5 L GF-3307/ha (1 to 2 x 75 g fenpicoxamid/ha + 150 g prothioconazole/ha) with a:

- 30 m NSZ + 10 m NSZ or
- 20 m NSZ + 10 m VFS + 75% DRN
- 10 m NSZ + 10 m VFS + 75% DRN

It should be also indicated that the same conclusion from the calculations considering Tier 1 data (see above) are concluded.

Chronic aquatic organism mixture toxicity Fenpicoxamid + Prothioconazole

Input Tox tab

Table A3-7: Active substance chronic Tier 1 data for fenpicoxamid (FPN) and prothioconazole (PTZ)

Active Substance (a.s.) standard data (Tier 1 EP)				
Active substance names		FPN		PTZ
Concentration in product [g a.s./L or g a.s./kg]	FPN	50		100
p(X) (fraction in product)	Species	0.33	Species	0.67
LC ₅₀ fish [mg a.s./L]	NOEC, ELS P. promelas	0.00091	NOEC, ELS O. mykiss	0.308
LC ₅₀ invertebrates [mg a.s./L]	Inv. Mesocosm	0.0001	NOEC, daph chronic	0.56
AF for RAC				
Fish	NOEC, ELS P. promelas	10	NOEC, ELS O. mykiss	10
Invertebrates	Inv. Mesocosm	3	NOEC, daph chronic	10
Algae		10		10
Macrophytes		10		10
RAC				
Fish	NOEC, ELS P. promelas	0.000091	NOEC, ELS O. mykiss	0.0308
Invertebrates	Inv. Mesocosm	0.000033	NOEC, daph chronic	0.056

Input PEC tab

Table A3-8: Active substance FOCUS Steps 1-3 maximum PECsw values for fenpicoxamid and prothioconazole_sugar beet

Used FO-CUS	FPN		PTZ	
Scenarios	PECs [µg/L]	Max PECsw	PECs [µg/L]	Max PECsw
Step 1	Step 1	7.1257	Step 1	32.5801
Step 2	Step 2		Step 2	
N-Europe	N-Europe	0.6898	N-Europe	1.3795
S-Europe	S-Europe	0.6898	S-Europe	1.3795
Step 3	Step 3		Step 3	
D1 Ditch	D1 Ditch		D1 Ditch	
D1 Stream	D1 Stream		D1 Stream	
D2 Ditch	D2 Ditch		D2 Ditch	
D2 Stream	D2 Stream		D2 Stream	
D3 Ditch	D3 Ditch	0.3863	D3 Ditch	0.7849
D4 Pond	D4 Pond	0.016	D4 Pond	0.0317
D4 Stream	D4 Stream	0.3211	D4 Stream	0.6529
D5 Pond	D5 Pond		D5 Pond	
D5 Stream	D5 Stream		D5 Stream	
D6 Ditch	D6 Ditch		D6 Ditch	

R1 Pond	R1 Pond	0.016	R1 Pond	0.032
R1 Stream	R1 Stream	0.265	R1 Stream	0.540
R2 Stream	R2 Stream		R2 Stream	
R3 Stream	R3 Stream	0.3775	R3 Stream	0.7672
R4 Stream	R4 Stream		R4 Stream	

Table A3-9: Fenpicoxamid and Prothioconazole FOCUS Step 4 maximum PECsw values at worst case 1 - 2 x 75 g a.s./ha with various mitigations_sugar beet

Active substance 1: FNP					Active substance 2: PTZ				
PECsw [µg/L]	Scenario	FOCUS Step 4			PECs w [µg/L]	Scenario	FOCUS Step 4		
Nozzle reduction	Vegetative strip [m]	10	10	10	Nozzle reduction	Vegetative strip [m]	10	10	10
	No spray buffer [m]	10	20	30		No spray buffer [m]	10	20	30
None	D3	0.06670		0.02340	None	D3	0.13640		0.04800
50%					50%				
75%		0.01660	0.0000		75%		0.03410	0.0177	
90%					90%				
None	D4 Pond	0.01010		0.00500	None	D4	0.02030		0.01030
50%					50%				
75%		0.00250	0.0003		75%		0.00510	0.0034	
90%					90%				
None	D4	0.07130		0.02500	None	D4	0.14570		0.05130
50%					50%				
75%		0.01770	0.0020		75%		0.03640	0.0189	
90%					90%				
None	R1 Pond	0.00980		0.00500	None	R1 Pond	0.02030		0.01030
50%					50%				
75%		0.00250	0.0054		75%		0.00510	0.0034	
90%					90%				
None	R1	0.05890		0.02060	None	R1	0.12050		0.05470
50%					50%				
75%		0.01460	0.0134		75%		0.05470	0.0547	
90%					90%				
None	R3	0.08380		0.02940	None	R3	0.17		0.06
50%					50%				
75%		0.02080	0.0204		75%		0.04	0.0222	
90%					90%				

Output: Mixture assessment, Step 8b, RQmix

Table A3-10: FOCUS Step 1, 2 and 3 Mixture assessment, Step 8b, RQmix_Fenpicoxamid + Prothioconazole

FOCUS Step 1, Step 2 and Step 3			
Fish		Invertebrates	
RQmix		RQmix	
Step 1	79.36	Step 1	214.35
Step 2		Step 2	
N-Europe	7.63	N-Europe	20.72
S-Europe	7.63	S-Europe	20.72
Step 3		Step 3	
D1 Ditch		D1 Ditch	
D1 Stream		D1 Stream	
D2 Ditch		D2 Ditch	
D2 Stream		D2 Stream	
D3 Ditch	4.27	D3 Ditch	11.60
D4 Pond	0.18	D4 Pond	0.48
D4 Stream	3.55	D4 Stream	9.64
D5 Pond		D5 Pond	
D5 Stream		D5 Stream	
D6 Ditch		D6 Ditch	
R1 Pond	0.17	R1 Pond	0.47
R1 Stream	2.93	R1 Stream	7.97
R2 Stream		R2 Stream	
R3 Stream	4.17	R3 Stream	11.34
R4 Stream		R4 Stream	

Table A3-11: Results FOCUS Step 4 Mixture assessment, Step 8b, RQmix _Fenpicoxamid + Prothioconazole

FOCUS Step 4									
Fish					Invertebrates				
FOCUS Step 4	Scenario	RQmix			FOCUS Step 4	Scenario	RQmix		
	Vegetative strip [m]	10	10	10		Vegetative strip [m]	10	10	10
Nozzle reduction	No spray buffer [m]	10	20	30	Nozzle reduction	No spray buffer [m]	10	20	30
None	D3 Ditch	0.74		0.26	None	D3 Ditch	2.00		0.70
50%					50%				
75%		0.18	0.00		75%		0.50	0.00	
90%					90%				
None	D4 Pond	0.11		0.06	None	D4 Pond	0.30		0.15
50%					50%				
75%		0.03	0.00		75%		0.08	0.01	
90%					90%				

None	D4 Stream	0.79		0.28	None	D4	2.14		0.75
50%					50%				
75%		0.20	0.02		75%		0.53	0.06	
90%					90%				
None	R1 Pond	0.11		0.06	None	R1 Pond	0.29		0.15
50%					50%				
75%		0.03	0.06		75%		0.08	0.16	
90%					90%				
None	R1 Stream	0.65		0.23	None	R1	1.77		0.62
50%					50%				
75%		0.16	0.15		75%		0.44	0.40	
90%					90%				
None	R3 Stream	0.93		0.33	None	R3	2.52		0.88
50%					50%				
75%		0.23	0.22		75%		0.62	0.61	
90%					90%				

Acceptable chronic risk is demonstrated for the mixture of fenpicoxamid + prothioconazole in sugar beet at 1 to 2 applications of 1.5 L GF-3307/ha (1 to 2 x 75 g fenpicoxamid/ha + 150 g prothioconazole/ha) with a:

- 30 m NSZ + 10 m NSZ and
- 20 m NSZ + 10 m VFS + 75% DRN
- 10 m NSZ + 10 m VFS + 75% DRN

zRMS comments:

Chronic mixture toxicity assessment: Fenpicoxamid + prothioconazole



AGD_AquaMix_v1.22_
1to2x_75gFPN+150gP

Acceptable chronic risk is demonstrated for the mixture of fenpicoxamid + prothioconazole in sugar beet at 1 to 2 applications of 1.5 L GF-3307/ha (1 to 2 x 75 g fenpicoxamid/ha + 150 g prothioconazole/ha) with a:

- 30 m NSZ + 10 m NSZ and
- 20 m NSZ + 10 m VFS + 75% DRN
- 10 m NSZ + 10 m VFS + 75% DRN

Chronic aquatic organism mixture toxicity Fenpicoxamid + JAU 6476-desthio

Input Tox tab

Table A3-12: Active substance chronic Tier 1 data for fenpicoxamid (FPN) and JAU 6476-desthio (PTZ-desthio)

Active Substance (a.s.) standard data (Tier 1 EP)				
Active substance names		FPN		PTZ-desthio
Concentration in product [g a.s./L or g a.s./kg]	FPN	50		100
p(X) (fraction in product)	Species	0.33	Species	0.67
LC ₅₀ fish [mg a.s./L]	NOEC, ELS	0.00091	NOEC, ELS	0.00334
LC ₅₀ invertebrates [mg a.s./L]	NOEC, inv.mesocosm	0.0001	NOEC, daph chronic	0.1
AF for RAC				
Fish	NOEC, ELS	10	NOEC, ELS	10
Invertebrates	NOEC, inv.mesocosm	3	NOEC, daph chronic	10
RAC				
Fish	NOEC, ELS	0.000091	NOEC, ELS	0.000334
Invertebrates	NOEC, inv.mesocosm	0.000033	NOEC, daph chronic	0.01

Input PEC tab

Table A3-13: Active substance FOCUS Steps 1-3 maximum PEC_{sw} values for fenpicoxamid and JAU 6476-desthio_sugar beet

FOCUS Step 1, Step 2 and Step 3				
Used FOCUS Scenarios	FPN		PTZ-desthio	
	PECs [µg/L]	Max PEC _{sw}	PECs [µg/L]	Max PEC _{sw}
Step 1	Step 1	7.1257	Step 1	58.5727
Step 2	Step 2		Step 2	
N-Europe	N-Europe	0.6898	N-Europe	2.745
S-Europe	S-Europe	0.6898	S-Europe	5.0409
Step 3	Step 3		Step 3	
D1 Ditch	D1 Ditch		D1 Ditch	
D1 Stream	D1 Stream		D1 Stream	
D2 Ditch	D2 Ditch		D2 Ditch	
D2 Stream	D2 Stream		D2 Stream	
D3 Ditch	D3 Ditch	0.3863	D3 Ditch	0.0798
D4 Pond	D4 Pond	0.016	D4 Pond	0.026
D4 Stream	D4 Stream	0.3211	D4 Stream	0.0424
D5 Pond	D5 Pond		D5 Pond	
D5 Stream	D5 Stream		D5 Stream	
D6 Ditch	D6 Ditch		D6 Ditch	

R1 Pond	R1 Pond	0.016	R1 Pond	0.053
R1 Stream	R1 Stream	0.265	R1 Stream	0.223
R2 Stream	R2 Stream		R2 Stream	
R3 Stream	R3 Stream	0.3775	R3 Stream	0.3583
R4 Stream	R4 Stream		R4 Stream	

Table A3-14: Fenpicoxamid and JAU 6476-desthio FOCUS Step 4 maximum PECsw values at worst case 1 - 2 x 75 g a.s./ha with various mitigations_sugar beet

Active substance 1: FNP					Active substance 2: PTZ-desthio				
PECsw [µg/L]	Scenario	FOCUS Step 4			PECs w [µg/L]	Scenario	FOCUS Step 4		
Nozzle	Vegetative strip [m]	10	10	10	Nozzle	Vegetative strip [m]	10	10	10
reduction	No spray buffer [m]	10	20	30	reduction	No spray buffer [m]	10	20	30
None	D3	0.06670		0.02340	None	D3	0.01380		0.00490
50%					50%				
75%		0.01660	0.0000		75%		0.00350	0.00180	
90%					90%				
None	D4 Pond	0.01010		0.00500	None	D4	0.01620		0.00950
50%					50%				
75%		0.00250	0.0003		75%		0.00740	0.00670	
90%					90%				
None	D4	0.07130		0.02500	None	D4	0.03570		0.03570
50%					50%				
75%		0.01770	0.0020		75%		0.03570	0.03570	
90%					90%				
None	R1 Pond	0.00980		0.00500	None	R1 Pond	0.02450		0.02110
50%					50%				
75%		0.00250	0.0054		75%		0.01950	0.01890	
90%					90%				
None	R1	0.05890		0.02060	None	R1	0.10100		0.10100
50%					50%				
75%		0.01460	0.0134		75%		0.10100	0.10100	
90%					90%				
None	R3	0.08380		0.02940	None	R3	0.1633		0.1633
50%					50%				
75%		0.02080	0.0204		75%		0.1633	0.1633	
90%					90%				

Output: Mixture assessment, Step 8b, RQmix

Table A3-15: FOCUS Step 1, 2 and 3 Mixture assessment, Step 8b, RQmix _Fenpicoxamid + JAU 6476-desthio

FOCUS Step 1, Step 2 and Step 3			
Fish		Invertebrates	
RQmix		RQmix	
Step 1	253.67	Step 1	219.63
Step 2		Step 2	
N-Europe	15.80	N-Europe	20.97
S-Europe	22.67	S-Europe	21.20
Step 3		Step 3	
D1 Ditch		D1 Ditch	
D1 Stream		D1 Stream	
D2 Ditch		D2 Ditch	
D2 Stream		D2 Stream	
D3 Ditch	4.48	D3 Ditch	11.60
D4 Pond	0.25	D4 Pond	0.48
D4 Stream	3.66	D4 Stream	9.64
D5 Pond		D5 Pond	
D5 Stream		D5 Stream	
D6 Ditch		D6 Ditch	
R1 Pond	0.33	R1 Pond	0.47
R1 Stream	3.58	R1 Stream	7.98
R2 Stream		R2 Stream	
R3 Stream	5.22	R3 Stream	11.36
R4 Stream		R4 Stream	

Table A3-16: Results FOCUS Step 4 Mixture assessment, Step 8b, RQmix _Fenpicoxamid + JAU 6476-desthio

FOCUS Step 4									
Fish					Invertebrates				
FOCUS Step 4	Scenario	RQmix			FOCUS Step 4	Scenario	RQmix		
	Vegetative strip [m]	10	10	10		Vegetative strip [m]	10	10	10
Nozzle reduction	No spray buffer [m]	10	20	30	Nozzle reduction	No spray buffer [m]	10	20	30
None	D3 Ditch	0.77		0.27	None	D3 Ditch	2.00		0.70
50%					50%				
75%		0.19	0.01		75%		0.50	0.00	
90%					90%				
None	D4 Pond	0.16		0.08	None	D4 Pond	0.30		0.15
50%					50%				
75%		0.05	0.02		75%		0.08	0.01	

90%					90%				
None	D4 Stream	0.89		0.38	None	D4	2.14		0.75
50%					50%				
75%		0.30	0.13		75%		0.53	0.06	
90%					90%				
None	R1 Pond	0.18		0.12	None	R1 Pond	0.30		0.15
50%					50%				
75%		0.09	0.12		75%		0.08	0.16	
90%					90%				
None	R1 Stream	0.95		0.53	None	R1	1.78		0.63
50%					50%				
75%		0.46	0.45		75%		0.45	0.41	
90%					90%				
None	R3 Stream	1.41		0.81	None	R3	2.53		0.90
50%					50%				
75%		0.72	0.71		75%		0.64	0.63	
90%					90%				

Acceptable chronic risk is demonstrated for the mixture of fenpicoxamid + JAU 6476-desthio in sugar beet at 1 to 2 applications of 1.5 L GF-3307/ha (1 to 2 x 75 g fenpicoxamid/ha + 150 g prothioconazole/ha) with a:

- 30 m NSZ + 10 m NSZ and
- 20 m NSZ + 10 m VFS + 75% DRN
- 10 m NSZ + 10 m VFS + 75% DRN

zRMS comments:

Chronic mixture toxicity assessment: Fenpicoxamid + JAU 6476-desthio



AGD_AquaMix_v1.22_
1to2x_75gFPN+150g

Acceptable chronic risk is demonstrated for the mixture of fenpicoxamid + JAU 6476-desthio in sugar beet at 1 to 2 applications of 1.5 L GF-3307/ha (1 to 2 x 75 g fenpicoxamid/ha + 150 g prothioconazole/ha) with a:

- 30 m NSZ + 10 m NSZ and
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- 10 m NSZ + 10 m VFS + 75% DRN