

REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: GF-4021

Product name: LaDiva

Chemical active substances:

Halauxifen-methyl, 10 g a.s./L (9.594 g a.e./L)

Picloram, 48 g a.s./L

Aminopyralid, 32 g a.s./L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(Authorisation)

Applicant: Corteva Agriscience (Dow AgroSciences)

Submission date: November 2020

MS Finalisation date: August 2022 (initial Core Assessment)

November 2022 (final Core Assessment)

Version history

When	What
November 2020	New submission of GF-4021 in the Central Zone.
March 2021	Addition of final chronic bee study for formulated product and updates of risk assessment
August 2022	<p>Initial zRMS assessment</p> <p>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.</p>
November 2022	<p>Final report (Core Assessment updated following the commenting period).</p> <p>Additional information/assessments included by the zRMS in the report in response to comments received from the cMS and the Applicant are highlighted in yellow. Information no longer relevant is struck through and shaded.</p>

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9 Ecotoxicology (KCP 10)

This Core assessment has been prepared to support a Central Zone decision on a possible authorisation of the product GF-4021 in the Central Zone for the uses listed below.

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

Table 3.11.1: Table of critical CRPs																				
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 (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g safener/ synergist per ha	Conclusion						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg a.s./ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	Poland Germany Czech Republic United Kingdom Slovakia Hungary Romania Slovenia	Winter oilseed rape	F	Broadleaf weeds (post-em)	Broadcast foliar spray	BBCH 12 to 19	a) 1 b) 1	NA	a) 0.25 l pr/ha b) 0.25 l pr/ha	a) b) 2.5 halauxifen- methyl+ 12 picloram+ 8 aminopyralid	100-300		Timing: 90% of crop has to be in BBCH 12	A	A	A	A	A	A	R

* 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

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:	<div> <div> (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 (<i>e.g.</i> 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 <u>and</u> EPPO-Codes of target pests/diseases/ weeds or when relevant the common names of the pest groups (<i>e.g.</i> 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, <i>e.g.</i> high volume spraying, low volume spraying, spreading, dusting, drench Kind, <i>e.g.</i> overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated </div> <div> (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, <i>e.g.</i>: 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 (<i>e.g.</i> 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 </div> </div>
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9.1.1 Overall conclusions

zRMS comments:

Conclusions of the Applicant presented in this point were amended accordingly or changed entirely, depending on the outcome of the evaluation for particular groups of non-target species. Unlike in other points of this report, not agreed information provided by the Applicant has been removed instead of being struck through in order to present overall conclusions in a most transparent way.

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

Regulatory testing has been conducted with halauxifen-methyl, picloram and aminopyralid in accordance with EU requirements. The acute risks of GF-4021 to birds and mammals were assessed based on the predicted toxicity endpoint and maximum predicted exposure based on the sum of the application rates of the active substances. To address the long-term combined risk the TERMix was calculated, as agreed in the Central Zone.

For the active substances and the mixture, the TERs calculated in the screening assessment all exceed the trigger values of 10 and 5 for acute and long-term risk, respectively, indicating acceptable risk to birds and mammals from application of GF-4021 according to the proposed Central Zone use pattern.

For halauxifen-methyl an acceptable risk from secondary poisoning to earthworm and fish-eating birds and mammals was shown. Due to the low potential for bioaccumulation ($\log Pow < 3$) the risk of secondary poisoning from halauxifen-methyl metabolites, picloram and aminopyralid is considered to be low.

Furthermore, the risk assessment for exposure *via* drinking water also showed acceptable risk for the active substances and their pertinent soil metabolites.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

Regulatory testing has been conducted with halauxifen-methyl, picloram, aminopyralid, their respective metabolites and the product in accordance with EU requirements. Based on the active substances and product, the acute and chronic risk assessment for aquatic organisms indicated an acceptable risk to aquatic organisms from the use of GF-4021 in winter oilseed rape without the need for mitigation measures.

9.1.1.3 Effects on bees (KCP 10.3.1)

Regulatory testing has been conducted with halauxifen-methyl, picloram and aminopyralid and the product in accordance with EU requirements. An acceptable acute and long-term risk to adult bees and bee larvae is concluded from the proposed use of GF-4021 in winter oilseed rape without the need for risk mitigation measures.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

Regulatory testing has been conducted with GF-4021 in accordance with EU requirements. An acceptable in- and off-field risk to non-target arthropods is concluded from the proposed use of GF-4021 in winter oilseed rape without the need for risk mitigation measures.

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

Regulatory testing has been conducted with halauxifen-methyl, aminopyralid, picloram and the product in accordance with EU requirements. The effects of halauxifen-methyl non-extractable residues were also investigated at the EU level. All long-term TER values were calculated to be in excess of the trigger value of 5, therefore an acceptable risk for non-target soil meso- and macrofauna was concluded for the intended Central Zone uses of GF-4021.

Similarly, an acceptable risk to soil micro-organisms is expected from the proposed uses of GF-4021 in winter oilseed rape.

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

Regulatory testing has been conducted with the product in accordance with EU requirements. Risk assessment was performed using standard and probabilistic approach. Overall, acceptable risk to non-target terrestrial plants could be concluded from the intended uses of GF-4021, provided that following risk mitigation measures are respected:

1. Standard risk assessment:

- 10 m unsprayed buffer zone to non-agricultural land combined with 50% drift reduction,
- 5 m unsprayed buffer zone to non-agricultural land combined with 75% drift reduction

2. Probabilistic risk assessment:

- 5 m unsprayed buffer zone to non-agricultural land, or
- 75% drift reduction.

Concerned Member States must decide on applicability of proposed risk mitigation measures in their countries.

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

No effects on other terrestrial organisms are anticipated if the previously proposed risk mitigations are implemented during applications of GF-4021 in winter oilseed rape.

9.1.2 Grouping of intended uses for risk assessment

In the Central Zone the intended use of GF-4021 in winter oilseed rape is at one application of the maximum rate (0.25 L product/ha). Therefore the risk assessment has been based on one application of the maximum proposed rate and no grouping of uses is necessary.

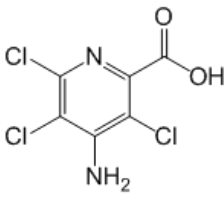
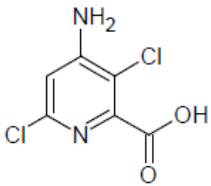
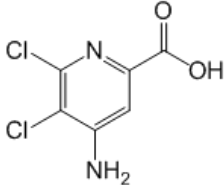
9.1.3 Consideration of metabolites

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-4021 is indicated in the tables.

Table 9.1-2 Metabolites of halauxifen-methyl relevant to the exposure assessment

Substance	Molar mass	Chemical structure	Maximum observed in compartments	Risk assessment required?
Halauxifen methyl (XDE-729 methyl X11393728)	345		Not Applicable	Yes, all compartments
Halauxifen acid (XDE-729 acid or X11393729)	331		Hydrolysis: 13.0% (pH 7), 99.3% (pH 9) at 25°C Aqueous photolysis: 10.7% Aerobic Soil: 72.7% Water/Sediment Water Phase: 20.0% Water/Sediment Total System: 23.5%	Yes, aquatic, sediment and soil organisms
X-757 (X11449757)	317		Aerobic Soil: 17.4% Water/Sediment Water Phase: 48.3% Water/Sediment Sediment Phase: 50.6% Water/Sediment Total System: 76.7%	Yes, aquatic, sediment and soil organisms
X-790 (X11406790)	331		Water/Sediment Water Phase: 16.5% Water/Sediment Sediment Phase: 10.6% Water/Sediment Total System: 33.4%	Yes, aquatic and sediment organisms
Deg 10	326		Aqueous photolysis: 12.6%	Yes, aquatic organisms
Deg 11	273		Aqueous photolysis: 15.7%	Yes, aquatic organisms
Deg 14	229		Aqueous photolysis: 11.5%	Yes, aquatic organisms

Table 9.1-3: Metabolites of picloram relevant for the exposure assessment

Substance	Molar mass	Chemical structure	Maximum observed in compartments	Risk assessment required?
Picloram	241.5		Not Applicable	Yes, all compartments
3,6-dichloro analogue of picloram (aminopyralid)	207		Water/Sediment Water Phase: 8.7% Water/Sediment Sediment Phase: 5.2% Water/Sediment Total System: 11.0%	Yes, aquatic and sediment organisms
5,6-dichloro analogue of picloram	207		Water/Sediment Water Phase: 1.1% Water/Sediment Sediment Phase: 19.0% Water/Sediment Total System: 22.1%	Yes, aquatic and sediment organisms

There are no metabolites of aminopyralid >5% AR.

zRMS comments:

Information regarding metabolites of halauxifen-methyl, picloram and aminopyralid is in general in line with EU agreed endpoints reported in EFSA Journal 2014;12(12):3913, EFSA Journal 2009;7(12):1390, and EFSA Journal 2013;11(9):3352, respectively.

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with halauxifen-methyl, picloram and aminopyralid. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on birds of GF-4021 were not conducted in accordance with EU data requirements. Endpoints for the formulation were calculated based on the active substances. The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

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

Species	Substance	Exposure System	Results	Reference
<i>Colinus virginianus</i>	Halauxifen-methyl	Oral 1 d acute	LD ₅₀ > 2250 mg/kg bw LD ₅₀ = 4248 mg a.s./kg bw (extrapolated)	EFSA Conclusion, 2014. (... /2011/ DAS 090026)
<i>Poephila guttata</i>	Halauxifen-methyl	Oral 1 d acute	LD ₅₀ > 2250 mg/kg bw LD ₅₀ = 4248 mg a.s./kg bw (extrapolated)	EFSA Conclusion, 2014. (... /2011/ DAS 090027)
<i>Colinus virginianus</i>	Halauxifen-methyl	Dietary 8 d short-term	LD ₅₀ > 1328 mg/kg bw/d LC ₅₀ = > 5260 mg/kg feed	EFSA Conclusion, 2014. (... /2011/DAS 090028)
<i>Anas platyrhynchos</i>	Halauxifen-methyl	Dietary 8 d short-term	LD ₅₀ > 2088 mg/kg bw/d LC ₅₀ > 5260 mg/kg feed	EFSA Conclusion, 2014. (... /2011/ DAS 090029)
<i>Colinus virginianus</i>	Halauxifen-methyl	Dietary reproductive toxicity	NOAEL = 36.9 mg/kg bw/d NOEC = 400 mg/kg feed	EFSA Conclusion, 2014. .../ 2011/ DAS 101137)
<i>Anas platyrhynchos</i>	Halauxifen-methyl	Dietary reproductive toxicity	NOAEL = 160.5 mg/kg bw/d NOEC = 1000 mg/kg feed	EFSA Conclusion, 2014. (... /2011/ DAS 101139)

EFSA Journal 2014;12(12):3913

According to EFSA/2009/1438, avian dietary (short term) risk assessments are only necessary on occasions when a dietary LD₅₀ (expressed in terms of a daily dose) is lower than the corresponding acute oral LD₅₀. In the case of halauxifen-methyl, the short-term LDD₅₀ (> 1328 mg a.s./kg bw/d) appears to be lower than the corresponding acute oral LD₅₀ (> 2250 mg a.s./kg bw), but this is an artifact that has arisen because both endpoints are greater-than values which exceed the highest dose administered in the respective studies. The acute toxicity endpoint should therefore be used in the risk assessment and a short-term risk assessment is not required.

According to EFSA/2009/1438, it is permissible to extrapolate an LD₅₀ value upwards in cases where there is no mortality or a single mortality at a limit dose in acute avian toxicity study. The endpoint was extrapolated endpoint based on no mortality in the acute bird study in accordance with EFSA/2009/1438. In the study with halauxifen-methyl, ten individuals were used per dose group in this study, so an extrapolation factor of 1.888 is appropriate and the resulting estimated acute LD₅₀ is 4248 mg a.s./kg bw.

According to EFSA/2009/1438, if the acute LD₅₀/10 is lower than the reproductive NOEL, then the acute LD₅₀/10 should be used in the long-term risk assessment. In the case of halauxifen-methyl, the

LD₅₀/10 is 424.8 mg a.s./kg bw/d which is not lower than the reproductive measured NOAEL 36.9 mg a.s./kg bw/d, therefore, the NOAEL is used in the long-term risk assessment.

Table 9.2-2: Endpoints and effect values relevant for the risk assessment for birds for picloram

Species	Substance	Exposure System	Results	Reference
<i>Anas platyrhynchos</i> (mallard duck)	picloram (as potassium salt)	Oral 14 d acute	LD ₅₀ >1944 mg a.e./kg bw (>2250 mg/kg bw as K-salt) LD ₅₀ = 3670.3 mg a.e./kg bw (extrapolated)	EFSA Conclusion, 2009 (... 1985 /DAS Report No. ES-DR-0049-3936-5)
<i>Colinus virginianus</i> (bobwhite quail)	picloram (as potassium salt)	Dietary 8 d short-term	LDD ₅₀ > 1904 mg ae/kg bw/d (>2204 mg/kg bw/day as K-salt)	EFSA Conclusion, 2009 (... 1985 /DAS 103-244)
<i>Colinus virginianus</i> (bobwhite quail)	picloram	Dietary reproductive toxicity	NOEL = 65 mg a.e./kg bw/d (% eggs laid)	EFSA Conclusion 2009 (.../2002/DAS 011172)

EFSA Journal 2009; 7(12):1390

According to EFSA/2009/1438, it is permissible to extrapolate an LD₅₀ value upwards in cases where there is no mortality or a single mortality at a limit dose in acute avian toxicity study. The endpoint was extrapolated endpoint based on no mortality in the acute bird study in accordance with EFSA/2009/1438. In the study with picloram, ten individuals were used per dose group in this study, so an extrapolation factor of 1.888 is appropriate and the resulting estimated acute LD₅₀ is 3670.3 mg a.e./kg bw.

According to EFSA/2009/1438, if the acute LD₅₀/10 is lower than the reproductive NOEL, then the acute LD₅₀/10 should be used in the long-term risk assessment. In the case of halauxifen-methyl, the LD₅₀/10 is 367.03 mg a.e./kg bw/d which is not lower than the reproductive measured NOAEL 65 mg a.s./kg bw/d, therefore, the NOAEL is used in the long-term risk assessment.

Table 9.2-3: Endpoints and effect values relevant for the risk assessment for birds for aminopyralid

Species	Substance	Exposure System	Results	Reference
<i>Colinus virginianus</i> (bobwhite quail)	aminopyralid	Oral 14 d acute	LD ₅₀ >2250 mg a.s./kg bw LD ₅₀ is 4248 mg a.s./kg bw (extrapolated)	EFSA Conclusion, 2013 (... , /2001 /DAS 011046)
<i>Colinus virginianus</i> (bobwhite quail)	aminopyralid	Dietary 8 d short-term	LDD ₅₀ >1457 mg a.s./kg bw/day	EFSA Conclusion, 2013 (... , /2001 /DAS 011047)
<i>Colinus virginianus</i> (bobwhite quail)	aminopyralid	Dietary reproductive toxicity	NOEL = 190.23 mg a.s./kg bw/d (highest level tested)	EFSA Conclusion, 2013 (.../2003/DAS 011271)

EFSA Journal 2013;11(9): 3352

According to EFSA/2009/1438, it is permissible to extrapolate an LD₅₀ value upwards in cases where there is no mortality or a single mortality at a limit dose in acute avian toxicity study. The endpoint was extrapolated endpoint based on no mortality in the acute bird study in accordance with EFSA/2009/1438. In the study with aminopyralid, ten individuals were used per dose group in this study, so an extrapolation factor of 1.888 is appropriate and the resulting estimated acute LD₅₀ is 4248 mg a.e./kg bw.

According to EFSA/2009/1438, avian dietary (short term) risk assessments are only necessary on

occasions when a dietary LD₅₀ (expressed in terms of a daily dose) is lower than the corresponding acute oral LD₅₀. In the case of aminopyralid, the short-term LDD₅₀ (> 1457 mg/kg bw/d, Table 9.2-3) appears to be lower than the corresponding acute oral LD₅₀ (> 2250 mg/kg bw), but this is an artefact that has arisen because both endpoints are greater-than values which exceed the highest dose administered in the respective studies. In the dietary bobwhite study seven treatment levels were tested (178, 316, 562, 1000, 1780, 3160 and 5620 mg/kg diet) and mortality was 0% in the control and all of the treatments. There were no clinical signs of toxicity noted at any of the concentrations tested and all birds were normal in appearance and behaviour throughout the test. When compared to the control group, there were no apparent treatment related effects on body weight among birds in any of the treatment groups at any body weight interval. In addition, there were no apparent treatment related effects on feed consumption at any of the concentrations tested. The short-term (8-day) LC50 value was determined to be >5620 mg/kg diet. The LD50 was >1457 mg/kg bw/day. The NOEC for mortality was 5620 mg/kg diet (NOEL = 1457 mg/kg bw/day). As no dose-related mortality or sub-lethal effects were observed in the dietary study for aminopyralid the acute LD₅₀ endpoint is therefore considered the most relevant for use in the acute risk assessment.

According to EFSA/2009/1438, if the acute LD₅₀/10 is lower than the reproductive NOEL, then the acute LD₅₀/10 should be used in the long-term risk assessment. In the case of aminopyralid, the LD₅₀/10 is 424.8 mg a.e./kg bw/d which is not lower than the reproductive measured NOAEL 190.23 mg a.e./kg bw/d, therefore, the NOAEL is used in the long-term risk assessment.

zRMS comments:

Avian toxicity data for halauxifen-methyl, picloram and aminopyralid provided in Tables 9.2-1 to 9.2-3 are in line with EU agreed endpoints reported in EFSA Journal 2014;12(12):3913, EFSA Journal 2009;7(12):1390, and EFSA Journal 2013;11(9):3352, respectively.

Since no mortality was observed in all studies and 10 birds were used in each test, it is justified to apply the extrapolation factor of 1.888 to the endpoints, in line with EFSA (2009).

Administration of the active compounds in the diet have not induced increased mortality and for this reason the acute risk assessment may be based on LD₅₀ values.

Endpoints derived from the reproductive toxicity studies are relevant for purposes of the long-term risk assessment, since LD₅₀/10 are higher than the NOEL values.

Combination toxicity assessment

The acute toxicity to birds has been estimated assuming dose additivity of the single active substances in the formulation with the following equation (EFSA Journal 2009; 7(12): 1438):

$$LD_{50}(mix) = \left(\sum_i \frac{X(a.s._i)}{LD_{50}(a.s._i)} \right)^{-1}$$

where: X(a.s.i) is the fraction of the active substance i in the mixture (the sum Σ(a.s.i) must be 1)
LD₅₀(a.s.i) is the acute toxicity for the active substance i.

Table 9.2-4: Acute combination toxicity endpoints of halauxifen-methyl, picloram and aminopyralid calculated from active substances toxicity endpoints of birds

	Halauxifen-methyl	Picloram	Aminopyralid
Content in the formulation GF-4021 (% w/w)	1.06%	5.07%	3.38%
Fraction in the a.s. mixture	11.15%	53.31%	35.54%
LD ₅₀ of a.s. [mg/kg bw]	4248	3670.3	4248
Fraction / LD ₅₀	0.00003	0.00015	0.00008
Sum	0.0003		
1/ sum = predicted LD ₅₀ (mix)	3919.13 mg mix/kg bw		
Contribution of the active to predicted toxicity	10.28%	56.93%	32.79%

None of the active substances are clearly driving the toxicity of the formulation (i.e. contribute to more than 90% of the toxicity), therefore, the predicted LD₅₀ (mix) of 3919.13 mg/kg bw and the sum of the application rates of the active substances (0.0225 kg/ha) will be used in the acute risk assessment of the mixture.

~~According to EFSA/2009/1438, it is currently not recommended to predict toxicity values for long-term reproductive effects of formulations containing more than one active substance. As a chronic exposure to the formulation is unlikely, it is more appropriate to address the long-term risk from the individual active substances. However, according to the Central Zone requirements a long-term combination assessment is provided following the concentration-addition model and guidance in the EFSA/2009/1438 Appendix B.~~

~~**Table 9.2-5: Long-term combination toxicity endpoints of halauxifen-methyl, picloram and aminopyralid calculated from active substances toxicity endpoints of birds**~~

	Halauxifen-methyl	Picloram	Aminopyralid
Content in the formulation GF-4021 (% w/w)	1.06%	5.07%	3.38%
Fraction in the a.s. mixture	11.15%	53.31%	35.54%
NOEL of a.s. [mg/kg bw]	36.9	65	190.23
Fraction / NOEL	0.00302	0.00820	0.00187
Sum	0.0131		
1/ sum = predicted NOEL (mix)	76.39 mg mix/kg bw		
Contribution of the active to predicted toxicity	23.07%	62.65%	14.27%

~~None of the active substances are clearly driving the toxicity of the formulation (i.e. contribute to more than 90% of the toxicity), therefore, the predicted NOEL (mix) of 76.39 mg/kg bw and the sum of the application rates of the active substances (0.0225 kg/ha) will be used in the long-term risk assessment of the mixture.~~

zRMS comments:

The LD₅₀mix calculated by the Applicant in Table 9.2-4 is agreed by the zRMS. Some minor differences between Applicants' (3919.13 mg/kg bw) and zRMS calculations (3920.9 mg/kg bw) are result of the rounding procedure.

In line with EFSA (2009), concentration addition approach is not relevant for the long-term endpoints which are based on effects on different parameters. Taking this into account, calculation of the NOELmix provided by the Applicant above is struck through and the combined long-term risk assessment will be addressed using simplified approach with calculation of the TERmix, as commonly agreed at the Central Zone level for formulations containing multiple active substances.

9.2.1.1 Justification for new endpoints

According to EFSA/2009/1438, it is permissible to extrapolate an LD₅₀ value upwards in cases where there is no mortality or a single mortality at a limit dose in acute avian toxicity study. The endpoints for halauxifen-methyl, aminopyralid and picloram were extrapolated based on no mortality in the acute bird study in accordance with EFSA/2009/1438.

zRMS comments:

Extrapolation procedure is in line with the guidance document and is not considered to generate new active substance endpoints, since extrapolation factor is applied to the EU agreed endpoints.

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 (EFSA Journal 2009; 7(12): 1438) hereafter referred to as EFSA/2009/1438. There is no requirement for the calculation of TER_{st} (short-term) for birds under the EFSA birds and mammals guidance document (EFSA Journal 2009; 7(12): 1438) and, consequently, a risk assessment for short-term toxicity has not been conducted.

9.2.2.1 First-tier assessment (screening)

Table 9.2-6: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of GF-4021 in winter oilseed rape – halauxifen-methyl

Intended use	Winter oilseed rape				
Active substance/product	Halauxifen-methyl				
Application rate (g/ha)	1 × 2.5				
Acute toxicity (mg/kg bw)	4248 (extrapolated endpoint)				
TER criterion	10				
Crop scenario Growth stage	Indicator species for screening	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Oilseed rape	Small omnivorous bird	158.8	1	0.397	10700
Reprod. toxicity (mg/kg bw/d)	36.9				
TER criterion	5				
Crop scenario Growth stage	Indicator species for screening	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Oilseed rape	Small omnivorous bird	64.8	1 x 0.53	0.086	429

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.

Table 9.2-7: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of GF-4021 in winter oilseed rape – picloram

Intended use	Winter oilseed rape				
Active substance/product	Picloram				
Application rate (g/ha)	1 × 12				
Acute toxicity (mg/kg bw)	3670.3 (extrapolated value)				
TER criterion	10				
Crop scenario Growth stage	Indicator species for screening	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Oilseed rape	Small omnivorous bird	158.8	1	1.91	1926
Reprod. toxicity (mg/kg bw/d)	65				
TER criterion	5				
Crop scenario Growth stage	Indicator species for screening	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_t
Oilseed rape	Small omnivorous bird	64.8	1 x 0.53	0.412	157.72

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.

Table 9.2-8: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of GF-4021 in winter oilseed rape - aminopyralid

Intended use	Winter oilseed rape				
Active substance/product	Aminopyralid				
Application rate (g/ha)	1 × 8				
Acute toxicity (mg/kg bw)	4248 (extrapolated value)				
TER criterion	10				
Crop scenario Growth stage	Indicator species for screening	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Oilseed rape	Small omnivorous bird	158.8	1	1.27 1.23	3343.8
Reprod. toxicity (mg/kg bw/d)	190.23				
TER criterion	5				
Crop scenario Growth stage	Indicator species for screening	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_t
Oilseed rape	Small omnivorous bird	64.8	1 x 0.53	0.275	692.4

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.

Table 9.2-9: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of GF-4021 in winter oilseed rape.

Intended use	Winter oilseed rape				
Active substance/product	GF-4021				
Application rate (g/ha)	1 × 22.5 (sum of a.s. application rates)				
Acute toxicity (mg/kg bw)	3919.13 (predicted value)				
TER criterion	10				
Crop scenario Growth stage	Indicator species for screening	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Oilseed rape	Small omnivorous bird	158.8	1	3.57	1097
Reprod. toxicity (mg/kg bw/d)	76.39 (predicted value)				
TER criterion	5				
Crop scenario Growth stage	Indicator species for screening	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Oilseed rape	Small omnivorous bird	64.8	1 × 0.53	1.46	52.3

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 TER_a and TER_{lt} values exceed the trigger of 10 and 5, respectively, indicating acceptable acute and chronic risks to birds from halauxifen-methyl, picloram, aminopyralid and GF-4021 following application at the proposed label rates.

zRMS comments:

The acute and long-term dietary risk assessment provided in tables above is agreed by the zRMS with exception of the long-term combined risk assessment, which was based on the estimated NOEL_{mix}, while the CA approach is not relevant for the long-term endpoints. For this reason the TER_{mix} was calculated by the zRMS, as agreed at the Central Zone level, however rather for formal reasons taking into account that the long-term TER for individual active compounds were far above the trigger. Results are presented below.

Compound						Σ1/TER	Σ1/TER ⁻¹	Trigger
Halauxifen-methyl		Picloram		Aminopyralid				
TER	1/TER	TER	1/TER	TER	1/TER			
429	0.00233	157.7	0.00634	692.4	0.00144	0.01012	98.8	5

Based on Applicants' and zRMS calculations, acceptable acute and long-term dietary risk to birds from exposure to halauxifen-methyl, picloram, aminopyralid and their mixture may be concluded following application of GF-4021 according to the intended Central Zone use pattern.

No metabolites were included in the risk assessment performed at the EU level for individual compounds and the same is applicable for evaluation of GF-4021.

9.2.2.2 Higher-tier risk assessment

Since acceptable acute and long-term risks have been concluded for birds exposed to halauxifen-methyl, picloram and aminopyralid at the screening level, a higher-tier risk assessment is not required for the proposed uses of GF- 4021.

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-4021 is not intended to be applied on crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not need 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 $K(f)_{oc}$ of 995 L/kg, halauxifen-methyl belongs to the group of more sorptive substances.

Effective application rate (g/ha) =	1 x 2.5			Trigger
Acute toxicity (mg/kg bw) =	4248	Quotient =	0.0006	3000
Reprod. toxicity (mg/kg bw/d) =	36.9	Quotient =	0.068	3000

With a $K(f)_{oc}$ of 19.6 L/kg, picloram belongs to the group of less sorptive substances.

Effective application rate (g/ha) =	1 x 12			Trigger
Acute toxicity (mg/kg bw) =	3670.3	Quotient =	0.003	50
Reprod. toxicity (mg/kg bw/d) =	65	Quotient =	0.185	50

With a $K(f)_{oc}$ of 5.14 L/kg, aminopyralid belongs to the group of less sorptive substances.

Effective application rate (g/ha) =	1 x 8			Trigger
Acute toxicity (mg/kg bw) =	4248	Quotient =	0.002	50
Reprod. toxicity (mg/kg bw/d) =	190.23	Quotient =	0.042	50

Since the ratios of effective application rate (g/ha) to relevant endpoint (mg/kg bw/d) do not exceed the critical value of 3000 or 50, a quantitative risk assessment (calculation of TER values) for halauxifen-methyl or picloram and aminopyralid, respectively, is not necessary.

zRMS comments:

The drinking water risk assessment performed for particular active substances above is agreed by the zRMS.

It is noted that the evaluation should also include pertinent soil metabolites of the active compounds. No relevant soil metabolites are formed from picloram and aminopyralid. However, halauxifen-methyl forms 2 relevant soil metabolites: halauxifen acid and X-757, which should be taken into account in the drinking water risk assessment. Comparison of the effective rate with toxicity endpoints for the parent resulted with very low quotients (0.0006 and 0.068 for acute and long-term risk, respectively). Taking this into account, with the worst case assumptions taken for metabolites (i.e. 10 times toxicity of the parent and parent application rate not adjusted for the maximum occurrence in soil and the molar ratio) the quotients would be 10 times higher (i.e. 0.006 and 0.68 for acute and long-term risk, respectively), i.e. considerably below 50 (trigger relevant for both metabolites due to $K_{foc} < 500$ mL/g) indicating acceptable risk to birds exposed to halauxifen acid and X-757 via the drinking water. Hence, further calculations are deemed not necessary.

Overall, no unacceptable risk to birds from exposure via drinking water is anticipated following uses of GF-4021 in line with the Central Zone GAP.

9.2.2.4 Effects of secondary poisoning

The log K_{ow} of halauxifen-methyl is 3.76 and thus exceeds the trigger value of 3, therefore, a risk assessment for effects due to secondary poisoning is required. The log K_{ow} of picloram is -1.92 at pH 7 at 20°C and log K_{ow} of aminopyralid is -2.87, thus do not exceed the trigger value of 3, therefore, a risk assessment for effects due to secondary poisoning not 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 the predicted concentrations in soil.

Table 9.2-10: Assessment of the risk for earthworm-eating birds due to exposure to halauxifen-methyl via bioaccumulation in earthworms (secondary poisoning) for the intended use in winter oilseed rape.

Parameter	Halauxifen-methyl	Comments
PEC _{soil} (21d TWA) (mg/kg)	0.0017	Section 8 Table 8.7-5
log P _{ow} / P _{ow}	3.76 / 5754	EFSA Conclusion, 2014
K _{oc}	995	Mean (n = 7, EFSA, 2014)
f _{oc}	0.02	Default
BCF _{worm}	3.51	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times P_{ow}) / f_{oc} \times K_{oc}$
PEC _{worm}	0.01	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.01	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	36.9	EFSA Conclusion, 2014
TER _{it}	5 886	

TER values shown in bold fall below the relevant trigger.

The TER_{it} for the assessment of the risk for worm-eating birds due to halauxifen-methyl exceeds the trigger TER value of 5, indicating acceptable risk to birds following applications of halauxifen-methyl to winter oilseed rape.

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.

Table 9.2-11: Assessment of the risk for fish-eating birds due to exposure to halauxifen-methyl via bioaccumulation in fish (secondary poisoning) for the intended use in winter oilseed rape.

Parameter	Halauxifen-methyl	comments
PEC _{sw} (STEP 1) (mg/L)	0.00043 0.00001595	Section 8, D2-ditch Table 8.9-7
BCF _{fish}	217	EFSA Conclusion, 2014 DAS 101135.
BMF	N/A	biomagnification factor (relevant for BCF ≥ 2000)
TWA	0.53	default value, EFSA Journal 2009; 7(12):1438
PEC _{fish}	0.0495 0.0018	$PEC_{fish} = PEC_{water} \times BCF_{fish} \times TWA$
Daily dietary dose (mg/kg bw/d)	0.0079 0.0003	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	36.9	EFSA Conclusion, 2014
TER _{it}	4693 126,512	

Since the maximum PEC_{sw} value is used, the equation for calculating PEC_{fish} includes a multiplication

by the TWA (default 0.53) in accordance with the EFSA/2009/1438. EFSA Journal 2014;12(12):3913
TER values shown in bold fall below the relevant trigger.

The TER_{lt} for the assessment of the risk for fish-eating birds due to halauxifen-methyl exceeds the trigger TER value of 5, indicating acceptable risk to birds following applications of halauxifen-methyl to winter oilseed rape.

zRMS comments:

Although the presented above evaluation of the risk of secondary poisoning for halauxifen-methyl was performed correctly, the calculations for fish-eating birds were amended by the zRMS with consideration of Step 1 PEC_{SW} for convenience of the cMS that do not accept Step 3 FOCUS modelling.

Evaluation of the risk of secondary poisoning was not triggered for picloram, aminopyralid (being also 3,6-analogue of picloram) and relevant aquatic metabolites of halauxifen-methyl (halauxifen acid, X-757 and X-790) due to log Pow being all <3. No information on log Pow of relevant aquatic photoproducts of halauxifen-methyl (Deg 10, Deg 11 and Deg 14) as well as 5,6-dichloro analogue of picloram is available, however in the course of the EU review these compounds were not included in the evaluation of the risk of secondary poisoning and the same conclusion is applicable for the zonal evaluation of GF-4021. No relevant surface water metabolites are formed from aminopyralid.

Overall, acceptable risk of secondary poisoning is concluded from the intended Central Zone uses of GF-4021.

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

Regulatory testing has been conducted with halauxifen-methyl, picloram and aminopyralid in accordance with EU requirements. The acute ~~and chronic~~ risks of GF-4021 to birds was assessed based on the predicted toxicity endpoint and maximum predicted exposure based on the sum of the application rates of the active substances. To address the long-term combined risk the TER_{mix} was calculated, as agreed in the Central Zone. ~~Acceptable acute and long-term risk is concluded based on the intended uses in winter oilseed rape.~~

For the active substances ~~and the mixture~~, the TERs calculated in the screening ~~and first-tier~~ assessment all exceed the trigger values of 10 and 5 for acute and long-term risk, respectively, indicating acceptable risk to birds from application of GF-4021 according to the proposed **Central Zone** use pattern.

For halauxifen-methyl an acceptable risk from secondary poisoning to earthworm and fish-eating birds was shown. Due to the low potential for bioaccumulation (log Pow < 3) the risk of secondary poisoning from ~~halauxifen-methyl metabolites~~, picloram and aminopyralid is considered to be low.

Furthermore, the risk assessment for exposure *via* drinking water also showed acceptable risk for the active substances ~~and their pertinent soil metabolites~~.

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 halauxifen-methyl, picloram, picloram TIPA salt and aminopyralid. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on mammals of GF-4021 were not conducted in accordance with EU data requirements. Endpoints for the formulation were calculated based on the active substances. The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

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

Species	Substance	Exposure System	Results	Reference
Rat	Halauxifen-methyl	Oral 1 d Acute	LD ₅₀ > 5000 mg/kg bw	EFSA Conclusion, 2014 (.../2011/ DAS 110543)
Rabbit	Halauxifen methyl	Dietary Developmental toxicity	NOAEL = 5.78 mg/kg bw/d	EFSA Conclusion, 2014 (.../2012/DAS 111137)

EFSA Journal 2014;12(12):3913

Table 9.3-2: Endpoints and effect values relevant for the risk assessment for mammals – picloram

Species	Substance	Exposure System	Results	Reference
Rat	picloram	Oral 1 d Acute	LD ₅₀ = 4012 mg ae/kg bw	EFSA Conclusion 2009 (.../1987/DAS Report No. K-038323-042A)
Rabbit	Picloram (as TIPA salt)	Oral Developmental toxicity	NOAEL = 300 mg a.e./kg bw/day	EFSA Conclusion 2009 (...1992/DAS K-049877-015)

EFSA Journal 2009; 7(12):1390

Table 9.3-3: Endpoints and effect values relevant for the risk assessment for mammals – aminopyralid

Species	Substance	Exposure System	Results	Reference
Rat	aminopyralid	Oral 1 d Acute	LD ₅₀ >5000 mg ae/kg bw	EFSA Conclusion 2013 (.../2001/DAS 011115)
Rabbit	aminopyralid	Oral Developmental toxicity	NOAEL = 26 mg ae/kg bw/day (Reduced body weight and delayed ossification)	EFSA Conclusion 2013 (.../2004/ 1992/DAS 031142)

EFSA Journal 2013;11(9): 3352

zRMS comments:

Mammalian toxicity data for halauxifen-methyl, picloram and aminopyralid provided in Tables 9.3-1 to 9.3-3 are in line with EU agreed endpoints reported in EFSA Journal 2014;12(12):3913, EFSA Journal 2009;7(12):1390, and EFSA Journal 2013;11(9):3352, respectively.

It is noted that in line with EFSA conclusions for halauxifen-methyl, the NOAEL of 5.78 mg a.s./kg bw/d was used to set ADI and for this reason is not a true reflection of the reproductive toxicity. For this reason refinement of this endpoint is possible in case unacceptable long-term risk is demonstrated.

For aminopyralid the NOAEL of 26 mg ae/kg bw/d is indicated to be relevant for the screening risk assessment, while for Tier 1 evaluation higher endpoints of 256 mg ae/kg bw/d is reported. In evaluation performed for GF-4021 the lower value was used as representing worst case.

Combination toxicity assessment

The acute toxicity to mammals has been estimated assuming dose additivity of the single active substances in the formulation with the following equation (EFSA Journal 2009; 7(12): 1438):

$$LD_{50}(mix) = \left(\sum_i \frac{X(a.s._i)}{LD_{50}(a.s._i)} \right)^{-1}$$

where: $X(a.s._i)$ is the fraction of the active substance i in the mixture (the sum $\Sigma(a.s._i)$ must be 1)
 $LD_{50}(a.s._i)$ is the acute toxicity for the active substance i .

Table 9.3-4: Acute combination toxicity endpoints of halauxifen-methyl, picloram and aminopyralid calculated from active substances toxicity endpoints of mammals

	Halauxifen-methyl	Picloram	Aminopyralid
Content in the formulation GF-4021 (% w/w)	1.06%	5.07%	3.38%
Fraction in the a.s. mixture	11.15%	53.31%	35.54%
LD ₅₀ of a.s. [mg/kg bw]	>5000	4012	>5000
Fraction / LD ₅₀	0.00002	0.00013	0.00007
Sum	0.0002		
1/ sum = predicted LD ₅₀ (mix)	4419.7 mg mix/kg bw		
Contribution of the active to predicted toxicity	9.85%	58.73%	31.42%

None of the active substances are clearly driving the toxicity of the formulation (i.e. contribute to more than 90% of the toxicity), therefore, the predicted LD₅₀ (mix) of 4419.7 mg mix/kg bw and the sum of the application rates of the active substances (0.0225 kg/ha) will be used in the acute risk assessment of the mixture.

According to EFSA/2009/1438, it is currently not recommended to predict toxicity values for long-term reproductive effects of formulations containing more than one active substance. As a chronic exposure to the formulation is unlikely, it is more appropriate to address the long term risk from the individual active substances. However, according to the Central Zone requirements a long term combination assessment is provided following the concentration addition model and guidance in the EFSA/2009/1438 Appendix B.

Table 9.3-5: Long term combination toxicity endpoints of halauxifen-methyl, picloram and aminopyralid calculated from active substances toxicity endpoints of mammals

	Halauxifen-methyl	Picloram	Aminopyralid
Content in the formulation GF-4021 (% w/w)	1.06%	5.07%	3.38%
Fraction in the a.s. mixture	11.15%	53.31%	35.54%
NOEL of a.s. [mg/kg bw]	5.78	300	26
Fraction / NOEL	0.01928	0.00178	0.01367
Sum	0.0347		
1/ sum = predicted NOEL (mix)	28.8 mg mix/kg bw		
Contribution of the active to predicted toxicity	55.52%	5.12%	39.36%

None of the active substances are clearly driving the toxicity of the formulation (i.e. contribute to more than 90% of the toxicity), therefore, the predicted NOEL (mix) of 28.8 mg/kg bw and the sum of the application rates of the active substances (0.0225 kg/ha) will be used in the long term risk assessment of the mixture.

zRMS comments:

The LD₅₀mix calculated by the Applicant in Table 9.3-4 is agreed by the zRMS. Some minor differences between Applicants' (4419.7 mg/kg bw) and zRMS calculations (4422.8 mg/kg bw) are result of the rounding procedure.

In line with EFSA (2009), concentration addition approach is not relevant for the long-term endpoints which are based on effects on different parameters. Taking this into account, calculation of the NOELmix provided by the Applicant above is struck through and the combined long-term risk assessment will be addressed using simplified approach with calculation of the TERmix, as commonly agreed at the Central Zone level for formulations containing multiple active substances.

9.3.1.1 Justification for new endpoints

Not relevant.

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 (EFSA Journal 2009; 7(12): 1438) hereafter referred to as EFSA/2009/1438. There is no requirement for the calculation of TER_{st} (short-term) for birds under the EFSA birds and mammals guidance document (EFSA Journal 2009; 7(12): 1438) and, consequently, a risk assessment for short-term toxicity has not been conducted.

9.3.2.1 First-tier assessment (screening)

Table 9.3-6: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of GF-4021 in winter oilseed rape- halauxifen-methyl

Intended use	Winter oilseed rape				
Active substance/product	Halauxifen-methyl				
Application rate (g/ha)	1 × 2.5				
Acute toxicity (mg/kg bw)	> 5000				
TER criterion	10				
Crop scenario	Indicator species for screening	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Growth stage					
Oilseed rape	Small herbivorous mammal	118.4	1	0.296	>16892
Reprod. toxicity (mg/kg bw/d)	5.78				
TER criterion	5				
Crop scenario	Indicator species for screening	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Growth stage					
Oilseed rape	Small herbivorous mammal	48.3	1 x 0.53	0.064	90

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.

Table 9.3-7: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of GF-4021 in winter oilseed rape – picloram

Intended use	Winter oilseed rape				
Active substance/product	Picloram				
Application rate (g/ha)	1 × 12				
Acute toxicity (mg/kg bw)	4012				
TER criterion	10				
Crop scenario Growth stage	Indicator species for screening	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
N/A	Small herbivorous mammal	118.4	1	1.421	2824
Reprod. toxicity (mg/kg bw/d)	300				
TER criterion	5				
Crop scenario Growth stage	Indicator species for screening	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{tt}
N/A	Small herbivorous mammal	48.3	1 x 0.53	0.307	977

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.

Table 9.3-8: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of GF-4021 in winter oilseed rape - aminopyralid

Intended use	Winter oilseed rape				
Active substance/product	Aminopyralid				
Application rate (g/ha)	1 × 8				
Acute toxicity (mg/kg bw)	>5000				
TER criterion	10				
Crop scenario Growth stage	Indicator species for screening	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
N/A	Small herbivorous mammal	118.4	1	0.9472	> 5279
Reprod. toxicity (mg/kg bw/d)	26				
TER criterion	5				
Crop scenario Growth stage	Indicator species for screening	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{tt}
N/A	Small herbivorous mammal	48.3	1 x 0.53	0.205	127

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.

Table 9.3-9: Screening assessment of the acute and chronic risk for mammals due to the use of GF-4021 in winter oilseed rape.

Intended use	Winter oilseed rape				
Active substance/product	GF-4021				
Application rate (g/ha)	1 × 22.5 (sum of a.s. application rates)				
Acute toxicity (mg/kg bw)	4419.7(estimated)				
TER criterion	10				
Crop scenario Growth stage	Indicator species for screening	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
N/A	Small herbivorous mammal	118.4	1	2.66	1659
Reprod. toxicity (mg/kg bw/d)	28.8 (estimated)				
TER criterion	5				
Crop scenario Growth stage	Indicator species for screening	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{tt}
N/A	Small herbivorous mammal	48.3	1 × 0.53	0.58	50

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.

zRMS comments:

The acute and long-term dietary risk assessment provided in tables above is agreed by the zRMS with exception of the long-term combined risk assessment, which was based on the estimated NOEL_{mix}, while the CA approach is not relevant for the long-term endpoints. For this reason the TER_{mix} was calculated by the zRMS, as agreed at the Central Zone level, however rather for formal reasons taking into account that the long-term TER for individual active compounds were far above the trigger. Results are presented below.

Compound						Σ1/TER	Σ1/TER ⁻¹	Trigger
Halauxifen-methyl		Picloram		Aminopyralid				
TER	1/TER	TER	1/TER	TER	1/TER			
90	0.01111	977	0.00102	127	0.00787	0.02001	50.0	5

Based on Applicants' and zRMS calculations, acceptable acute and long-term dietary risk to mammals from exposure to halauxifen-methyl, picloram, aminopyralid and their mixture may be concluded following application of GF-4021 according to the intended Central Zone use pattern.

No metabolites were included in the risk assessment performed at the EU level for individual compounds and the same is applicable for evaluation of GF-4021.

9.3.2.2 Higher-tier risk assessment

Since acceptable acute and long-term risks have been concluded for mammals exposed to halauxifen-methyl, picloram and aminopyralid at the Tier 1 level, a higher tier risk assessment is not required for the proposed uses of GF-4021.

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 $K(f)_{oc}$ of 995 L/kg, halauxifen-methyl belongs to the group of more sorptive substances.

Effective application rate (g/ha) =	1 x 2.5			Trigger
Acute toxicity (mg/kg bw) =	>5000	Quotient =	<0.0005	3000
Reprod. toxicity (mg/kg bw/d) =	5.78	Quotient =	0.43	3000

With a $K(f)_{oc}$ of 19.6 L/kg, picloram belongs to the group of less sorptive substances.

Effective application rate (g/ha) =	1 x 12			Trigger
Acute toxicity (mg/kg bw) =	4012	Quotient =	0.003	50
Reprod. toxicity (mg/kg bw/d) =	300 30	Quotient =	0.04 0.4	50

With a $K(f)_{oc}$ of 5.14 L/kg, aminopyralid belongs to the group of less sorptive substances.

Effective application rate (g/ha) =	1 x 8			Trigger
Acute toxicity (mg/kg bw) =	>5000	Quotient =	<0.0016	50
Reprod. toxicity (mg/kg bw/d) =	26	Quotient =	0.31	50

Since the ratios of effective application rate (g/ha) to relevant endpoint (mg/kg bw/d) do not exceed the critical value of 3000 or 50, a quantitative risk assessment (calculation of TER values) for halauxifen-methyl or picloram and aminopyralid, respectively, is not necessary.

zRMS comments:

The drinking water risk assessment performed for particular active substances above is in general agreed by the zRMS with correction of the long-term drinking water risk assessment for picloram (most probably due to the typing error not correct endpoint was considered by the Applicant).

It is noted that the evaluation should also include pertinent soil metabolites of the active compounds. No relevant soil metabolites are formed from picloram and aminopyralid. However, halauxifen-methyl forms 2 relevant soil metabolites: halauxifen acid and X-757, which should be taken into account in the drinking water risk assessment. Comparison of the effective rate with toxicity endpoints for the parent resulted with very low quotients (<0.0005 and 0.43 for acute and long-term risk, respectively). Taking this into account, with the worst case assumptions taken for metabolites (i.e. 10 times toxicity of the parent and parent application rate not adjusted for the maximum occurrence in soil and the molar ratio) the quotients would be 10 times higher (i.e. 0.005 and 4.3 for acute and long-term risk, respectively), i.e. considerably below 50 (trigger relevant for both metabolites due to $K_{foc} < 500$ mL/g) indicating acceptable risk to mammals exposed to halauxifen acid and X-757 via the drinking water. Hence, further calculations are deemed not necessary.

Overall, no unacceptable risk to mammals from exposure via drinking water is anticipated following uses of GF-4021 in line with the Central Zone GAP.

9.3.2.4 Effects of secondary poisoning

The log K_{ow} of halauxifen-methyl is 3.76 and thus exceeds the trigger value of 3, therefore, a risk assessment for effects due to secondary poisoning is required. The log K_{ow} of picloram is -1.92 at pH 7 at 20°C and log K_{ow} of aminopyralid is -2.87, thus do not exceed the trigger value of 3, therefore, a

risk assessment for effects due to secondary poisoning not 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.

Table 9.3-10: Assessment of the risk for earthworm-eating mammals due to exposure to halauxifen-methyl via bioaccumulation in earthworms (secondary poisoning) for the intended use in winter oilseed rape.

Parameter	Halauxifen-methyl	Comments
PEC _{soil} (TWA = 21 d) (mg/kg)	0.0017	Section 8 Table 8.7-5
log P _{ow} / P _{ow}	3.76 / 5754	EFSA Conclusion, 2014
Koc	995	Mean (n = 7, EFSA, 2014)
foc	0.02	Default
BCF _{worm}	3.512	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC _{worm}	0.006	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.008	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	5.78	EFSA Conclusion, 2014
TER _{lt}	756	

TER values shown in bold fall below the relevant trigger.

Risk assessment for fish-eating mammals via secondary poisoning

According to EFSA/2013/3290, the risk for piscivorous mammals is assessed for a mammal of 3000 g body weight with a daily food consumption of 415 g fish/day, which gives a multiplication factor of 0.138.

Table 9.3-11: Assessment of the risk for fish-eating mammals due to exposure to halauxifen-methyl via bioaccumulation in fish (secondary poisoning) for the intended use in winter oilseed rape.

Parameter	Halauxifen-methyl	comments
PEC _{sw} (STEP 1) (mg/L)	0.00043 0.00001595	Section 8, D2 ditch , Table 8.9-7
BCF _{fish}	217	EFSA Conclusion, 2014 (2011) DAS 101135.
BMF	N/A	biomagnification factor (relevant for BCF ≥ 2000)
TWA	0.53	default value, EFSA Journal 2009; 7(12):1438
PEC _{fish}	0.0495 0.0018	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.007 0.0005	$DDD = PEC_{fish} \times 0.142$ 0.138
NOEL (mg/kg bw/d)	5.78	EFSA Conclusion, 2014
TER _{lt}	826 42,104	

TER values shown in bold fall below the relevant trigger.

The TER_{lt} for the assessment of the risk for fish-eating mammals due to halauxifen-methyl exposure via bioaccumulation in fish does not fall below the relevant trigger TER value of 5, indicating low risk to mammals following applications of halauxifen-methyl to winter oilseed rape.

zRMS comments:

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

Applicants' calculations for fish-eating mammals could not be reproduced by the zRMS (it seems that incorrect FIR/bw was used by the Applicant, but it is not clear what value was taken into account) and were thus corrected

by the zRMS. For convenience of the cMS that do not accept Step 3 FOCUS modelling, Step 1 PEC_{SW} was used.

Evaluation of the risk of secondary poisoning was not triggered for picloram, aminopyralid (being also 3,6-analogue of picloram) and relevant aquatic metabolites of halauxifen-methyl (halauxifen acid, X-757 and X-790) due to log Pow being all <3. No information on log Pow of relevant aquatic photoproducts of halauxifen-methyl (Deg 10, Deg 11 and Deg 14) as well as 5,6-dichloro analogue of picloram is available, however in the course of the EU review these compounds were not included in the evaluation of the risk of secondary poisoning and the same conclusion is applicable for the zonal evaluation of GF-4021. No relevant surface water metabolites are formed from aminopyralid.

Overall, acceptable risk of secondary poisoning is concluded from the intended Central Zone uses of GF-4021.

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

Regulatory testing has been conducted with halauxifen-methyl, picloram and aminopyralid in accordance with EU requirements. The acute ~~and long-term~~ risks of GF-4021 to mammals was assessed based on the predicted toxicity endpoint and maximum predicted exposure based on the sum of the application rates of the active substances. To address the long-term combined risk the TER_{mix} was calculated, as agreed in the Central Zone. ~~Acceptable acute and long-term risk was concluded based on the intended uses in winter oilseed rape.~~

For the active substances ~~and the mixture~~, the TERs calculated in the screening ~~and first-tier~~ assessment all exceed the trigger values of 10 and 5 for acute and long-term risk, respectively, indicating acceptable risk to mammals from application of GF-4021 according to the proposed **Central Zone** use pattern.

For halauxifen-methyl an acceptable risk from secondary poisoning to earthworm and fish-eating mammals was shown. Due to the low potential for bioaccumulation (log Pow < 3) the risk of secondary poisoning from ~~halauxifen-methyl metabolites~~, picloram and aminopyralid is considered to be low.

Furthermore, the risk assessment for exposure *via* drinking water also showed acceptable risk for the active substances ~~and their pertinent soil metabolites~~.

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

According to the data requirements under regulation 1107/2009 (Commission Regulations (EU) 283/2013¹ and 284/2013²), the risk to amphibians and reptiles shall be addressed. However, there is no EU guidance or validated regulatory protocol yet available, neither on the type of the necessary regulatory testing nor on how to conduct a risk assessment for amphibians and reptiles. Accordingly, specific toxicity tests for amphibian and reptile species are not requested and therefore no data on reptiles and terrestrial amphibians are available for the halauxifen-methyl, picloram and aminopyralid. In the EU, there is no guidance or validated regulatory protocols yet available either on the type of regulatory testing necessary or how to conduct a risk assessment for amphibian and reptiles.

According to EFSA Journal 2013; 11(7): 3290, amphibians should be included in the aquatic and terrestrial risk assessment. In the absence of GLP studies, the assessment should be based on any existing relevant information (testing of amphibian is not recommended initially due to animal welfare reasons and to the absence of standard guidelines for amphibian testing). With regards to the aquatic risk assessment, several data analyses indicate that the risk assessment for aquatic organisms (and fish in particular) covers the risk assessment for aquatic phases of amphibians (Fryday and Thompson, 2009, 2012³; Weltje *et al.*, 2013⁴). Based on these extensive data reviews, it can be concluded that the acute and chronic risk to aquatic life stages of amphibians is covered by the currently requested and conducted risk assessment for aquatic organisms (see Section 9.5.2 in this document).

Acceptable risk acute risk to fish is shown for each of the active substances and formulation. As such no adverse effects or risks are expected for aquatic life stages of amphibians exposed to applications of GF 4021 at rates up to and including 1×0.25 L/ha.

With regards to the terrestrial vertebrate risk assessment, in the absence of a specific framework, the data and risk assessment for birds and mammals are considered an adequate surrogate for other terrestrial vertebrates. In the few cases where terrestrial stages of amphibians were tested in studies comparable to those on birds and mammals, amphibians were generally less sensitive than the latter two vertebrate groups (Tables 12 and 13 in Fryday and Thompson, 2012⁵). It can be concluded that the acute and chronic risk to terrestrial life stages of amphibians is covered by the current risk assessment for terrestrial vertebrates.

In the case of reptiles there is even less information available than for amphibians (see the review by Fryday and Thompson, 2009). The risk from dietary exposure can be assumed to be lower for reptiles than for birds and mammals (Fryday and Thompson 2009), because reptiles are poikilotherms (i.e. do not maintain a constant body temperature) and as a result, feeding activity will peak on warm days and will be zero during hibernation or on cold days. In contrast, birds and mammals will have to maintain a constant body temperature and, hence, will need to be active and feed every day (Fryday and Thompson 2009). There is no indication from 'read-across' that reptiles either could be particularly sensitive or would not be covered by the available vertebrate data and risk assessments.

¹ Commission Regulation (EU) No 283/2013 setting out data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. Official Journal of the European Union: 1st March 2013.

² Commission Regulation (EU) No 284/2013 setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. Official Journal of the European Union: 1st March 2013.

³ Fryday S. and Thompson H. (2009): Literature reviews on ecotoxicology of chemicals with a special focus on plant protection products. Lot 1. Exposure of reptiles to plant protection products. EFSA (CFT/EFSA/PPR/2008/01).

⁴ Weltje L., Simpson P., Gross M., Crane M., Wheeler J.R. (2013): Comparative acute and chronic sensitivity of fish and amphibians: a critical review of data. Environmental Toxicology and Chemistry, Vol. 32, No. 5, pp. 984-994

⁵ Fryday S. and Thompson, H. (2012): Toxicity of pesticides to aquatic and terrestrial life stages of amphibians and occurrence, habitat use and exposure of amphibian species in agricultural; Food and Environment research agency, UK

No overt toxicity has been observed in any of the avian and mammalian studies relevant for the ecotoxicological risk assessment. In addition, acceptable acute and long term risks were concluded for birds and mammals under the very conservative assumptions of the screening level approach with a high margin of safety. As such no adverse effects or risks are expected for reptiles and terrestrial amphibians exposed via applications of GF 4021 at rates up to and including 0.25 L prod/ha.

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.

Information provided by the Applicant above has been thus not validated by the zRMS and is struck through and shaded.

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 halauxifen-methyl, picloram, aminopyralid and their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents as well as in Appendix 2 of this document (new studies).

Effects on aquatic organisms of GF-4021 were not evaluated as part of the EU assessment of halauxifen-methyl, picloram or aminopyralid. 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 is in line with the results of the EU review process.

Table 9.5-1: Endpoints and effect values relevant for the risk assessment for aquatic organisms – halauxifen-methyl and major metabolites

Species	Substance	Exposure System	Results	Reference
Fish				
<i>Oncorhynchus mykiss</i>	Halauxifen-methyl	96 h, s	LC ₅₀ = 2.01 mg a.s./L _{nom}	EFSA conclusion 2014 (....2011 /DAS 090187)
<i>Pimephales promelas</i>	Halauxifen-methyl	96 h, s	LC ₅₀ > 3.22 mg a.s./L _{mm}	EFSA conclusion 2014 (....2011 /DAS 090186)
<i>Cyprinodon variegatus</i>	Halauxifen-methyl	96 h, s	LC ₅₀ > 1.33 mg a.s./L _{mm}	EFSA conclusion 2014 (...../2011 /DAS 090188)
<i>Oncorhynchus mykiss</i>	Halauxifen acid	96 h, s	LC ₅₀ > 107 mg metabolite/L _{mm}	EFSA conclusion 2014 (...../2011 /DAS 101152)
<i>Oncorhynchus mykiss</i>	X11449757	96 h, s	LC ₅₀ > 120 mg metabolite/L _{nom}	EFSA conclusion 2014 (..../2011 /DAS 101166)
<i>Oncorhynchus mykiss</i>	X11406790	96 h, s	LC ₅₀ > 30 mg metabolite/L _{nom}	EFSA conclusion 2014 (...../2011 /DAS 120020)
<i>Pimephales promelas</i>	Halauxifen-methyl	28 d ELS, ft	NOEC = 0.259 mg a.s./L _{mm}	EFSA conclusion 2014 (...../2011 /DAS 101134)
<i>Cyprinodon variegatus</i>	Halauxifen-methyl	28 d ELS, ft	NOEC = 0.0115 mg a.s./L _{mm}	EFSA conclusion 2014 (.....2012 /DAS 120017)
<i>Pimephales promelas</i>	Halauxifen acid	28 d ELS, ft	NOEC = 11.8 mg metabolite/L _{mm}	EFSA conclusion 2014 (...../2011 /DAS 101151)
<i>Pimephales promelas</i>	X11449757	28 d ELS, ft	NOEC = 8.9 mg metabolite/L _{mm}	EFSA conclusion 2014 (...2012 /DAS 101165)
<i>Pimephales promelas</i>	Halauxifen-methyl	21 d reproduction assay	NOEC = 0.078 mg a.s./L _{mm}	EFSA conclusion 2014 ... /DAS 102125)
<i>Pimephales promelas</i>	Halauxifen acid	21 d reproduction assay	NOEC = 12 mg metabolite/L _{mm}	EFSA conclusion 2014 (.../2012 /DAS 120535)
Invertebrates				
<i>Daphnia magna</i>	Halauxifen-methyl	48 h, s	EC ₅₀ = 2.12 mg a.s./L _{mm}	EFSA conclusion 2014 (Rebstock, M. A./2011 /DAS 090185)
<i>Daphnia magna</i>	Halauxifen acid	48 h, s	EC ₅₀ > 106 mg metabolite/L _{mm}	EFSA conclusion 2014 (Bergfield, A./2011 /DAS 101149)
<i>Daphnia magna</i>	X11449757	48 h, s	EC ₅₀ > 120 mg metabolite/L _{nom}	EFSA conclusion 2014 (Bergfield, A./2011 /DAS 101163)

Species	Substance	Exposure System	Results	Reference
<i>Daphnia magna</i>	X11406790	48 h, s	EC ₅₀ > 30 mg metabolite/L _{nom}	EFSA conclusion 2014 (Gaertner, K./2012 /DAS 120019)
<i>Daphnia magna</i>	Halauxifen-methyl	21 d, ss	NOEC = 0.144 mg a.s./L _{mm}	EFSA conclusion 2014 (Bergfield, A./2011 /DAS 101133)
<i>Daphnia magna</i>	Halauxifen acid	21 d, ss	NOEC = 100 mg metabolite/L _{nom}	EFSA conclusion 2014 (Bergfield, A./2011 /DAS 101150)
Aquatic Insects – Sediment Dwelling				
<i>Chironomus riparius</i>	Halauxifen-methyl	28 d, ss	NOEC = 1.26 mg a.s./L _{im}	EFSA conclusion 2014 (Gerke, A./2011 /DAS 101130)
<i>Chironomus dilutes riparius</i>	Halauxifen-methyl	28 d, 10d, s	NOEC EC ₅₀ = 89.3 mg a.s./kg (sediment treated) sed- (dw)-nom	EFSA conclusion 2014 (Gerke, 2011, /DAS 090183)
Other Aquatic Organisms				
<i>Americamysis bahia</i>	Halauxifen-methyl	96 h, s	LC ₅₀ > 1.30 mg a.s./L _{mm}	EFSA conclusion 2014 (Bergfield, A. /2011 /DAS 090184)
<i>Crassostrea virginica</i>	Halauxifen-methyl	96 h, s	EC ₅₀ > 1.21 mg a.s./L _{mm}	EFSA conclusion 2014 (Hicks, S. L./2011 /DAS 090120)
<i>Xenopus laevis</i>	Halauxifen-methyl	96 h	LC ₅₀ > 2 mg a.s./L _{nom}	EFSA conclusion 2014 (Dinehart, S. A./2012 /DAS 090121)
<i>Leptocheirus plumulosus</i>	Halauxifen-methyl	10 d	LC ₅₀ > 58.1 mg a.s./kg (sediment treated)	EFSA conclusion 2014 (Gerke, A./2011 /DAS 101132)
<i>Xenopus laevis</i>	Halauxifen-methyl	21 d	NOEC > 0.38 mg a.s./L _{nom}	EFSA conclusion 2014 ... /DAS 102126)
<i>Americamysis bahia</i>	Halauxifen-methyl	28 d, ss	NOEC = 0.152 mg a.s./L _{mm}	EFSA conclusion (Hicks, S.L. /2011 /DAS 101131)
Algae				
<i>Pseudokirchneriella subcapitata</i>	Halauxifen-methyl	96 h 72 h	ErC ₅₀ > 0.245 mg a.s./L _{mm} EyC ₅₀ > 0.245 mg a.s./L _{mm} ErC ₅₀ > 0.855 mg a.s./L _{mm} EyC ₅₀ > 0.855 mg a.s./L _{mm}	EFSA conclusion 2014 (Weber, K./2011 /DAS 090173)
<i>Skeletonema costatum</i>	Halauxifen-methyl	96 h 72 h	ErC ₅₀ > 1.85 mg a.s./L _{mm} EyC ₅₀ = 1.07 mg a.s./L _{mm} ErC ₅₀ = 1.80 mg a.s./L _{mm} EyC ₅₀ = 0.904 mg a.s./L _{mm}	EFSA conclusion 2014 (Rebstock, M./2011 /DAS 090176)
<i>Anabaena flos-aquae</i>	Halauxifen-methyl	96 h 72 h	ErC ₅₀ > 0.775 mg a.s./L _{mm} EyC ₅₀ > 0.775 mg a.s./L _{mm} ErC ₅₀ = 1.13 mg a.s./L _{mm} EyC ₅₀ = 1.13 mg a.s./L _{mm}	EFSA conclusion 2014 (Weber, K./2011 /DAS 090175)
<i>Navicula pelliculosa</i>	Halauxifen-methyl	96 h 72 h	ErC ₅₀ = 1.26 mg a.s./L _{mm} EyC ₅₀ = 0.663 mg a.s./L _{mm} ErC ₅₀ = 1.50 mg a.s./L _{mm} EyC ₅₀ = 0.822 mg a.s./L _{mm}	EFSA conclusion 2014 (Rebstock, M./2011 /DAS 090174)
<i>Pseudokirchneriella subcapitata</i>	Halauxifen acid	72 h	ErC ₅₀ = 63 mg/L _{nom} EyC ₅₀ = 23 mg/L _{nom}	EFSA conclusion 2014 (Rebstock, M./2011 /DAS 102027)

Species	Substance	Exposure System	Results	Reference
<i>Skeletonema costatum</i>	Halauxifen acid	96 h 72 h	ErC ₅₀ = 77 mg/L _{nom} EyC ₅₀ = 66 mg/L _{nom} ErC ₅₀ = 78 mg/L _{nom} EyC ₅₀ = 68 mg/L _{nom}	EFSA conclusion 2014 (Rebstock, M./2011 /DAS 102028)
<i>Anabaena flos-aquae</i>	Halauxifen acid	72 h	ErC ₅₀ = 55 mg/L _{nom} EyC ₅₀ = 49 mg/L _{nom}	EFSA conclusion 2014 (Rebstock, M./2011 /DAS 101144)
<i>Navicula pelliculosa</i>	Halauxifen acid	72 h	ErC ₅₀ = 56 mg/L _{nom} EyC ₅₀ = 50 mg/L _{nom}	EFSA conclusion 2014 (Rebstock, M./2011 /DAS 102029)
<i>Pseudokirchneriella subcapitata</i>	X11449757	72 h	ErC ₅₀ >15.8 mg/L _{mm} EyC ₅₀ = 4.13 mg/L _{mm}	EFSA conclusion 2014 (Rebstock, M./2011 /DAS 101158)
<i>Pseudokirchneriella subcapitata</i>	X11406790	72 h	ErC ₅₀ >5.7 mg/L _{mm} EyC ₅₀ = 1.8 mg/L _{mm}	EFSA conclusion 2014 (Rebstock, M./2012 /DAS 120021)
Higher Plant				
<i>Lemna gibba</i>	Halauxifen-methyl	7 d, ss	ErC ₅₀ > 2.27 mg a.s./L _{mm} EyC ₅₀ = 2.13 mg a.s./L _{mm}	EFSA conclusion 2014 (Rebstock, M./2011 /DAS 090182)
<i>Lemna gibba</i>	Halauxifen acid	7 d, ss	ErC ₅₀ > 50 mg/L _{mm} EyC ₅₀ = 15 mg/L _{mm}	EFSA conclusion 2014 (Rebstock, M./2011 /DAS 101145)
<i>Lemna gibba</i>	X11449757	7 d, ss	ErC ₅₀ > 92.9 mg/L _{mm} EyC ₅₀ > 92.9 mg/L _{mm}	EFSA conclusion 2014 (Rebstock, M./2011 /DAS 101159)
<i>Lemna gibba</i>	X11406790	7 d, ss	ErC ₅₀ > 12 mg/L _{mm} EyC ₅₀ > 12 mg/L _{mm}	EFSA conclusion 2014 (Rebstock, M./2012 /DAS 120022)
<i>Myriophyllum spicatum</i>	Halauxifen-methyl	14 d	ErC ₅₀ = 0.000393 mg a.s./L _{nom} EyC ₅₀ = 0.000149 mg a.s./L _{nom}	EFSA conclusion 2014 (Gonsior, G./2012 /DAS 102023)
<i>Myriophyllum spicatum</i>	Halauxifen acid	14 d 14 d	ErC ₅₀ = 0.00158 mg/L _{nom} EyC ₅₀ = 0.00080 mg/L _{nom}	EFSA conclusion 2014 (Gonsior, G./2012 /DAS 120533)
<i>Myriophyllum spicatum</i>	X11449757	14 d 14 d	ErC ₅₀ > 0.1 mg/L _{nom} EyC ₅₀ > 0.1 mg/L _{nom}	EFSA conclusion 2014 (Gonsior, G./2012 /DAS 102015)
<i>Myriophyllum spicatum</i>	X11406790	14 d 14 d	ErC ₅₀ > 0.1 mg/L _{nom} EyC ₅₀ > 0.1 mg/L _{nom}	EFSA conclusion 2014 (Gonsior, G./2012 /DAS 120534)
Higher-tier studies (micro- or mesocosm studies) N/A				

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
EFSA Journal 2014;12(12):3913

zRMS comments:

Aquatic toxicity data for halauxifen-methyl provided in Table 9.5-1 are in line with EU agreed endpoints reported in EFSA Journal 2014;12(12):3913.

Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – picloram and its metabolites

Species	Substance	Exposure System	Results	Reference
Fish				
<i>Oncorhynchus mykiss</i>	picloram	96 h, s	LC ₅₀ = 8.8 mg a.e./L _{mm}	EFSA Conclusion 2009 (...2001/ DAS 379A-103)

Species	Substance	Exposure System	Results	Reference
<i>Oncorhynchus mykiss</i> , <i>Lepomis macrochirus</i>	3,6-dichloro- picloram (aminopyralid)	96 h, f	LC ₅₀ > 100 mg a.e./L _{nom}	EFSA Conclusion 2013 (... 2001/DAS 011078; /2002/DAS 011225)
<i>Salmo gairdneri</i>	picloram	70 d, f	NOEC = 0.55 mg a.e./L _{mm}	EFSA Conclusion 2009 (.... /1983/ DAS ES-DR- 0114-1351-8)
<i>Cypripinodon variegatus</i>	3,6-dichloro- picloram (aminopyralid)	28 d (ELS), f	NOEC = 0.1 mg a.e./L _{nom} (complete time to hatch)	EFSA Conclusion 2013 (.... /2011/ DAS 101582)
Invertebrates				
<i>Daphnia magna</i>	picloram	48 h, s	EC ₅₀ = 44.2 mg a.e./L _{mm}	EFSA Conclusion 2009 (Drottar, K.R. <i>et al.</i> /2001/DAS No 379A- 101B)
<i>Daphnia magna</i>	3,6-dichloro- picloram (aminopyralid)	48 h, s	EC ₅₀ > 100 mg a.e./L _{mm}	EFSA Conclusion 2013 (... 2001/DAS 011079)
<i>Crassostrea virginica</i>	3,6-dichloro- picloram (aminopyralid)	48 h, s	EC ₅₀ > 89 mg a.e./L _{mm}	EFSA Conclusion 2013 (... / DAS 011268)
<i>Daphnia magna</i>	picloram	21 d, ss	NOEC = 6.79 mg ae/L _{mm}	EFSA Conclusion 2009 (Boeri, R.L <i>et al.</i> /2002/ DAS 021029)
<i>Daphnia magna</i>	3,6-dichloro- picloram (aminopyralid)	21 d, ss	NOEC = 100 mg a.e./L _{nom}	EFSA Conclusion 2013 (Henry, K.S. <i>et al.</i> /2003/ DAS 021085)
Sediment dwellers				
<i>Chironomus riparius</i>	picloram	28 d, spiked water	NOEC = 100 mg a.e./L _{nom}	EFSA Conclusion 2009 (Putt, A.E. /2002/ DAS 12550.6157)
<i>Chironomus riparius</i>	3,6-dichloro- picloram (aminopyralid)	28 d, spiked water	NOEC = 130 mg a.e./L _{nom}	EFSA Conclusion 2013 (Putt, A.E. /2002/ DAS 011277)
<i>Chironomus riparius</i>	5,6-dichloro- picloram	28 d, spiked water	NOEC = 50 mg/L _{nom}	EFSA Conclusion 2009 (Putt, A.E. /2002/ DAS 040372)
Algae				
<i>Pseudokirchneriella subcapitata</i>	picloram	96 h, s	EC ₅₀ = 60.2 mg a.e./L _{mm} EbC ₅₀ = 63.4 mg a.e./L _{mm} ErC ₅₀ > 78.7 mg a.e./L _{mm}	EFSA Conclusion 2009 and DAR (Desjardins, D <i>et al.</i> /2001/ DAS 011197)
<i>Anabaena flos-aquae</i>	picloram	120 h, s	ErC ₅₀ = 51.2 mg a.e./L _{mm} EbC ₅₀ = 38.2 mg a.e./L _{mm}	EFSA Conclusion 2009 and DAR (Kirk, H.D. <i>et al.</i> (2001/ DAS 001153)
<i>Navicula pelliculosa</i>	3,6-dichloro- picloram (aminopyralid)	72 h, s	EC ₅₀ = 21 mg a.e./L _{mm} ErC ₅₀ = 21 mg a.e./L _{mm} EbC ₅₀ = 18 mg a.e./L _{mm}	EFSA Conclusion 2013 (Hoberg, J.R./2002/ DAS 011278)
Higher plant				
<i>Lemna gibba</i>	picloram	14 d, ss	EC ₅₀ = 102 mg a.e./L _{mm} (fronds)	EFSA Conclusion 2009 (Drottar, K.R. <i>et al.</i> /2001/ DAS 011198)
<i>Lemna gibba</i>	3,6-dichloro- picloram (aminopyralid)	7 and 14 d, ss	EC ₅₀ > 88 mg a.e./L _{mm} (fronds)	EFSA Conclusion 2013 (Hoberg, J.R./2002/ DAS 011223)
<i>Myriophyllum spicatum</i>	picloram	14 d, s	ErC ₅₀ = 0.458 mg a.e./L _{nom} ^{1) 2)} EyC ₅₀ = 0.192 mg a.e./L _{nom} ^{1) 2)} ErC ₅₀ = 0.558 mg a.e./L _{nom} EyC ₅₀ = 0.234 mg a.e./L _{nom}	Banman, C. S. and S. Moore, S./2015/ DAS 140737

Species	Substance	Exposure System	Results	Reference
<i>Myriophyllum spicatum</i>	3,6-dichloro-picloram (aminopyralid)	14 d, s	ErC _{50,fresh weight} = 0.363 a.e./L _{nom} EyC _{50,fresh weight} = 0.188 mg a.e./L _{nom}	EFSA Conclusion 2013 (Wenzel, A/2012/ DAS 120759)
<i>Myriophyllum spicatum</i>	5,6-dichloro-picloram	14 d, s	ErC ₅₀ = 61.9 mg/L _{mm} ²⁾ EyC ₅₀ = 32.0 mg/L _{mm} ²⁾ ErC ₅₀ = 78.2 mg/L _{nom} EyC ₅₀ = 40.4 mg/L _{nom}	Gonsior, G./2015/ DAS 150390
Higher-tier studies (micro- or mesocosm studies)				
Not relevant				

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; The metabolite 3,6-dichloro-picloram is aminopyralid. The risk from this metabolite has been taken into consideration with the risk assessment of the active substance aminopyralid.

EFSA Journal 2009; 7(12):1390 (picloram)

EFSA Journal 2013;11(9): 3352 (aminopyralid)

¹⁾ Corrected for the test item purity (82.1%)

²⁾ Endpoints not fully reliable since the fresh and dry weight were determined for roots and shoots combined, while in line with OECD TG 239 only shoots should be considered in determination of these parameters. Taking this into account, provided endpoints are used for comparative purposes and an illustrative risk assessment only.

zRMS comments:

Aquatic toxicity data for picloram provided in Table 9.5-2 are in general line with EU agreed endpoints reported in EFSA Journal 2009;7(12):1390.

Two new studies on toxicity of picloram and its 5,6-dichloro analogue were submitted by the Applicant in support of the zonal evaluation of GF-4021. Although in general new active substance data should not be generated at the zonal level, the zRMS is of the opinion that these two studies were necessary in order to demonstrate that *Myriophyllum spicatum* is the aquatic species most sensitive to particular active substances (this comparison would be not possible without the new data since no endpoints for *M. spicatum* are reported in EFSA conclusion for picloram of 2009). Without new endpoints for 5,6-dichloro analogue the risk assessment would need to be performed with assumption of 10 times toxicity of the parent which would lead to necessity for risk mitigation measures. The new data clearly show that the metabolite is considerably less toxic than the parent. Both studies were evaluated by the zRMS and significant deviations from the OECD TG 239 were noted (the fresh and dry weight were determined for roots and shoots combined although the guideline indicates that only shoots should be considered in determination of these parameters). Nevertheless, in opinion of the zRMS despite some uncertainty over the endpoints, both studies still may be used as a source of additional information confirming that *Myriophyllum spicatum* is the species most sensitive to all three active compounds, that halauxifen-methyl is driving the risk to aquatic macrophytes (see point 9.5.1.1 below) and that 5,6-dichloro analogue of picloram is considerably less toxic than the parent compound. The endpoints reported in Table 9.5-2 were amended in line with the outcome of the zRMS assessment, but should be treated as indicative only until reliable toxicity data are available from the EU renewal process of picloram. Details of evaluation together with summaries of the studies may be found in Appendix 2.

Table 9.5-3: Endpoints and effect values relevant for the risk assessment for aquatic organisms – aminopyralid

Species	Substance	Exposure System	Results	Reference
Fish				
<i>Oncorhynchus mykiss</i> , <i>Lepomis macrochirus</i>	aminopyralid	96 h, f	LC ₅₀ > 100 mg a.e./L _{nom}	EFSA Conclusion 2013 (... 2001/DAS 011078; ... /2002/DAS 011225)
<i>Cypirnodon variegatus</i>	aminopyralid	28 d (ELS), f	NOEC = 0.1 mg a.e./L _{nom} (complete time to hatch)	EFSA Conclusion 2013 (... /2011/ DAS 101582)
Invertebrates				
<i>Daphnia magna</i>	aminopyralid	48 h, s	EC ₅₀ > 100 mg a.e./L _{mm}	EFSA Conclusion 2013 (... 2001/DAS 011079)
<i>Crassostrea virginica</i>	aminopyralid	48 h, s	EC ₅₀ > 89 mg a.e./L _{mm}	EFSA Conclusion 2013 (.../ DAS 011268)
<i>Daphnia magna</i>	aminopyralid	21 d, ss	NOEC = 100 mg a.e./L _{nom}	EFSA Conclusion 2013 (Henry, K.S. <i>et al.</i> /2003/ DAS 021085)
Sediment dwellers				
<i>Chironomus riparius</i>	aminopyralid	28 d, spiked water	NOEC = 130 mg a.e./L _{nom}	EFSA Conclusion 2013 (Putt, A.E. /2002/ DAS 011277)
Algae				
<i>Pseudokirchneriella subcapitata</i>	aminopyralid	72 h, s	EC ₅₀ = 32 mg a.e./L _{nom} ErC ₅₀ = 33 mg a.e./L _{nom} EbC ₅₀ = 35 mg a.e./L _{nom}	RAR aminopyralid (Hober, J.R./2002/ DAS 011222)
<i>Navicula pelliculosa</i>	aminopyralid	72 h, s	EC ₅₀ = 21 mg a.e./L _{mm} ErC ₅₀ = 21 mg a.e./L _{mm} EbC ₅₀ = 18 mg a.e./L _{mm}	EFSA Conclusion 2013 (Hoberg, J.R./2002/ DAS 011278)
Higher plant				
<i>Lemna gibba</i>	aminopyralid	7 and 14 d, ss	EC ₅₀ > 88 mg a.e./L _{mm} (fronds)	EFSA Conclusion 2013 (Hoberg, J.R./2002/ DAS 011223)
<i>Myriophyllum spicatum</i> (rooted in sediment)	aminopyralid	14 d, s	ErC _{50, fresh weight} = 0.363 a.e./L _{nom} EyC _{50, fresh weight} = 0.188 mg a.e./L _{nom}	EFSA Conclusion 2013 (Wenzel, A/2012/ DAS 120759)
Higher-tier studies (micro- or mesocosm studies)				
Not relevant				

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
EFSA Journal 2013;11(9): 3352

zRMS comments:

Aquatic toxicity data for aminopyralid provided in Table 9.5-3 are in line with EU agreed endpoints reported in EFSA Journal 2013;11(9):3352 with some minor corrections introduced by the zRMS.

It is noted that endpoints for *P. subcapitata* were not reported in the EFSA Journal which is not clear, since the study by Hober (2002, DAS 011222) was agreed by the RMS in the RAR with endpoints as provided by the Applicant in Table 9.5-3.

Table 9.5-4: Endpoints and effect values relevant for the risk assessment for aquatic organisms – GF-4021

Species	Substance	Exposure System	Results	Reference
<i>Pseudokirchneriella subcapitata</i>	GF-4021	72 h, s	ErC ₅₀ = 0.15 mg/L _{mm} * (corresponding to 0.01422 mg	Goudie, O. /2020/ DAS 190111

Species	Substance	Exposure System	Results	Reference
			sum of a.s./L **) E _y C ₅₀ = 0.081 mg/L _{mm} * NOE _r C = 0.038 mg/L _{mm}	
<i>Myriophyllum spicatum</i>	GF-4021	14 d, ss	E _r C ₅₀ = 0.00817 mg/L _{mm} * (corresponding to 0.000775 mg sum of a.s./L **) E _y C ₅₀ = 0.00568 mg/L _{mm} * NOE _r C = 0.00141 mg/L _{mm}	Eser, S./2020/ DAS 190151

Higher-tier studies (micro- or mesocosm studies)

N/A

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations

* mean measured concentration based on the least stable active substance (halauxifen-methyl)

** based on analysed concentration of active substances in batch of formulation used for testing

Testing with the product was conducted only on algae and *Myriophyllum spicatum*. Based on the active substance data, *Myriophyllum spicatum* and algae are more sensitive than *Lemna gibba*. The endpoints from algae and *Myriophyllum spicatum* are expected to drive the risk assessment and therefore the requirements for mitigation measures. *Lemna* testing was not included since it is not expected to be driving the risk assessment for the product. Predicted toxicity based on concentration addition calculation estimated the lowest endpoint for *Myriophyllum spicatum* with EC_{50mix-CA} = 0.001 mg/L while for *Lemna gibba*, the predicted endpoint was estimated as EC_{50mix-CA} = 17.18 mg/L, almost three orders of magnitude higher than for *Myriophyllum spicatum*.

Testing on fish and daphniids was not conducted since the registered product is an herbicide and aquatic plants (*Myriophyllum spicatum*) are more sensitive (factor of 10 difference) than fish and daphniids (EFSA Journal 2013;11(7):3290).

zRMS comments:

Studies on toxicity of GF-4021 to *P. subcapitata* and *M. spicatum* were evaluated and agreed by the zRMS. Endpoints reported in Table 9.5-4 above are confirmed to be correct. For summaries of the studies and details of the evaluation, please refer to Appendix 2. NOEC values were added by the zRMS as being relevant for the CLP classification purposes.

Aquatic toxicity data available for individual active compounds clearly indicate that *Myriophyllum spicatum* is the most sensitive species, much more sensitive than *Lemna gibba* and algae. Taking this into account, testing with formulation could be limited to this single species, which will definitely driving the risk.

9.5.1.1 Justification for new endpoints

Studies assessing the toxicity of the picloram and its metabolite 5,6-dichloro-picloram to the aquatic plant *Myriophyllum spicatum* have been conducted and can be considered in the risk assessment. Summaries of these studies are provided at Appendix 2.

zRMS comments:

For zRMS comments on consideration of the new active substance data for picloram, please refer to commenting box under the Table 9.5-2.

Combination toxicity assessment

The decision scheme presented in the EFSA Guidance document (2013) is used to assess the mixture toxicity, step by step. The initial assessment is conducted with FOCUS Step 1, followed by FOCUS Step 2 and 3 and 4 if necessary. The data used in the assessment is summarized below.

Table 9.5-5: Mixture toxicity assessment for GF-4021

Organism	% (w/w)			LC ₅₀ /EC ₅₀ (mg/L)			ECx (mg/L)	ECx _{mix-CA} (mg/L)
	Halauxifen-methyl	Picloram	Aminopyralid	Halauxifen-methyl	Picloram	Aminopyralid	PPP ¹	Predicted mixture toxicity
Algae				>0.245	>78.7	33	0.15	2.095
<i>M. spicatum</i>	1.06	5.07	3.38	0.000393	0.458 ¹⁾ 0.558	0.363	0.00817	0.004

PPP: Measured mixture toxicity of GF-4021; Density of the product considered in assessment is 0.946 g/cm³

¹⁾ Endpoint should be treated as indicative only due to uncertainties resulting from the study design deviating from the OECD TG 239 (for more details, see zRMS comments in point 9.5.1 above)

zRMS comments:

Calculation of the combined toxicity was corrected with consideration of the *M. spicatum* endpoint for picloram agreed by the zRMS in the course of evaluation of the new study submitted by the Applicant (for details, please refer to Appendix 2). Slightly lower endpoint had no significant impact on the calculated ECx_{mix-CA} (0.0035 mg/L was derived by the zRMS which would be 0.004 mg/L after rounding).

Slight difference between ECx_{mix-CA} calculated by the zRMS for algae (2.143 mg/L) comparing to this calculated by the Applicant (2.095 mg/L) is a result of different rounding.

Algae and *Myriophyllum spicatum* assessment

Step 1. Are measured toxicity data (ECx) available for the given endpoint (typically chronic data available only for a.s.)?

Only for the a.s. (ECxa.s.): Go to 7;

For both formulation (ECxPPP) and a.s. (ECxa.s.): Go to 2

Answer: Measured toxicity data for the formulation and the a.s. are available for algae and aquatic macrophyte *Myriophyllum spicatum*. → **Go to 2**

Step 2. Check the plausibility of the measured formulation toxicity (ECx_{PPP}) against the calculated mixture toxicity ECx_{mix-CA} (assuming CA, Equation 13) for exactly the mixture composition of the a.s. in the formulation (ECx_{PPP}) by means of the model deviation ratio ($MDR = ECx_{mix-CA}/ECx_{PPP}$).

If $MDR = 0.2-5$ (CA approximately holds for the mixture): Go to 3

If $MDR > 5$ (mixture more toxic than CA): Go to 10

If $MDR < 0.2$ (mixture less toxic than CA): Go to 9

Answer: The model deviation ratio (MDR) has been calculated and is presented in the table below.

Table 9.5-6: Overview of Step 2 of the combination toxicity assessment

Test species	Toxicity of the product (a.s. based) (EC _{xPPP}) [mg a.s./L]	Calculated mixture toxicity (a.s. in product) (EC _{x mix-CA} = $1/\sum (TU_i)$) [mg a.s./L]	MDR (EC _{x mix-CA} / EC _{xPPP})
Algae	0.014	2.095 2.08	148.76
<i>Myriophyllum spicatum</i>	0.001	0.004	4.52

Myriophyllum spicatum

The calculated MDR value for *Myriophyllum spicatum* is between 0.2 and 5, indicating that the observed and calculated mixture toxicities are in agreement. No synergisms or additional toxicity occurs due to the co-formulants. → **Go to 3**

Step 3. Check whether the mixture composition in the formulation study giving the measured mixture toxicity (EC_{xPPP}) in terms of the relative proportions of the individual a.s. is similar to the mixture composition at the PEC_{mix}. As a direct comparison on the basis of the relative proportions of the a.s. at the EC_{xPPP} with the relative proportion at the PEC_{mix} is not informative as such, the comparison is done based on calculated mixture toxicity (assuming CA) for both mixture compositions. Therefore, calculate EC_{x mix-CA} (see Equation 13) for the mixture composition of the a.s. at the PEC_{mix} and compare with the estimate calculated for the formulation (as already done in step 2 above).

If EC_{x mix-CA} (a.s. in PPP)/EC_{x mix-CA} (a.s. in PEC_{mix}) = 0.8–1.2 (mixture similar): Go to 4

If not (mixture not similar): Go to 5

Table 9.5-7: Overview of Step 3 of the combination toxicity assessment for *Myriophyllum spicatum*

Organism	Exposure scenario	EC _{x mix-CA} (a.s. in product)/EC _{x mix-CA} (a.s. in PEC _{mix})	Go to
<i>Myriophyllum spicatum</i>	FOCUS Step 1	0.544	5
	FOCUS Step 2 NZ	0.480	5
	FOCUS Step 2 SZ	0.480	5

Answer: The mixture is not similar → **Go to 5**

Step 5. Check whether one mixture component clearly drives the toxicity if considering the measured mixture toxicity (EC_{xPPP}), that is, does the largest part of the sum of toxic units (Equation 14) calculated for the formulation ($\geq 90\%$) comes from a single a.s. (TU_i)⁵³?

Yes (single ‘driver’ of mixture toxicity identified): Go to 6

No: Go to 8

Table 9.5-8: Overview of Step 5 of the combination toxicity assessment for *Myriophyllum spicatum*

Organism	Active substance	Toxicity per fraction (1/TU _i) [mg a.s./L]	Deviation from mixture toxicity = $1 - \text{EC}_{x \text{ mix-CA}} \times (1/\text{EC}_{x \text{ mix-CA-TU}_i})$ [%]	>=90% for no a.s.
<i>Myriophyllum spicatum</i>	Halauxifen-methyl	0.004	99.3%	Yes → Go to Step 6
	Picloram	1.046 ¹⁾	0.3%	
	Aminopyralid	1.021	0.3%	

¹⁾ Endpoint used for this calculation is not fully reliable due to uncertainties resulting from the study design deviating from the OECD TG 239. Nevertheless, the study was considered by the zRMS as sufficient to confirm that picloram is not more toxic to *M. spicatum* than halauxifen-methyl which is driving the risk to aquatic macrophytes (for more details, see zRMS comments in point 9.5.1 above).

Answer: Halauxifen-methyl clearly appears as the driver of the mixture toxicity to *Myriophyllum spicatum*. Therefore, the risk assessment based on halauxifen-methyl could be considered sufficient for these organisms. → Go to Step 6.

Step 6. Conduct a RA based on single-substance toxicity data (EC₅₀s.) for the identified ‘driver’ of mixture toxicity, with the exposure-toxicity ratio (ET_Rs.) being defined as the PEC₅₀s. divided by the measured EC₅₀s. and compare the outcome with the acceptability criterion (trigger value) decisive for the specific endpoint/exposure scenario combination.

Answer: See the risk assessment for the active substance (halauxifen-methyl) below, where the PEC/RAC ratios are presented. Acceptable risk is concluded to *Myriophyllum spicatum*.

The combination assessment for *Myriophyllum spicatum* is complete, risk is covered by the active substance assessment.

Algae

The calculated MDR value for algae is > 5 (Table 9.5-7), indicating that the observed and calculated mixture toxicities are not in agreement. → Go to 10

Step 10. Carefully recheck the apparent synergism as observed in the measured mixture toxicity data (EC₅₀PPP) regarding potential impacts of heterogeneous input data (a.s.) and of co-formulants ignored in the CA calculation. Does the apparent synergism remain?

Yes: Go to 3, if measured data are not available (see section 7.5.2), or if the assessment in point 3 indicates that the mixtures are not similar, **go to 8** (use modified ETR trigger values, see 10.3.4)

No: Go to 3

Answer: No, with the inclusion of a co-formulant at the concentration of 27.2% w/w (258 g/L) to the mixture toxicity assessment the apparent synergism does not remain (Table 9.5-10). Please refer to Part C of the dossier for the details on the co-formulant. → Go to 3

Table 9.5-9: Overview of the revised mixture toxicity assessment for algae including a co-formulant

Organism	% (w/w)				LC ₅₀ /EC ₅₀ (mg/L)				EC ₅₀ PPP (mg a.s./L)	EC ₅₀ mix-CA (mg/L)	MDR
	Halauxifen-methyl	Picloram	Aminopyralid	Co-formulant A*	Halauxifen-methyl	Picloram	Aminopyralid	Co-formulant A*	Toxicity of the product (a.s. based)	Calculated mixture toxicity (a.s. based)	(EC ₅₀ mix-CA / EC ₅₀ PPP)
Algae	1.06	5.07	3.38	27.3	>0.245	>78.7	33	0.131*	0.057	0.178	3.13

*Please refer to Part C on the details of the co-formulant present at 27.3% w/w (co-formulant A), the 72 h toxicity endpoint for algae was obtained from the co-formulant REACH dossier (a range of endpoints was reported and the lowest was applied for the combination toxicity assessment).

zRMS comments:

Calculations performed with consideration of one of the co-formulants presented in Table 9.5-9 above are agreed by the zRMS. Some minor differences between Applicants’ and zRMS calculations were due to different rounding. It should be, however, noted that the actual toxicity of the co-formulant A is greater than value (no effects on algae were observed at 0.131 mg/L), therefore it may be questionable if contribution of this particular co-formulant to the mixture toxicity is actually significant.

Overview of SDS for particular co-formulants indicated that another compound of the mixture may significantly contribute to the toxicity of the formulated product (co-formulant “B”, present in formulation at 11.2%, with E₀₁C₅₀ for algae in range of >0.001-0.01 mg/L). For this reason respective calculations were performed by the zRMS with consideration of this second co-formulant. Results are presented below.

Organism	% (w/w)				LC ₅₀ /EC ₅₀ (mg/L)				EC _x PPP (mg a.s./L)	EC _x mix-CA (mg/L)	MDR
	HM	Pic	Amin	Co-B	HM	Pic	Amin	Co-B	Toxicity of the product (a.s. based)	Calculated mixture toxicity (a.s. based)	(EC _x mix-CA / EC _x PPP)
Algae	1.06	5.07	3.38	11.2	>0.245	>78.7	33	>0.001	0.031	0.0018	0.06
	1.06	5.07	3.38	11.2	>0.245	>78.7	33	0.01	0.031	0.0184	0.59

FM: Halauxifen-methyl; Pic: Picloram; Amin: Aminopyralid

Organism	% (w/w)					LC ₅₀ /EC ₅₀ (mg/L)					EC _x PPP (mg a.s./L)	EC _x mix-CA (mg/L)	MDR
	HM	Pic	Amin	Co-A	Co-B	HM	Pic	Amin	Co-A	Co-B	Toxicity of the product (a.s. based)	Calculated mixture toxicity (a.s. based)	(EC _x mix-CA / EC _x PPP)
Algae	1.06	5.07	3.38	27.2	11.2	>0.245	>78.7	33	>0.131	>0.001	0.072	0.004	0.06
	1.06	5.07	3.38	27.2	11.2	>0.245	>78.7	33	>0.131	0.01	0.072	0.036	0.5

FM: Halauxifen-methyl; Pic: Picloram; Amin: Aminopyralid

When the lower value of the range of toxicity of Co-B is taken into account (i.e. >0.001 mg/L), the MDR value are below 0.2 indicating less-than additive mixture toxicity, when the lower value of the range of its toxicity is taken into account. When the higher value of the range is considered (i.e. 0.01 mg/L), the MDR is >0.5 (and <5) demonstrating that the estimated and measured mixture toxicity is comparable.

Based on indication of the EFSA (2013) it is not clear how to proceed in such a case since such situation is not foreseen in the guidance. Therefore, the risk assessment for algae from mixture will be performed using the measured toxicity endpoint expressed in terms of the active compounds (0.01422 mg/L) and PEC_{mix} calculated with consideration of PEC_{sw} for individual active compounds. Although the considered endpoint is expressed in terms of the sum of active substances, it also accounts for toxicity of the co-formulants, since it was derived in the study performed with the product. The co-formulants PEC_{sw} cannot be included in calculation of PEC_{mix} since no EU agreed endpoints are available for co-formulants and for this reason they are not included in exposure assessment performed in area of Section 8. Nevertheless, in order to cover the exposure to co-formulants at least partially, the measured formulation endpoint expressed in terms of the formulation will be compared with the formulation PEC_{sw} calculated using Spray Drift Calculator.

Assessment using FOCUS Step 1-2

Step 3. Check whether the mixture composition in the formulation study giving the measured mixture toxicity (EC_xPPP) in terms of the relative proportions of the individual a.s. is similar to the mixture composition at the PEC_{mix}. As a direct comparison on the basis of the relative proportions of the a.s. at the EC_xPPP with the relative proportion at the PEC_{mix} is not informative as such, the comparison is done based on calculated mixture toxicity (assuming CA) for both mixture compositions. Therefore, calculate EC_xmix-CA (see Equation 13) for the mixture composition of the a.s. at the PEC_{mix} and compare with the estimate calculated for the formulation (as already done in step 2 above).

If EC_xmix-CA (a.s. in PPP)/EC_xmix-CA (a.s. in PEC_{mix}) = 0.8–1.2 (mixture similar): Go to 4

If not (mixture not similar): Go to 5

Table 9.5-10: Overview of Step 3 of the combination toxicity assessment for aquatic plants (algae)

Organism	Exposure scenario	ECx mix-CA (a.s. in product)/ECx mix-CA (a.s. in PECmix)	Go to
Algae	FOCUS Step 1	0.047	5
	FOCUS Step 2 NZ	0.042	5
	FOCUS Step 2 SZ	0.042	5

Step 5. Check whether one mixture component clearly drives the toxicity if considering the measured mixture toxicity (ECxPPP), that is, does the largest part of the sum of toxic units (Equation 14) calculated for the formulation ($\geq 90\%$) comes from a single a.s. (TUi)⁵³?

Yes (single ‘driver’ of mixture toxicity identified): Go to 6

No: Go to 8

Table 9.5-11: Overview of Step 5 of the combination toxicity assessment for algae

Organism	Active substance	Toxicity per fraction (1/TUi) [mg a.s./L]	Deviation from mixture toxicity = $1 - \frac{ECx \text{ mix-CA} \times (1/ECx \text{ mix-CA-TUi})}{[\%]}$	$\geq 90\%$ for no a.s.
Algae	Halauxifen-methyl	8.771	2.0%	Yes → Go to Step 6
	Picloram	586.97	0.05%	
	Aminopyralid	369.188	0.05%	
	Co-formulant	0.182	97.9%	

Answer: The co-formulant appears as the driver for the toxicity to algae. However, since the co-formulant is not assessed in the dossier and no PEC_{sw} values are available, the risk assessment for the mixture is based on the predicted toxicity endpoint and the sum of PEC_{sw} of the active substances as a worst-case approach (Step 8).

zRMS comments:

When E_rC₅₀ of 0.001 mg/L is considered for co-formulant B, it is the toxicity driver with TU of 99.96% when only Co-B is taken into account or TU of 98.1% when Co-A and Co-B are considered.

When E_rC₅₀ of 0.01 mg/L is considered for co-formulant B, the TU of 99.6% is calculated when Co-A is excluded. When both co-formulants are taken into account, no toxicity driver may be found (TU of 83.8% is calculated for Co-B in such case).

Step 8. Conduct a mixture RA based on calculated mixture toxicity according to 10.3.8:

If ETR_{mix-CA} < trigger: Low risk

If ETR_{mix-CA} > trigger: Low risk not demonstrated, check single-substance refinement options

Table 9.5-12: Overview of Step 8 of the combination toxicity assessment for algae using FOCUS Step 1 to Step 2 PEC_{sw}

Oilseed rape			
Exposure scenario	PEC _{mix} (mg/L)	ETR _{mix-CA}	Conclusion
Algae, Trigger = 0.1			
FOCUS Step 1	0.002	0.0003	Acceptable risk
FOCUS Step 2	0.0021	<0.001	Acceptable risk
FOCUS Step 2	0.0017	<0.001	Acceptable risk

Based on calculated mixture toxicity acceptable risk to algae can be concluded at FOCUS Step 1 and 2. Assessment for algae is complete.

In conclusion, acceptable risk can be concluded based on the mixture toxicity assessment without the need for mitigation measures than those already applied to active substances.

zRMS comments:

The Applicants' calculations performed in Table 9.5-12 above are not agreed by the zRMS since it is not clear what exactly endpoint was considered. Furthermore, the PEC_{mix} based on FOCUS Step 1 and 2 PEC_{SW} for individual active compounds are not in line with surface water exposure agreed in area of Section 8.

Respective calculations were thus performed by the zRMS, as described in one of the commenting boxes above (i.e. the formulation endpoint expressed in terms of the sum of active substances will be compared with the PEC_{mix} calculated as the sum of PEC_{SW} for the active substances while formulation endpoint expressed as the formulation will be compared with the surface water exposure calculated using Spray Drift Calculator). At Step 3 the maximum PEC_{SW} of scenarios relevant for Central Zone was considered

Oilseed rape			
Exposure scenario	PEC _{mix} (mg/L)	PEC/RAC	Acceptable risk?
Algae, RAC = 1.422 µg sum a.s./L			
FOCUS Step 1	7.2	5.1	No
FOCUS Step 2 (NE)	2.08	1.5	No
FOCUS Step 2 (SE)	1.7	1.2	No
FOCUS Step 3, scenario D2 *	2.88	2.0	No
FOCUS Step 3, scenario D4 **	0.77	0.54	Yes
Algae, RAC = 15 µg product/L			
Spray Drift Calculator, ditch ***	1.52	0.10	Yes

* Scenario not relevant for the Central Zone, included for illustrative purposes only

** Maximum Step 3 PEC_{SW} of scenarios relevant for the Central Zone

*** Maximum formulation PEC_{SW} calculated using Spray Drift Calculator for various water bodies

Based on the above calculation acceptable risk to algae from the mixture may be concluded with Step 3 PEC_{SW} (when scenarios relevant for the Central Zone are considered). Acceptable risk may be also concluded when formulation endpoint and exposure expressed in terms of the formulation are compared.

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} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the tables below.

In the following table, 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 9.5-13: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for halauxifen-methyl for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GF-4021 in winter oilseed rape.

Calculations for the use of G1-4021 in winter onseed rape.										
Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged	Higher Plant		Sed. dwell. prolonged
Test species		<i>Cyprinodon variegatus</i>	<i>Cyprinodon variegatus</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Pseudokirchn. subcapitata</i>	<i>Chironomus riparius</i> Spiked water	<i>Myriophyllum spicatum</i>		<i>Chironomus riparius</i> Spiked sediment
Endpoint		LC ₅₀	NOEC	EC ₅₀	NOEC	E _r C ₅₀	NOEC	E _r C ₅₀		NOEC
(µg/L)		> 1330	11.5	2120	144	> 245	1260	0.393		89 300
AF		100	10	100	10	10	10	10		10
RAC (µg/L)		> 13.3	1.15	21.2	14.4	> 24.5	126	0.0393		8930
FOCUS Scenario	PEC _{sw} max (µg/L)	PEC/RAC Ratio							PEC _{sed} (µg/kg)	PEC/RAC Ratio
Step 1										
	0.43	<0.03	0.37	0.02	0.03	<0.02	<0.01	10.94	3.22	<0.01
Step 2										
N-Europe	0.11	<0.01	0.10	0.01	0.01	<0.01	<0.01	2.8	0.88	<0.01
S-Europe	0.09	<0.01	0.08	<0.01	0.01	<0.01	<0.01	2.29	0.71	<0.01
Step 3										
D2/ditch	0.01595	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	0.41	0.02438	<0.01
D2/stream	0.01428	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	0.36	0.0219	<0.01
D3/ditch	0.01581	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	0.40	0.01198	<0.01
D4/pond	0.000497	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.001167	<0.01
D4/stream	0.0137	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	0.35	0.00279	<0.01
D5/pond	0.000497	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.001024	<0.01
D5/stream	0.01478	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	0.38	0.003849	<0.01
R1/pond	0.000584	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.001903	<0.01
R1/stream	0.01047	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	0.27	0.004536	<0.01
R3/stream	0.01465	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	0.37	0.03017	<0.01

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

For the intended uses in winter oilseed rape, calculated PEC/RAC ratios did indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for higher plants as characterised by an E_rC₅₀ for species of aquatic macrophytes in connection with an assessment factor of 10) in FOCUS 3 scenarios.

Metabolites

Table 9.5-14: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for halauxifen acid for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GF-4021 in winter oilseed rape.

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Higher Plant
Test species		<i>Cyprinodon variegatus</i>	<i>Pimephales promelas</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Anabaena flos-aquae</i>	<i>Myriophyllum spicatum</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	NOEC	ErC ₅₀	ErC ₅₀
(µg/L)		> 107 000	11 800	> 106 000	100 000	55 000	1.58
AF		100	10	100	10	10	10
RAC (µg/L)		> 1070	1180	> 1060	10 000	5 500	0.158
FOCUS Scenario	PEC _{sw max} (µg/L)						
Step 1							
	0.47	<0.01	<0.01	<0.01	<0.01	<0.01	2.97
Step 2							
N-Europe	0.11	<0.01	<0.01	<0.01	<0.01	<0.01	0.70
S-Europe	0.09	<0.01	<0.01	<0.01	<0.01	<0.01	0.57
Step 3							
D2/ditch	0.6056	<0.01	<0.01	<0.01	<0.01	<0.01	0.44
D2/stream	0.3778	<0.01	<0.01	<0.01	<0.01	<0.01	0.29
D3/ditch	0.0317	<0.01	<0.01	<0.01	<0.01	<0.01	0.02
D4/pond	0.0219	<0.01	<0.01	<0.01	<0.01	<0.01	0.03
D4/stream	0.03843	<0.01	<0.01	<0.01	<0.01	<0.01	0.06
D5/pond	0.01542	<0.01	<0.01	<0.01	<0.01	<0.01	0.02
D5/stream	0.02963	<0.01	<0.01	<0.01	<0.01	<0.01	0.04
R1/pond	0.001098	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
R1/stream	0.02100	<0.01	<0.01	<0.01	<0.01	<0.01	0.04
R3/stream	0.1250	<0.01	<0.01	<0.01	<0.01	<0.01	0.08

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-15: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite, X11449757 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GF-4021 in winter oilseed rape.

Group		Fish acute	Fish prolonged	Inverteb. acute	Algae	Higher Plant
Test species		<i>Oncorhynchus mykiss</i>	<i>Pimephales promelas</i>	<i>Daphnia magna</i>	<i>Pseudokirchn. subcapitata</i>	<i>Myriophyllum spicatum</i>
Endpoint (µg/L)		LC ₅₀ > 120 000	NOEC 8900	EC ₅₀ > 120 000	E _r C ₅₀ 15 800	E _r C ₅₀ /E _y C ₅₀ > 100
AF		100	10	100	10	10
RAC (µg/L)		> 1200	890	> 1200	413	> 10
FOCUS Scenario	PEC ^{gl-max} (µg/L)	PEC/RAC Ratio				
Step 1						
	0.65	<0.01	<0.01	<0.01	<0.01	0.065
Step 2						
N-Europe	0.11	<0.01	<0.01	<0.01	<0.01	0.011
S-Europe	0.09	<0.01	<0.01	<0.01	<0.01	0.009

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-16: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite, X11406790 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GF-4021 in winter oilseed rape.

Group		Fish acute	Inverteb. acute	Algae	Higher Plant
Test species		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Pseudokirchn. subcapitata</i>	<i>Myriophyllum spicatum</i>
Endpoint		LC ₅₀	EC ₅₀	E _r C ₅₀	E _r C ₅₀ / E _y C ₅₀
(µg/L)		> 30 000	> 30 000	> 5700	> 100
AF		100	100	10	10
RAC (µg/L)		> 300	> 300	> 570	> 10
FOCUS Scenario	PEC ^{gl-max} (µg/L)	PEC/RAC Ratio			
Step 1					
	0.29	<0.01	<0.01	<0.01	0.03
Step 2					
N-Europe	0.04	<0.01	<0.01	<0.01	<0.01
S-Europe	0.03	<0.01	<0.01	<0.01	<0.01

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

No acute toxicity studies were available with the aqueous photolysis metabolites Deg 10, Deg 11 and Deg 14. However, considering that all fall below 10% AR within four hours and that in the toxicity study with aquatic plants (most sensitive species) the time to onset of effects was observed after seven days, the risk from photolysis metabolites is considered addressed by the parent risk assessment (EFSA Journal 2014;12(12):3913).

Photolysis was the major, and rapid, route of degradation of halauxifen methyl in the algae tests; exposure to photolysis metabolites occurred *in situ*; consequently, any toxic contribution of the photolysis metabolites is reflected in the reported endpoints for halauxifen methyl. To assess the risk of photolysis metabolites to fish and invertebrates the EFSA Journal 2013;11(7):3290 risk assessment schemes on metabolites was used.

Step 1. Is the exposure to the metabolite in the toxicity test with the a.s. measured in the course of the test and adequate for assessing the potential effect of the metabolite (see section 10.2.5)?

Yes: Go to 2

No: Go to 3

Answer: Yes, the aqueous photolysis metabolites → Go to 2

Step 2. Perform the RA assuming all the effects observed in the test with the a.s. can be attributed to the metabolite (see section 10.2.4). Is $RAC_{sw;ac} > PEC_{sw}$ and $RAC_{sw;ch} > PEC_{sw}$? (Table 9.5-17 below)

Yes: Low risk

No: Go to 3

Answer: Yes, therefore low risk can be concluded.

Table 9.5-17: Aquatic organisms: acceptability of risk ($PEC/RAC < 1$) for the photoproducts of halauxifen-methyl for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GF-4021 in winter oilseed rape

Group	-	Fish acute	Inverteb. acute
Test species	-	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>
Endpoint	-	LC ₅₀	EC ₅₀
(µg/L)	-	≥1330	2120
AF	-	100	100
RAC (µg/L)	-	≥13.3	≥21.2
FOCUS Scenario	PEC _{sw} (µg/L)	PEC/RAC Ratio	
Step 1*			
DEG-10	0.05	0.04	0.02
DEG-11	0.05	0.04	0.02
DEG-14	0.03	0.02	0.01
Step 2 N Europe*			
DEG-10	0.01	<0.01	<0.01
DEG-11	0.01	<0.01	<0.01
DEG-14	0.01	<0.01	<0.01
Step 2 S Europe*			
DEG-10	0.01	<0.01	<0.01
DEG-11	0.01	<0.01	<0.01
DEG-14	0.01	<0.01	<0.01

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

For applications to winter oilseed rape, acceptable risk to aquatic organisms from the metabolites and photoproducts of halauxifen-methyl, is observed following the use of GF-4021.

zRMS comments:

The aquatic risk assessment performed by the Applicant for halauxifen-methyl and its metabolites above is in general agreed by the zRMS with following comments:

1. Step 3 PEC_{sw} for halauxifen acid reported in Table 9.5-14 are not in line with values agreed in area of Section 8. However, correction was not necessary since acceptable risk to aquatic species from halauxifen acid could be concluded already for Step 2 PEC_{sw} and for this reason PEC/RAC derived on the basis if Step 3 PEC_{sw} are struck through in Table 9.5-14.
2. For metabolites X11449757 and X11406790 higher Step 2 PEC_{sw} were agreed in area of Section 8. However, correction was not necessary since acceptable risk to aquatic species from both compounds could be concluded already for Step 1 PEC_{sw} and for this reason PEC/RAC derived on the basis if Step 2 PEC_{sw} are struck through in Tables 9.5-15 and 9.5-16.
3. Since in EFSA Journal 2014;12(12):3913 it is concluded that the risk from aqueous photolysis metabolites Deg 10, Deg 11 and Deg 14 is covered by the evaluation performed for the parent, additional evaluation performed by the Applicant was not validated by the zRMS and is struck through above.

Overall, acceptable risk to aquatic species may be concluded from halauxifen-methyl and its relevant aquatic metabolites following intended Central Zone uses of GF-4021 with no need for risk mitigation measures.

Picloram and its metabolites

Table 9.5-18: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for picloram for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GF-4021 in winter oilseed rape.

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Algae	Sed. dwell. prolonged	Higher Plant
Test species		<i>Oncorhynchus mykiss</i>	<i>Salmo gairdneri</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Anabaena flos-aquae</i>	<i>Chironomus riparius</i>	<i>Myriophyllum spicatum</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	NOEC	E _r C ₅₀	E _r C ₅₀	NOEC	E _r C ₅₀
(µg/L)		8800	550	44 200	6790	>78 700	51 200	100 000	458 ^b 558
AF		100	10	100	10	10	10	10	10
RAC (µg/L)		88	55	442	679	>7,870	5,120	10,000	45.8 ^b 55.8
FOCUS Scenario	PEC ^{gl-max} (µg/L)	PEC/RAC Ratio							
Step 1									
	4.01	0.05	0.07	0.01	0.01	<0.01	<0.01	<0.01	0.09 ^b 0.07
Step 2									
N-Europe	1.24	0.01	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	0.02
S-Europe	1.01	0.01	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	0.02
Step 3									
D2/ditch	1.819	0.02	0.03	<0.01	<0.01	<0.01	<0.01	<0.01	0.03
D2/stream	1.141	0.01	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	0.02
D3/ditch	0.3163	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01
D4/pond	0.5932	0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01
D4/stream	0.3361	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01
D5/pond	0.2959	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01
D5/stream	0.1702	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
R1/pond	0.002595	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
R1/stream	0.05031	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
R3/stream	0.2744	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

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

^b) Endpoint should be treated as indicative only due to uncertainties resulting from the study design deviating from the OECD TG 239 (for more details, see zRMS comments in point 9.5.1 above).

Metabolites

Table 9.5-19: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite, 5,6-dichloro-picloram for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GF-4021 in winter oilseed rape.

Group		Sed. dwell. prolonged	Aquatic plants
Test species		<i>Chironomus riparius</i>	<i>Myriophyllum spicatum</i>
Endpoint		NOEC	ErC ₅₀
(µg/L)		50 000	61 900 ¹⁾ 78 200
AF		10	10
RAC (µg/L)		5000	6190 7820
FOCUS Scenario	PEC ^{gl-max} (µg/L)	PEC/RAC Ratio	
Step 1			
	0.77	<0.01	<0.01
Step 2			
N-Europe	0.24	<0.01	<0.01
S-Europe	0.20	<0.01	<0.01

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

¹⁾ Endpoint should be treated as indicative only due to uncertainties resulting from the study design deviating from the OECD TG 239 (for more details, see zRMS comments in point 9.5.1 above).

For applications to winter oilseed rape, acceptable risk to aquatic organisms from picloram and its metabolites is observed following the use of GF-4021. The picloram metabolite 3,6-dichloro-picloram is aminopyralid and covered by the aminopyralid risk assessment (below).

zRMS comments:

The aquatic risk assessment performed by the Applicant for picloram and its metabolites above is in general agreed by the zRMS with following comments:

1. Risk assessment for picloram based on Step 2 and 3 PEC_{SW} was not necessary since acceptable risk could be concluded already for Step 1 PEC_{SW}. For this reason PEC/RAC derived on the basis of Step 2 and 3 PEC_{SW} are struck through in Table 9.5-18.
2. The risk assessment for *M. spicatum* from 5,6-dichloropicloram was amended with consideration of an endpoint agreed by the zRMS during evaluation of the new metabolite study (for details, see Appendix 2). Furthermore, calculations based on Step 2 PEC_{SW} were not validated since acceptable risk could be concluded already with Step 1 PEC_{SW}.
3. The zRMS agrees that the risk assessment from 3,6-dichloropicloram (aminopyralid) is addressed below.

As already indicated in the footnotes to Tables 9.5-18 and 9.5-19 above, the endpoints for *Myriophyllum spicatum* derived from studies performed with picloram and its 5,6-dichloro analogue are not fully reliable due to significant deviations of the test design from indications of OECD TG 239 (the fresh and dry weight were determined for roots and shoots combined, although in line with the guideline only shoots should be used for determination of these parameters). Nevertheless, both studies were considered by the zRMS as sufficient source of additional information that could be used for purposes of the informative risk assessment. It should be also noted that even if the endpoints from the studies were 10 times lower, the risk from picloram and 5,6-dichloro analogue would be still acceptable with Step 1 PEC_{SW}.

Overall, acceptable risk to aquatic species may be concluded from picloram aminopyralid and its relevant aquatic metabolites following intended Central Zone uses of GF-4021 with no need for risk mitigation measures.

Aminopyralid

Table 9.5-20: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for aminopyralid for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GF-4021 in winter oilseed rape.

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged	Higher Plant
Test species		<i>Oncorhynchus mykiss</i>	<i>Cypirinodeon variegatus</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Navicula pelliculosa</i>	<i>Chironomus riparius</i>	<i>Myriophyllum spicatum</i>
Endpoint		LC50	NOEC	EC50	NOEC	ErC50/EyC50	NOEC	ErC50
(µg/L)		>100 000	100	>100 000	100 000	21 000	130 000	363
AF		100	10	100	10	10	10	10
RAC (µg/L)		>100	10	>100	10 000	2100	13 000	36.3
FOCUS Scenario	PEC ^{gl-max} (µg/L)	PEC/RAC Ratio						
Step 1								
	3.11 2.72	0.03	0.31 0.27	0.03	<0.01	<0.01	<0.01	0.09 0.07
Step 2								
N-Europe	0.73	0.01	0.07	0.01	<0.01	<0.01	<0.01	0.02
S-Europe	0.60	0.01	0.06	0.01	<0.01	<0.01	<0.01	0.02
Step 3								
D2/ditch	1.049	0.01	0.10	0.01	<0.01	<0.01	<0.01	0.03
D2/stream	0.6653	0.01	0.07	0.01	<0.01	<0.01	<0.01	0.02
D3/ditch	0.1497	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
D4/pond	0.177	<0.01	0.02	<0.01	<0.01	<0.01	<0.01	<0.01
D4/stream	0.1202	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
D5/pond	0.07663	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
D5/stream	0.06855	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
R1/pond	0.001797	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
R1/stream	0.03346	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
R3/stream	0.1311	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01

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

For applications to winter oilseed rape, acceptable risk to aquatic organisms from aminopyralid is observed following the use of GF-4021.

zRMS comments:

The RAC values considered in the aquatic risk assessment performed by the Applicant for aminopyralid are agreed by the zRMS. However, in opinion of the zRMS the surface water exposure being the sum of PEC_{SW} for aminopyralid and 3,6-dichloropicloram (aminopyralid) should be considered for calculation of PEC/RAC values, since PEC_{SW} calculated for aminopyralid only does not cover formation of this compound from picloram. The risk assessment above was thus amended by the zRMS. Since acceptable risk could be concluded with Step 1 PEC_{SW} values, calculations based on Step 2 and Step 3 were struck through in Table 9.5-20 above.

Overall, acceptable risk to aquatic species may be concluded from aminopyralid (also formed from picloram) following intended Central Zone uses of GF-4021 with no need for risk mitigation measures.

Table 9.5-21: Aquatic organisms: acceptability of risk (PEC/RAC < 1) of GF-4021 for each organism group based on SWASH calculations for the use winter oilseed rape.

Group		Algae
Test species		<i>Pseudokirchneriella subcapitata</i>
Endpoint		E_rC_{50}
(µg/L)		150
AF		10
RAC (µg/L)		15
SWASH	PEC _{gl-max} (µg/L)	PEC/RAC Ratio
Default NSZ		
Ditch (1 m)	1.5194	0.01
Pond (3.5 m)	0.0518	<0.01
Stream (1.5 m)	1.1276	0.01

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

According to the combination toxicity assessment, halauxifen-methyl is contributing to more than 90% of the toxicity in the formulation when considering *Myriophyllum spicatum*, therefore, acceptable risk can be concluded based on the active substance assessment. Considering algae, the co-formulant was found to contribute to more than 90% of the toxicity, however, since no PEC_{sw} values are available for the co-formulant the risk assessment was concluded based on the predicted mixture toxicity and sum of PEC_{sw} of the active substances and is further supported by the measured product toxicity and the generated product PEC_{sw} values.

No potential risks are identified following application of GF-4021 to winter oilseed rape without the need for risk mitigation measures.

zRMS comments:

Calculations presented in Table 9.5-21 above are agreed by the zRMS and are considered to cover effects caused by toxic co-formulants in the formulated product as well as exposure of aquatic species to these compounds (see also evaluation performed in point 9.5.1.1).

9.5.3 Overall conclusions

Regulatory testing has been conducted with halauxifen-methyl, picloram, aminopyralid and their relevant metabolites and GF-4021 in accordance with EU requirements.

Based on the active substances, the acute and chronic risk assessment for aquatic organisms indicated acceptable risk (PEC/RAC <1) at FOCUS Step scenarios relevant to the Central Zone. The risk from the mixture is also considered to be acceptable.

Overall, acceptable risk to aquatic organisms may be concluded from the intended Central Zone uses of GF-4021 with no need for risk mitigation measures. ~~there is no unacceptable risk to aquatic organisms expected for the use of GF-4021 in winter oilseed rape.~~

The following text is added due to agreements during the Central Zone harmonisation meetings. It should be noted that this text has no impact on the outcome of zonal evaluation of formulation GF-4021, which was performed in line with the EU agreed methodology.

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

9.6 Effects on bees (KCP 10.3.1)

9.6.1 Toxicity data

Studies on the toxicity to bees have been carried out with halauxifen-methyl, picloram and aminopyralid. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on bees of GF-4021 were not evaluated as part of the EU assessment of either halauxifen-methyl, picloram and aminopyralid. 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 -halauxifen-methyl

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i>	Halauxifen-methyl	Oral	LD ₅₀ > 108 µg/bee	EFSA Conclusion, 2014 (Schmitzer, S./2011/DAS 101128 & 101129)
<i>Apis mellifera</i>	Halauxifen-methyl	Contact	LD ₅₀ > 98.1 µg/bee	EFSA Conclusion, 2014 (Schmitzer, S./2011/DAS 101128 & 101129)
<i>Apis mellifera</i>	Halauxifen-methyl	10-d feeding test adult	NOEDD ≥ 5.07 µg/bee/day LDD ₅₀ > 5.07 µg/bee/day	Oberrauch, S./ 2018/DAS 170071
<i>Apis mellifera</i>	Halauxifen-methyl	Repeat exposure larvae	NOED ≥ 23.1 µg/larva	Oberrauch, S./2018/DAS 170073
Higher-tier studies (tunnel test, field studies)				
N/A				

EFSA Journal 2014;12(12):3913

Table 9.6-2: Endpoints and effect values relevant for the risk assessment for bees-picloram

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i>	picloram	Oral	LD ₅₀ > 74 µg/bee	EFSA Conclusion 2009 (Hoberg, J.R. /2001/ DAS 011173; 011174)
<i>Apis mellifera</i>	picloram	Contact	LD ₅₀ >100 µg/bee	EFSA Conclusion 2009 (Hoberg, J.R. /2001/ DAS 011173; 011174)
<i>Apis mellifera</i>	picloram	10-d feeding test adult	NOED = 49.61 µg/bee/day LDD ₅₀ >49.61 µg/bee/day	Leonard, J and Moore, S /2017/DAS 170090
<i>Apis mellifera</i>	picloram	Repeat exposure larvae	NOED = 62.7 µg/larva	Leonard, J and Moore, S /2017/DAS 170091
Higher-tier studies (tunnel test, field studies)				
Not relevant				

EFSA Conclusion: EFSA Journal 2009; 7(12):1390

Table 9.6-3: Endpoints and effect values relevant for the risk assessment for bees- aminopyralid

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i>	aminopyralid	Oral	LD ₅₀ >120 µg/bee	EFSA Conclusion 2013 (Aufderheide, J./2001/ DAS 011045)
<i>Apis mellifera</i>	aminopyralid	Contact	LD ₅₀ >100 µg/bee	EFSA Conclusion 2013 (Aufderheide, J./2004/ DAS 011044R)
<i>Apis mellifera</i>	aminopyralid	10-d feeding test adult	NOED = 74.2 µg/bee/day LDD ₅₀ >74.2 µg/bee/day	Leonard, J and Moore, S /2017/DAS 170092
<i>Apis mellifera</i>	aminopyralid	Repeat exposure larvae	NOED = 100 µg/larva	Leonard, J and Moore, S /2017/DAS 170413

Higher-tier studies (tunnel test, field studies)

Not relevant

EFSA Journal 2013;11(9): 3352

Table 9.6-4: Endpoints and effect values relevant for the risk assessment for bees- GF-4021

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i>	GF-4021	Oral	LD ₅₀ >87.5 µg/bee	Tomé, H.V.V, Porch, J.R./ 2020/ DAS 190458
<i>Apis mellifera</i>	GF-4021	Contact	LD ₅₀ >250 µg/bee	Tomé, H.V.V, Porch, J.R./ 2020/ DAS 190458
<i>Apis mellifera</i>	GF-4021	10-d feeding test adult	NOED = 13.8 µg/bee/day LDD ₅₀ = 56.1 µg/bee/day	Wendling, K./2021/ DAS 200622
<i>Apis mellifera</i>	GF-4021	Repeat exposure larvae	NOED = 80.1 µg/bee/day	Wendling, K./2021/ DAS 200623

zRMS comments:

Bee toxicity data for halauxifen-methyl, picloram and aminopyralid provided in Tables 9.6-1 to 9.6-3 are in line with EU agreed endpoints reported in EFSA Journal 2014;12(12):3913, EFSA Journal 2009;7(12):1390, and EFSA Journal 2013;11(9):3352, respectively.

In support of the zonal evaluation of GF-4021 the Applicant submitted also studies on chronic toxicity of particular active compounds to adult bees and bee larvae. However, these new active substance studies were not required to finalise the risk assessment at the zonal level, sine relevant studies performed with the formulated product were submitted in order to fulfil the data requirements. New active substance endpoints were struck through in tables above, since they should be generated in the course of the EU renewal processes of particular active compounds.

Studies on acute and chronic toxicity of GF-4021 to adult bees and larvae were evaluated and agreed by the zRMS. Summaries of the studies together with zRMS evaluation are presented in Appendix 2. Endpoints reported in Table 9.6-4 are confirmed to be correct.

9.6.1.1 Justification for new endpoints

Chronic larvae and adult honeybee studies are available for the active substances and therefore included for risk assessment purposes. Summaries of these studies are provided at Appendix 2.

zRMS comments:

The new active substance studies were not required to finalise the risk assessment at the zonal level, sine relevant studies performed with the formulated product were submitted in order to fulfil the data requirements. Taking this into account, the new studies were not validated by the zRMS and new endpoints were not included in the risk assessment.

9.6.2 Risk assessment

The Applicant recognizes the need to review the bee pollinator risk assessment based on scientific progress. The EFSA Draft Bee Guidance Document issued in 2013 has not been noted and is currently being revised. Therefore, the risk assessment below has been conducted following the EPPO 2010a and b⁶ 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.

zRMS comments:

In opinion of the zRMS, in case the bee risk assessment is performed according in line with other GD than SANCO/10329/2002 rev 2 final, it should be conducted in line with indications of the guidance currently used at the EU level (i.e. EFSA, 2013), especially the EPPO PP3 (2010) Standard Environmental risk assessment schemes for plant protection products (including chapter 10: Honeybees) are withdrawn by January 2019, so their recommendations should not be followed in the risk assessment.

9.6.2.1 Hazard quotients for bees

The acute risk to honey bees from use of GF-4021 0.25 L prod/ha was assessed using the maximum single application rate and the LD₅₀ values to calculate hazard quotients. 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 9.6-5: First-tier assessment of the risk for bees due to the use of GF-4021 in winter oilseed rape

Intended use	Winter oilseed rape		
Active substance	Halauxifen-methyl		
Application rate (g a.s./ha)	1 × 2.5		
Test design	LD ₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	>108	2.5	<0.023
Contact toxicity	>98.1		<0.025
Active substance	Picloram		
Application rate (g a.s./ha)	1 × 12		
Test design	LD ₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	>74	12	<0.16 <0.13
Contact toxicity	>100		<0.12
Active substance	Aminopyralid		
Application rate (g a.s./ha)	1 × 8		
Test design	LD ₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	>120	8	<0.07 <0.06
Contact toxicity	>100		<0.08

⁶ 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.

Product	GF-4021		
Application rate (g/ha)	1 × 0.25 L/ha equals to 236.5 g product/ha (formulation density of 0.946 g/mL)		
Test design	LD₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q_{HO}, Q_{HC} criterion: Q_H ≤ 50
Oral toxicity	>87.5	236.5	<2.7
Contact toxicity	>250		<0.946

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

Risk assessment based on available chronic or repeated exposure studies

This risk assessment is based upon the EPPO 2010⁶ risk assessment for systemic substances which is cited in the regulation as a current risk assessment scheme. The maximum application rate of GF 4021 is 0.25 L/ha with a maximum 1 application per season (application window 15 Aug to December). The proposed crop on the label is winter oilseed rape at BBCH 12-19.

Risk assessment for honey bee larvae

Worst case data from Rortais et al., 2005⁷ as proposed in the EPPO scheme have been used to estimate the consumption by **honeybee larvae**. Based on the data in this publication, a worker larva consumes 59.4 mg sugar in 5 days. Assuming a 30% sugar content of nectar, the resulting worst case consumption for a worker larva is: $59.4/0.30 = 198 \text{ mg nectar in 5 days}$ (larval development). In addition, a worker larva is considered to consume 2 mg pollen during its development phase (EFSA 2013). Thus, considering the mean RUD values for nectar (i.e. 2.9 mg/kg) and pollen (i.e. 6.1 mg/kg) from foliar sprays in EFSA 2013 (Appendix F), exposure can be estimated for the whole larval development period of 5 days. The final estimated exposure levels deriving from nectar and pollen consumption can be compared to the available larval NOEL values for GF 4021 and its active substances. The EPPO 2010 scheme proposes a trigger of 1 for assessment of the risk to honeybees. Results are presented in the following table.

Table 9.6-6: Assessment of the risk for bee larvae due to the use of GF-4021 in winter oilseed rape

Intended use	Winter oilseed rape			
Active substance	Halauxifen-methyl			
Application rate (kg a.s./ha)	1 × 0.0025			
NOEL (µg/bee/developmental period)	23.1			
Food item	Consumption (kg/bee/developmental period)	RUD (µg/kg/kg/ha)	Dietary dose (µg/bee/developmental period)	TER criterion: TER ≥ 1
Nectar	198×10^{-6}	2.9×10^3	0.00144	15757
Pollen	2×10^{-6}	6.1×10^3	0.00003	
Total			0.00147	

⁷ 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

Active substance Application rate (kg a.s./ha)	Pictoram 1×0.012			
NOEL (µg/bee/developmental period)	62.7			
Food item	Consumption (kg/bee/developmental period)	RUD (µg/kg/kg/ha)	Dietary dose (µg/bee/developmental period)	TER criterion: TER ≥ 1
Nectar	198×10^{-6}	2.9×10^3	0.00689	8910
Pollen	2×10^{-6}	6.1×10^3	0.00015	
Total			0.00704	
Active substance Application rate (kg a.s./ha)	Aminopyralid 1×0.008			
NOEL (µg/bee/developmental period)	400			
Food item	Consumption (kg/bee/developmental period)	RUD (µg/kg/kg/ha)	Dietary dose (µg/bee/developmental period)	TER criterion: TER ≥ 1
Nectar	198×10^{-6}	2.9×10^3	0.00459	21316
Pollen	2×10^{-6}	6.1×10^3	0.00010	
Total			0.00469	
Product Application rate (kg a.s./ha)	GF 4021 1×0.25 L/ha equals to 0.2365 kg product/ha (formulation density of 0.946 g/mL)			
NOEL (µg/bee/developmental period)	80.1			
Food item	Consumption (kg/bee/developmental period)	RUD (µg/kg/kg/ha)	Dietary dose (µg/bee/developmental period)	TER criterion: TER ≥ 1
Nectar	198×10^{-6}	2.9×10^3	0.136	578
Pollen	2×10^{-6}	6.1×10^3	0.0029	
Total			0.139	

TER values shown in bold breach the relevant trigger.

All the TER values above greatly exceed the EPPO trigger of 1, indicating that the proposed uses of GF 4021 in winter oilseed rape pose an acceptable risk to bee larval development.

Risk assessment for chronic exposure of honey bee adult

The risk assessment for chronic exposure of **adult honeybees** 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) and derives a TER value. Worst case data from Rortais et al., 2005 indicates a sugar need of 128 mg/bee/day for a bee feeding exclusively from nectar containing 30% sugar. This results in a worst case consumption for an adult honeybee is: $128/0.30 = 427$ mg nectar/day. Considering the mean RUD value for nectar from foliar sprays (i.e. 2.9 mg/kg) in EFSA 2013 (Appendix F), the daily dietary exposure for adult honeybees can be estimated and it can be compared to the available chronic adult NOEDD values for GF 4021 and its active substances. The EPPO 2010 scheme proposes a trigger of 1 for assessment of the risk to honeybees. Results are presented in the following table.

Table 9.6-7: Assessment of the chronic risk for adult bees due to the use of GF 4021 in winter oilseed rape

Intended use	Winter oilseed rape			
Active substance	Halauxifen-methyl			
Application rate (kg a.s./ha)	1×0.0025			
NOEDD (µg/bee/day)	5.07			
Food item	Consumption (kg/bee/day)	RUD (µg/kg/kg/ha)	Dietary dose (µg/bee/day)	TER criterion: $TER \geq 1$
Nectar	427×10^{-6}	2.9×10^3	0.00310	1638
Active substance	Picoloram			
Application rate (kg a.s./ha)	1×0.012			
NOEDD (µg/bee/day)	49.61			
Food item	Consumption (kg/bee/day)	RUD (µg/kg/kg/ha)	Dietary dose (µg/bee/day)	TER criterion: $TER \geq 1$
Nectar	427×10^{-6}	2.9×10^3	0.0149	3338
Active substance	Aminopyralid			
Application rate (kg a.s./ha)	1×0.008			
NOEDD (µg/bee/day)	74.2			
Food item	Consumption (kg/bee/day)	RUD (µg/kg/kg/ha)	Dietary dose (µg/bee/day)	TER criterion: $TER \geq 1$
Nectar	427×10^{-6}	2.9×10^3	0.00991	7490
Product	GF 4021			
Application rate (kg a.s./ha)	1×0.25 L/ha equals to 0.2365 kg product/ha (formulation density of 0.946 g/mL)			
NOEDD (µg/bee/day)	13.8			
Food item	Consumption (kg/bee/day)	RUD (µg/kg/kg/ha)	Dietary dose (µg/bee/day)	TER criterion: $TER \geq 1$
Nectar	427×10^{-6}	2.9×10^3	0.293	47.1

TER values shown in bold breach the relevant trigger.

All the TER values above greatly exceed the EPPO trigger of 1, indicating that the proposed uses of GF 4021 in winter oilseed rape pose an acceptable chronic risk to adult bees.

zRMS comments:

The acute risk assessment based on indications of SANCO/10329/2002 rev 2 final is agreed by the zRMS. Based on performed calculations, acceptable risk to bees from the intended Central Zone uses of GF-4021 may be concluded.

As already indicated in point 9.6.2 above, in case the risk assessment is performed not in line with SANCO guidance document, it should follow indications of the guidance currently used at the EU level (i.e. EFSA, 2013), especially EPPO PP3 (2010) Standard Environmental risk assessment schemes for plant protection products are withdrawn by January 2019. Taking this into account, the risk assessment performed by the Applicant in line with EPPO recommendations was not validated by the zRMS and is struck through.

Instead, the risk assessment performed in line with EFSA (2013) has been performed by the zRMS and is presented below. Calculations were performed using EFSA Bee-Tool v. 3

Screening step risk assessment (oilseed rape, BBCH 12-19, 1×0.2365 kg product/ha)

Contact route of exposure				
"calculation factor" (linked with dust)		HQ	Trigger	Risk indicator
HB	1	0.9	42	OK
Oral route of exposure (pollen and nectar)				
"calculation factor" (Ef x SV)		ETR	Trigger	Risk indicator
HB - acute	7.6	0.02	0.2	OK
HB - chronic	7.6	0.032	0.03	!
HB - larvae	4.4	0.01	0.2	OK

Tier 1 chronic risk assessment

Crop	Category	Scenario	Ef	SV HB	TWA HB	ETR HB	Trigger	Risk indicator
Oilseed rape BBCH 12-19	chronic	treated crop	1	5.8	0.72	0.018	0.03	OK
	chronic	weeds	1	2.9	0.72	0.009	0.03	OK
	chronic	field margin	0.0092	2.9	0.72	0.000	0.03	OK
	chronic	adjacent crop	0.0033	5.8	0.72	0.000	0.03	OK
	chronic	next crop	1	0.54	0.72	0.002	0.03	OK

Based on calculations performed in line with indications of EFSA (2013), acceptable acute oral and contact risk to adult bees as well as chronic risk to larvae may be concluded from the intended uses of GF-4021 already at the screening step. The chronic risk was unacceptable at the screening step (with the ETR marginally above the trigger) and Tier 1 evaluation was performed which demonstrated acceptable chronic risk to bees in all relevant scenarios.

Overall, acceptable acute, chronic and larvae risk to bees may be concluded from the intended uses of GF-4021.

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

Since acceptable acute risks have been concluded for bees exposed to GF-4021 at the Tier 1 level, a higher-tier risk assessment is not required for the proposed uses of GF-4021.

9.6.3 Effects on bumble bees

No data available.

9.6.4 Effects on solitary bees

No data available.

9.6.5 Overall conclusions

Regulatory testing has been conducted with halauxifen-methyl, picloram, aminopyralid and the product GF-4021 in accordance with EU requirements. All HQ and ETR TER values were indicative of acceptable low acute and chronic risk to adult bees and bee larvae based on a single maximum application rate of 0.25 L GF-4021/ha to winter oilseed rape (BBCH 12-19).

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

Effects on non-target arthropods of GF-4021 were not evaluated as part of the EU assessment of either halauxifen-methyl, picloram or aminopyralid. 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

Species	Substance	Exposure System	Results	Reference
<i>Typhlodromus pyri</i> (protonymphs)	GF-4021	Laboratory test glass plates (2D)	7 d LR ₅₀ > 250 mL/ha 7-14 d ER ₅₀ > 250 mL/ha	Fallowfield, L. /2020/ DAS 190467
<i>Aphidius rhopalosiphi</i> (adults)	GF-4021	Laboratory test glass plates (2D)	48 h LR ₅₀ = 192.1 mL/ha 13 d ER ₅₀ > 192.1 mL/ha	Stevens, J. /2020/ DAS 190464
Field or semi-field tests				
N/A				

zRMS comments:

Studies on effects of GF-4021 on non-target arthropods were evaluated and agreed by the zRMS. Endpoints reported in Table 9.7-1 above are confirmed to be correct. For summaries of the studies and details of the evaluation, please refer to Appendix 2.

9.7.1.1 Justification for new endpoints

Data on the toxicity of the formulation to non-target arthropods is available and is used in the risk assessment. Summaries of these studies are provided at Appendix 2.

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 in-field exposure (predicted environmental rate (PER)) is calculated according to ESCORT 2. The potential risk of GF-4021 to in-field non-target arthropods was assessed by calculation of the hazard quotients ($HQ_{in-field} = \text{exposure/toxicity}$) with the predicted environmental rate ($PER_{in-field}$) and the lowest lethal rate (LR₅₀) values according ESCORT 2.

Table 9.7-2: First -tier assessment of the in-field risk for non-target arthropods due to the use of GF-4021 in winter oilseed rape

Intended use	winter oilseed rape		
Active substance/product	GF-4021		
Application rate (mL/ha)	1 x 250		
MAF	N/A		
Test species Tier I	LR₅₀ (lab.) (mL/ha)	PER_{in-field} (mL/ha)	HQ_{in-field} criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	>250	250	<1
<i>Aphidius rhopalosiphi</i>	192.2		1.3

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.

zRMS comments:

The in-field risk assessment presented in Table 9.7-2 is agreed by the zRMS.

Based on calculations performed with consideration of the Tier I laboratory data acceptable in-field risk to non-target arthropods from the intended uses of GF-4021 may be concluded.

9.7.2.2 Risk assessment for off-field exposure

Risk assessment of areas immediately surrounding the crop is considered important since these areas represent a natural reservoir for immigration, emigration, and reproduction of arthropod populations and provide increased species diversity. Exposure of non-target arthropods living in off-field areas to GF-4021 will mainly be due to spray drift from field applications. Off-field areas are assumed to be densely vegetated and thus spray drift is unlikely to reach bare ground. Therefore, evaluation of exposure *via* soil residues in off-field areas was not considered. Off-field foliar PER values were calculated from in-field foliar PERs in conjunction with drift values published by the Rautmann *et al.* (2000)⁸.

The potential risk of GF-4021 to off-field non-target arthropods was assessed by calculation of the hazard quotients (HQ) with the predicted environmental rate (PER_{off-field}) and the lowest lethal rate (LR₅₀) values according multiplied by a correction factor.

Table 9.7-3: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of GF-4021 in winter oilseed rape.

Intended use	winter oilseed rape				
Active substance/product	GF-4021				
Application rate (mL/ha)	1 x 250				
MAF	N/A				
vdf	5 (Tier 1)				
Test species Tier I	LR₅₀ (lab.) (mL/ha)	Drift rate	PER_{off-field} (mL/ha)	CF	HQ_{off-field} criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	>250	2.77%	1.385	10	<0.05
<i>Aphidius rhopalosiphi</i>	192.2				0.07

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate;

⁸ Rautmann, D., Streloke, M., Winkler, R. (2001). New basic drift values in the authorisation procedure for plant protection products. In Forster, R., Streloke, M. Workshop on Risk Assessment and Risk Mitigation Measures in the Context of the Authorization of Plant Protection Products (WORMM). Mitt. Biol. Bundesanst. Land-Forstwirtschaft. Berlin-Dahlem, Heft 381.

CF: Correction factor; HQ: Hazard quotient. ¹ The drift listed for “field crops” in Rautmann *et al.* (2001) (i.e. 2.77% at 1 m) can be used.

zRMS comments:

The off-field risk assessment presented in Table 9.7-3 is agreed by the zRMS.

The Applicant considered VDF of 5 has been considered, since available investigations indicate that VDF of 10 recommended by ESCORT 2 guidance document is not appropriate and may lead to underestimation of the exposure. It should be, however, noted that according to EFSA Supporting publication 2019:EN-1673, VDF of 5 should be considered as the interim solution that will be reflected in the SANCO/10329/2002 rev 2 final with its implementation considered further. Since use of VDF of 5 was not reflected in the current SANCO terrestrial guidance, its use is not yet mandatory. Nevertheless, the risk assessment performed with VDF of 5 is more protective and is thus agreed by the zRMS.

Based on calculations performed with consideration of the Tier I laboratory data acceptable off-field risk to non-target arthropods from the intended uses of GF-4021 may be concluded with no need for risk mitigation measures.

9.7.2.3 Additional higher-tier risk assessment

Since acceptable acute risks have been concluded for non-target arthropods exposed to GF-4021 at the Tier 1 level, an additional higher-tier risk assessment is not required for the proposed uses of GF-4021.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

Regulatory testing has been conducted with GF-4021 ~~the product~~ in accordance with EU requirements. All in-field and off-field HQ values were calculated to be less than the trigger of 2, indicating a low risk to non-target arthropods within the treated fields, and adjacent untreated habitat with no need for risk mitigation measures.

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

9.8.1 Toxicity data

Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with halauxifen-methyl, picloram, aminopyralid and their relevant metabolites. Full details of these studies are provided in the respective EU DAR.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of GF-4021 were not evaluated as part of the EU assessment of either halauxifen-methyl, picloram and aminopyralid. 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)

Species	Substance	Exposure System	Results	Reference
<i>Eisenia fetida</i>	Halauxifen-methyl	Mixed in to substrate 14 d, acute 10 % peat content	LC _{50,corr} = > 500 mg/kg dw *	EFSA conclusion 2014 (Witte, B./2010 (Amendment 2011)/DAS 090099)
<i>Eisenia fetida</i>	Halauxifen-acid	Mixed in to substrate 14 d, acute 10 % peat content	LC ₅₀ = > 1000 mg/kg dw	EFSA conclusion 2014 (Witte, B./2010/DAS 101141)
<i>Eisenia fetida</i>	X11449757	Mixed in to substrate 14 d, acute 10 % peat content	LC ₅₀ = > 1000 mg/kg dw	EFSA conclusion 2014 (Witte, B./2010/DAS 101155)
<i>Eisenia fetida</i>	Halauxifen-methyl	Mixed into substrate 56 d, chronic 5 % peat content	NOEC _{corr} = 5 mg/kg dw*	EFSA conclusion 2014 (Witte, B./2010 /Amendment 2011/ /DAS 090100)
<i>Eisenia fetida</i>	Halauxifen acid	Mixed into substrate 56 d, chronic 5 % peat content	NOEC = 10 mg/kg dw	EFSA conclusion 2014 (Witte, B./2010/DAS 101142)
<i>Eisenia fetida</i>	X11449757	Mixed into substrate 56 d, chronic 5 % peat content	NOEC = 10 mg/kg dw	EFSA conclusion 2014 (Witte, B./2010/DAS 101156)
<i>Eisenia fetida</i>	Non-extractable residues of halauxifen-methyl	Mixed into substrate 56 d, chronic Freshly collected natural soils – M802 (German) and M803 (UK)	NOEC = 7.10 mg/kg dw	EFSA conclusion 2014 (McCormac, A./2012/DAS 110605)
<i>Folsomia candida</i>	Halauxifen-methyl	Mixed into substrate 28 d, chronic 5 % peat content	NOEC _{corr} = 500 mg/kg dw*	EFSA conclusion 2014 (Gerke, A./2011/DAS 090181)
<i>Folsomia candida</i>	Halauxifen acid	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 25 mg/kg dw	EFSA conclusion 2014 (Witte, B./2011/DAS 102024)
<i>Folsomia candida</i>	X11449757	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 2.5 mg/kg dw	EFSA conclusion 2014 (Gerke, A./2011/DAS 101153)
<i>Hypoaspis aculeifer</i>	Halauxifen-methyl	Mixed into substrate 14 d, chronic 5 % peat content	NOEC _{corr} = 12.5 mg/kg dw*	EFSA conclusion 2014 (Luhrs, U./2011/DAS 110280)
<i>Hypoaspis aculeifer</i>	Halauxifen acid	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 12.5 mg/kg dw	EFSA conclusion 2014 (Witte, B./2011/DAS 102025)

Species	Substance	Exposure System	Results	Reference
<i>Hypoaspis aculeifer</i>	X11449757	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 25 mg/kg dw	EFSA conclusion 2014 (Witte, B./2011/DAS 101154)
Field studies				
N/A				
Litter bag test				
N/A				

*Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002, since the Log K_{ow} of halauxifen-methyl is higher than 2 (log K_{ow} = 3.76). EFSA Journal 2014;12(12):3913

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

Species	Substance	Exposure System	Results	Reference
<i>Eisenia fetida</i>	picloram	Mixed into substrate 14 d, acute 10 % peat content	LC ₅₀ > 4475 mg ae/kg dw	EFSA Conclusion 2009 (Boeri, R. L. and Ward, T.J. /2002/ DAS 011175)
<i>Eisenia fetida</i>	picloram	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 0.167 mg ae/kg dw	EFSA Conclusion 2009 (Mallett, M.J. /2001/ DAS GHE T-1148)
Litter bag test				
Not needed				

EFSA Journal 2009; 7(12):1390

Table 9.8-3: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) – aminopyralid

Species	Substance	Exposure System	Results	Reference
<i>Eisenia fetida</i>	aminopyralid	Mixed into substrate 14 d, acute 10 % peat content	LC ₅₀ > 1000 mg a.e./kg dw	EFSA Conclusion 2013 (Ward, T.J. and Boeri, R.L. /2001/ DAS 011049)
<i>Eisenia fetida</i>	aminopyralid	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 3.2 mg ae/kg dw	EFSA Conclusion 2013 (Davies, N. /2004/ DAS 040285)
Litter bag test				
Not needed				
Field studies				
Not needed				

EFSA Journal 2013;11(9): 3352

Table 9.8-4: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) – GF-4021

Species	Substance	Exposure System	Results	Reference
<i>Eisenia fetida</i>	GF-4021	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 40 mg/kg dw NOEC _{corr} = 20 mg/kg dw	McCormac, A/ 2020 /DAS 190475
Litter bag test				
Not needed				
Field studies				
Not needed				

Acceptable risk was concluded in the NTA assessment at Tier 1, therefore, testing on soil mites and collembola were not conducted.

zRMS comments:

Toxicity data for halauxifen-methyl, picloram and aminopyralid provided in Tables 9.8-1 to 9.8-3 are in line with EU agreed endpoints reported in EFSA Journal 2014;12(12):3913, EFSA Journal 2009;7(12):1390, and EFSA Journal 2013;11(9):3352, respectively. Information on acute toxicity has been struck through in tables above as being no longer a data requirement.

Study on chronic toxicity of GF-4021 to earthworms was evaluated and agreed by the zRMS. Study summary together with zRMS evaluation are presented in Appendix 2. Endpoint reported in Table 9.8-4 is confirmed to be correct, however due to log Pow of halauxifen-methyl being >2, corrected NOEC is considered relevant for the risk assessment purposes.

Endpoints for *Folsomia candida* and *Hypoaspis aculeifer* are available only from the EU review of halauxifen methyl. Studies on effects of picloram and aminopyralid on these species were not required at the EU level. No study was also performed with the formulation GF-4021. It should be, however, noted that in line with data requirements set by the Commission Regulation (EU) No 284/2013:

For plant protection products applied as a foliar spray, data on the relevant two non target arthropod species might be taken into account for a preliminary risk assessment. If effects do occur on either species, testing on Folsomia candida and Hypoaspis aculeifer shall be required (see point 10.4.2.1).

As acceptable in- and off-field risk to *Typhlodromus pyri* and *Aphidius rhopalosiphii* from GF-4021 (within this submission) and picloram and aminopyralid (at the EU level) could be concluded based on the Tier I data with no concerns and GF-4021 is not applied directly to soil, in line with the current legislation studies performed with *Folsomia candida* and *Hypoaspis aculeifer* are not mandatory and their waiving is justified.

9.8.1.1 Justification for new endpoints

Not relevant.

9.8.2 Risk assessment

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

9.8.2.1 First-tier risk assessment

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3. According to the assessment of environmental-fate data, multi-annual accumulation in soil should be considered for halauxifen acid and X11449757.

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-4021 in winter oilseed rape – halauxifen-methyl

Intended use	Winter oilseed rape, BBCH 12-19, 1 x 2.5 g a.s./ha		
Acute effects on earthworms			
Product/active substance	LC ₅₀ (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _a (criterion TER ≥ 10)
Halauxifen-methyl	> 500 _{corr}	0.0020	250000
Halauxifen acid	> 1000	0.0009*	111111
X11449757	> 1000	0.0003*	333333
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Halauxifen-methyl	5 _{corr}	0.0020	2500
Halauxifen acid	10	0.0009*	11111
X11449757	10	0.0003*	33333
NER	7.1	0.0017	4176
Chronic effects on other soil macro- and mesofauna			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Folsomia candida			
Halauxifen-methyl	500 _{corr}	0.0020	250000
Halauxifen acid	25	0.0009*	27778
X11449757	2.5	0.0003*	8333
Hypoaspis aculeifer			
Halauxifen-methyl	12.5 _{corr}	0.0020	6250
Halauxifen acid	12.5	0.0009*	13889
X11449757	25	0.0003*	83333

TER values shown in bold fall below the relevant trigger.

*PECaccumulation value used in assessment

Table 9.8-6: 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-4021 in winter oilseed rape – picloram

Oilseed rape - picloram			
Intended use	Winter oilseed rape, BBCH 12-19, 1 x 12.0 g a.s./ha		
Acute effects on earthworms			
Product/active substance	LC ₅₀ (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _a (criterion TER ≥ 10)
Picloram	4475	0.0096	466146
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Picloram	0.167	0.0096	17

TER values shown in bold fall below the relevant trigger.

*PECaccumulation value used in assessment

Table 9.8-7: 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-4021 in winter oilseed rape – aminopyralid

Intended use	Winter oilseed rape, BBCH 12-19, 1 x 8.0 g a.s./ha		
Acute effects on earthworms			
Product/active substance	LC ₅₀ (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _a (criterion TER ≥ 10)
Aminopyralid	>1000	0.0064	156250
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Aminopyralid	3.2	0.0064	500

TER values shown in bold fall below the relevant trigger.

Table 9.8-8: 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-4021 in winter oilseed rape

Intended use	Winter oilseed rape, BBCH 12-19, 1 x 236.5 g product/ha (0.25 L/ha)		
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
GF-4021	2040	0.1892	106.211

TER values shown in bold fall below the relevant trigger.

zRMS comments:

The risk assessment for soil macro- and meso-fauna performed for particular active compounds and their relevant metabolites in Tables 9.8-5 to 9.8-7 is agreed by the zRMS. Due to formation of high level of non-extractable residues and available EU agreed endpoint for earthworms, the risk assessment for halauxifen-methyl NER was added to Table 9.8-5.

The risk assessment for the formulated product presented in Table 9.8-8 was amended accordingly, since due to halauxifen-methyl log Pow >2, the corrected formulation endpoint should have been used. Since none of the active compounds is expected to accumulate in soil, it was justified to base the risk assessment on formulation endpoints and initial formulation PEC_{SOIL} instead of the endpoint expressed in terms of the sum of active substances compared with PEC_{mix} calculated as the sum of the initial and/or accumulated PEC_{SOIL} for particular active compounds.

Overall, acceptable risk to soil macro- and meso-fauna from particular active compounds, their metabolites, halauxifen-methyl non-extractable residues and formulation may be concluded for the intended Central Zone uses of GF-4021.

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

Regulatory testing has been conducted with halauxifen-methyl, aminopyralid, picloram and the product in accordance with EU requirements. All ~~acute and~~ long-term TER values were calculated to be in excess of the accepted trigger value of ~~10 and 5 respectively~~ therefore, an acceptable risk for non-target soil meso- and macrofauna was concluded for the intended Central Zone uses of GF-4021.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

Studies on effects soil microorganisms have been carried out with halauxifen-methyl, picloram, aminopyralid and their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on soil microorganisms of GF-4021 were not evaluated as part of the EU assessment of halauxifen-methyl, picloram and aminopyralid. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2. According to the assessment of environmental-fate data, multi-annual accumulation in soil should be considered for halauxifen acid and X11449757.

Table 9.9-1: Endpoints and effect values relevant for the risk assessment for soil microorganisms – halauxifen-methyl

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Halauxifen-methyl	28 d, aerobic soil type	Nitrate formation rate < 25 % at 0.0535 mg/kg soil dw	EFSA Conclusion, 2014 (Feil, N. /2011/ DAS 101127)
C-mineralisation	Halauxifen-methyl	28 d, aerobic soil type	CO ₂ -formation rate < 25 % at 0.0535 mg/kg soil dw	EFSA Conclusion, 2014 (Feil, N. /2011/ DAS 101127)
N-mineralisation	Halauxifen acid	28 d, aerobic soil type	Nitrate formation rate < 25 % at 0.05 mg/kg soil dw	EFSA Conclusion, 2014 (Feil, N. /2010/ DAS 101143)
C-mineralisation	Halauxifen acid	28 d, aerobic soil type	CO ₂ -formation rate < 25 % at 0.05 mg/kg soil dw	EFSA Conclusion, 2014 (Feil, N. /2010/ DAS 101143)
N-mineralisation	X11449757	28 d, aerobic soil type	Nitrate formation rate < 25 % at 0.052 mg/kg soil dw	EFSA Conclusion, 2014 (Feil, N. /2011/ DAS 101157)
C-mineralisation	X11449757	28 d, aerobic soil type	CO ₂ -formation rate < 25 % at 0.052 mg/kg soil dw	EFSA Conclusion, 2014 (Feil, N. /2011/ DAS 101157)

EFSA Journal 2014;12(12):3913

Table 9.9-2: Endpoints and effect values relevant for the risk assessment for soil microorganisms - picloram

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	picloram	28 d, aerobic sandy-loam soil	Nitrate formation rate < 25 % at 0.167 mg ae/kg soil dw	EFSA Conclusion 2009 (Mallett M.J., 2001/DAS GHE T-1158)
C-mineralisation	picloram	28 d, aerobic sandy-loam soil	CO ₂ -formation rate < 25 % at 0.167 mg ae/kg dw	EFSA Conclusion 2009 (Mallett M.J., 2001/DAS GHE T-1158)

EFSA Journal 2009; 7(12):1390

Table 9.9-3: Endpoints and effect values relevant for the risk assessment for soil microorganisms - aminopyralid

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	aminopyralid	28 d, aerobic sandy-loam soil	No effects at aminopyralid rates up to 100 times (8.4 mg a.s./kg dry soil (6000 g a.s./ha) the treatment rate for grasslands. Effects <25% on nitrogen mineralisation (nitrogen transformation and nitrate production) at 28 days. Endpoint calculated in terms of nitrogen transformation rates not available. Nitrate formation rate <25 % at 8.4 mg ae/kg soil dw	EFSA Conclusion 2013 (McMurray, A, 2002/DAS GHE T-1180)
C-mineralisation	aminopyralid	28 d, aerobic sandy-loam soil	CO ₂ -formation rate <25 % at 8.4 mg ae/kg soil dw	EFSA Conclusion 2013 (McMurray, A, 2002/DAS GHE T-1180)

EFSA Journal 2013;11(9): 3352

Table 9.9-4: Endpoints and effect values relevant for the risk assessment for soil microorganisms – GF-4021

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	GF-4021	28 d, aerobic sandy-loam soil	Nitrate formation rate < 25 % at 1.58 mg prod./kg soil dw	Hammesfahr, U./ 2020/ DAS 190194
C-mineralisation	GF-4021	28 d, aerobic sandy-loam soil	CO ₂ -formation rate <25 % at 1.58 mg prod./kg soil dw	Hammesfahr, U./ 2020/ DAS 190194

zRMS comments:

Toxicity data for halauxifen-methyl, picloram and aminopyralid provided in Tables 9.9-1 to 9.9-3 are in line with EU agreed endpoints reported in EFSA Journal 2014;12(12):3913, EFSA Journal 2009;7(12):1390, and EFSA Journal 2013;11(9):3352, respectively. Data reported for aminopyralid was amended accordingly to be in line with conclusions presented in the EFSA report. Information on effects on carbon mineralisation has been struck through in tables above as being no longer a data requirement.

Study on effects of GF-4021 soil nitrogen transformation was evaluated and agreed by the zRMS. Study summary studies together with zRMS evaluation are presented in Appendix 2. Endpoint reported in Table 9.9-4 is confirmed to be correct.

9.9.1.1 Justification for new endpoints

Not relevant.

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 (Environmental Fate), Chapter 8.7.2, Table 8.7-3 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.8).

Table 9.9-5: Assessment of the risk for effects on soil micro-organisms due to the use of GF-4021 in winter oilseed rape – halauxifen-methyl

Intended use		Winter oilseed rape	
N-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
Halauxifen-methyl	0.0535 (at 28 d)	0.0020	yes
Halauxifen acid	0.05 (at 28 d)	0.0009*	yes
X11449757	0.052 (at 28 d)	0.0003*	yes
C-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
Halauxifen-methyl	0.0535 (at 28 d)	0.0020	yes
Halauxifen acid	0.05 (at 28 d)	0.0009*	yes
X11449757	0.052 (at 28 d)	0.0003*	yes

*PECaccumulation value used in assessment

Table 9.9-6: Assessment of the risk for effects on soil micro-organisms due to the use of GF-4021 in winter oilseed rape - picloram

Intended use	Winter oilseed rape		
N-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg ae/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
Picloram	8.4 (at 28 d)	0.0064	yes
C-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg ae/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
Picloram	8.4 (at 28 d)	0.0064	yes

Table 9.9-7: Assessment of the risk for effects on soil micro-organisms due to the use of GF-4021 in winter oilseed rape - aminopyralid

Intended use		Winter oilseed rape	
N-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg ae/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
Aminopyralid	0.167 (at 28 d)	0.0096	yes
C-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg ae/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
Aminopyralid	0.167 (at 28 d)	0.0096	yes

Table 9.9-8: Assessment of the risk for effects on soil micro-organisms due to the use of GF-4021 in winter oilseed rape – GF-4021

Intended use		Winter oilseed rape	
N-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
GF-4021	1.58 (at 28 d)	0.1892	yes
C-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
GF-4021	1.58 (at 28 d)	0.1892	yes

zRMS comments:

The risk assessment for soil microorganisms performed for particular active compounds, their relevant metabolites and formulation GF-4021 in Tables 9.9-5 to 9.9-8 is agreed by the zRMS.

Since none of the active compounds is expected to accumulate in soil, it was justified to base the risk assessment on formulation endpoints and initial formulation PEC_{SOIL} instead of the endpoint expressed in terms of the sum of active substances compared with PEC_{mix} calculated as the sum of the initial and/or accumulated PEC_{SOIL} for particular active compounds.

Overall, no unacceptable effects of particular active compounds, their metabolites and formulation on the soil microbial activity are expected following the intended Central Zone uses of GF-4021.

9.9.3 Overall conclusions

Regulatory testing with soil microorganisms has been conducted with halauxifen-methyl, picloram, aminopyralid and the product in accordance with EU requirements. Results from these studies, indicate that all effect values were well above the PEC_{soil} values, therefore, a low risk for soil microorganisms was concluded.

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 the major soil metabolites of halauxifen-methyl (halauxifen-acid and X11449757). Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target terrestrial plants of GF-4021 were not evaluated as part of the EU assessment of halauxifen-methyl, picloram or aminopyralid. 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
<i>Daucus carota</i> (Carrot) 8 dicot + 3 monocot species tested	Halauxifen acid	21 d Seedling emergence	ER ₅₀ = 0.3835 g ae /ha	EFSA Conclusion, 2014 (Rockliff, C./2011/DAS 101955)
8 dicot + 3 monocot species tested	X11449757	21 d Seedling emergence	ER ₅₀ > 15.0 g ae /ha*	EFSA Conclusion, 2014 (Rockliff, C./2011/101956)
<i>Lycopersicon esculentum</i> (Tomato)	GF-4021	21 d Seedling emergence	ER ₅₀ = 14.1 mL/ha fresh weight ER ₅₀ = 17.2 mL/ha visual injury	Bramby-Gunary, J./2020/DAS 190546
<i>Lycopersicon esculentum</i> (Tomato)	GF-4021	21 d Vegetative Vigour	ER ₅₀ = 2.68 mL/ha fresh weight ER ₅₀ = 4.07 mL/ha visual injury	Bramby-Gunary, J./2020/DAS 190545
4 monocotyledon and 7 dicotyledon species tested	GF-4021	21 d Vegetative vigour	HR ₅ = 3.21 mL prod./ha fresh weight HR ₅ = 1.70 mL prod./ha visual injury	Calculated by the Applicant

*All species tested showed no effect at the highest tested application rate of 15 g ae/ha.

zRMS comments:

Toxicity data for halauxifen-acid and metabolite X11449757 provided in Table 9.10-1 are in line with EU agreed endpoints reported in EFSA Journal 2014;12(12):3913.

Studies on effects of GF-4021 on seedling emergence and vegetative vigour of non-target terrestrial plants were evaluated and agreed by the zRMS. Summaries of the studies together with zRMS evaluation are presented in Appendix 2. Endpoints (ER₅₀ values) reported in Table 9.10-1 are confirmed to be correct.

The HC₅ reported in Table 9.10-1 deviate from values provided in point 9.10.2.3 and are thus struck through. For details of derivation of relevant HC₅ for probabilistic risk assessment, please refer to point 9.10.2.3 below. Please note that the eventually used endpoints were these derived by the zRMS and not Applicants' values.

9.10.1.1 Justification for new endpoints

Data on the toxicity of the formulation to terrestrial non target plants is available and is used in the risk assessment.

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)

The risk assessment is based on the “Guidance Document on Terrestrial Ecotoxicology”, (SANCO/10329/2002 rev. 2 final, 2002). It is restricted to off-field situations, as non-target plants are non-crop plants located outside the treated area.

Table 9.10-2: Assessment of the risk for non-target plants due to the use of GF-4021 in winter oilseed rape.

Intended use		Winter oilseed rape		
Active substance/product		GF-4021		
Application rate (mL/ha)		1 x 250		
MAF		1		
Test species	ER₅₀ (mL/ha)	Drift rate	PER_{off-field} (mL/ha)	TER criterion: TER ≥ 5
Tomato, shoot fresh weight Seedling emergence	14.1	2.77%	6.925	2.04
Tomato, shoot fresh weight Vegetative vigour	2.68	2.77%	6.925	0.39

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:

The risk assessment for non-target terrestrial plants presented in Table 9.10-2 above is agreed by the zRMS.

TER values based on the lowest ER₅₀ values for vegetative vigour and seedling emergence are below the trigger of 5 indicating potentially unacceptable risk. Further evaluation is performed in points 9.10.2.3 and 9.10.2.4 below.

9.10.2.3 Higher-tier risk assessment

A sufficient number of endpoints (i.e. at least six) is available from the seedling emergence and vegetative vigour studies with GF-4021 to use a probabilistic risk assessment approach for these datasets (Guidance Document on Terrestrial Ecotoxicology, SANCO/10329/2002). Probabilistic methods that make use of species sensitivity distributions (SSD) may be used when at least 6-10 species have been tested and the SSD toxicity data fit a log-normal distribution. For a few of the test species in the GF-4021 studies the ER₅₀ values were determined to be >500 mL/ha. In these cases, in line with the recommendations of the Guidance on tiered risk assessment for edge-of-field surface waters (EFSA Journal 2013;11(7):3290; section 8.4.2 Criteria for the selection of toxicity data to construct species sensitivity distributions), one value of 500 mL/ha has been included in the derivation of the SSD. The SSD was built using ETX v. 2.1 developed by RIVM (Rijksinstituut voor Volksgezondheid en Milieu, The Netherlands). The data was tested for the Goodness of Fit prior to the analysis and resulted normally distributed according to the three tests available in the software (i.e. Kolmogorov Smirnov, Cramer Von Mises and Anderson Darling). After the SSD was built, the HC₅ in the distribution was determined. HC₅ values were derived based on the fresh weight and visual injury data from the vegetative vigour and seedling emergence studies and are summarized in the following table. For brevity only the graph of the SSD resulting in the lowest HC₅ is shown below.

Table 9.10-3: Results of HC₅ determination for non-target terrestrial plants exposed to GF-4021 (value used in the risk assessment in bold)

Substance	Study type	Parameter	HC ₅ estimates (mL/ha)		
			Lower	Median	Upper
GF-4021	Vegetative vigour	Visual injury	0.458	3.35	10.7
	Vegetative vigour	Fresh weight	0.380	2.81	8.35
	Seedling emergence	Visual injury	3.34	13.3	29.1
	Seedling emergence	Fresh weight	2.38	12.3	30.0

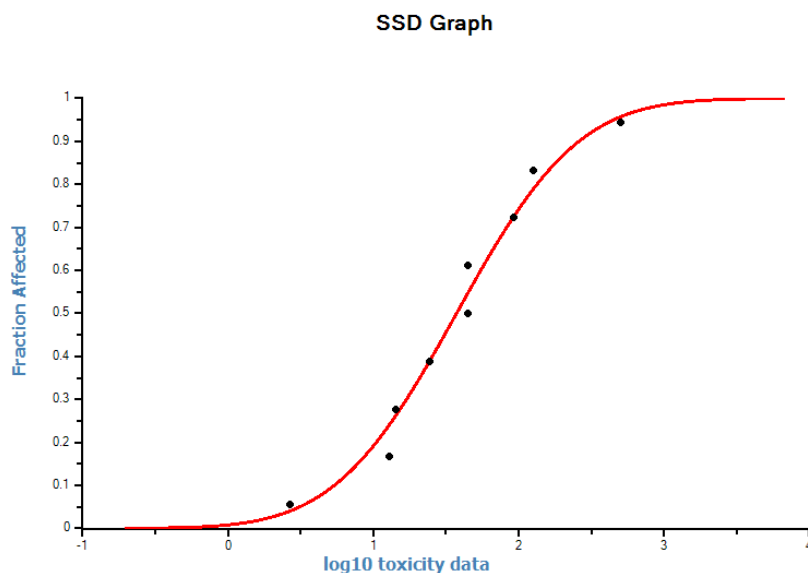


Figure 9.10-1: Species Sensitivity Distribution for fresh weight ER₅₀ from the vegetative vigour for GF-4021

The Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002) states that if the calculated 5th percentile ER₅₀ from the SSD is above the predicted exposure level, the level of risk to terrestrial plant populations adjacent to the treated fields is considered acceptable. Therefore, if expressed in terms of a TER, which is based on use of the 5th percentile ER₅₀ from the SSD as the toxicity value, a TER ≥ 1 indicates that risk to terrestrial non-target plants is within an acceptable level. TER values are calculated based on the lowest HC₅ above and accounting for different risk mitigation options in Section 9.10.2.4.

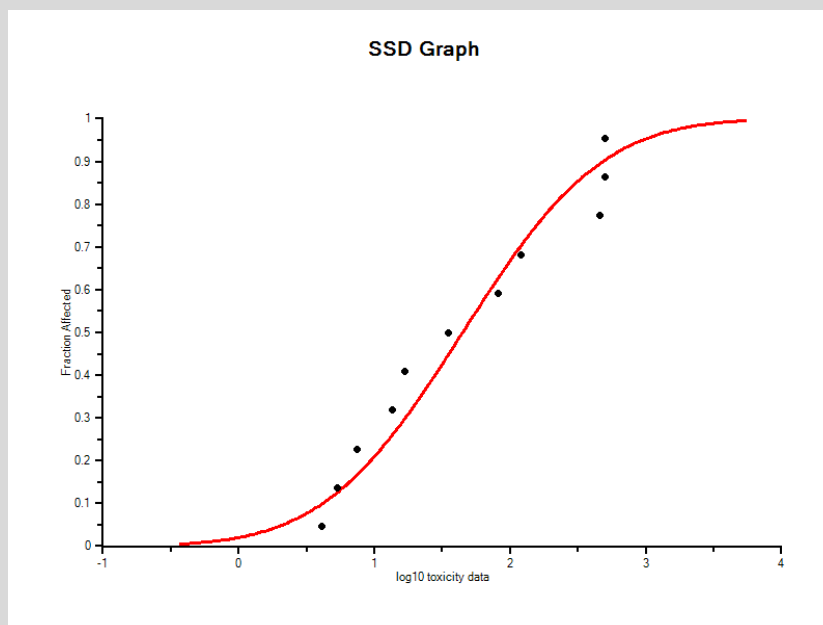
zRMS comments:

The HR₅ values derived by the Applicant were independently validated by the zRMS using the same tool (ETX 2.3).

All data passed the tests for normality (Anderson-Darling, Kolmogorov-Smirnov and Cramer von Mises) build in the ETX 2.3 tool.

The same HR₅ as reported in Table 9.10-3 were estimated by the zRMS for the fresh weight (vegetative vigour and seedling emergence) and phytotoxicity (seedling emergence). However, based on phytotoxicity endpoints for particular species in the vegetative vigour study lower HR₅ was obtained by the zRMS (see table below).

Substance	Study type	Parameter	HR ₅ estimates (mL/ha)		
			Lower	Median	Upper
GF-4021	Vegetative vigour	Visual injury	0.242	1.930	6.499



Due to differences between Applicants' and zRMS results, performed calculations were double checked and each time the median HR₅ of 1.93 mL/ha was obtained by the zRMS. The reason for this difference is unknown, especially from the above description it seems that the same inputs were used. It should be, however, noted that in Table 9.10-1 even lower HR₅ of 1.70 mL/ha was reported by the Applicant for phytotoxicity from vegetative vigour test, so there must have been some mistake made in the calculations performed later by the Applicant and inserted in point 9.10.2.3 of this report.

Overall, the median HR₅ of 1.93 mL/ha calculated by the zRMS for phytotoxicity in vegetative vigour test is considered relevant for purposes of the probabilistic risk assessment.

9.10.2.4 Risk mitigation measures

In order to reduce the off-field exposure, risk mitigation measures can be implemented. These correspond to unsprayed in-field buffer strips of a given width and/or the usage of drift reducing nozzles. The results of the risk assessment using typical mitigation measures (no-spray buffer zones of 5 or 10 m; drift-reducing nozzles with reduction by 50 %, 75 %, or 90 %) are summarised in the following table.

Table 9.10-4: Probabilistic risk assessment for non-target terrestrial plants due to the use of GF-4021 in winter oilseed rape considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Winter oilseed rape			
Active substance/product		GF-4021			
Application rate (mL/ha)		1 × 250			
MAF		1			
Buffer strip (m)	Drift rate (%)	PER_{off-field} (mL/ha)	PER_{off-field} 50% drift red. (mL/ha)	PER_{off-field} 75% drift red. (mL/ha)	PER_{off-field} 90% drift red. (mL/ha)
1	2.77	6.93	3.46	1.73	0.69
5	0.57	1.43	0.71	0.36	0.14
10	0.29	0.73	0.36	0.18	0.07
Toxicity value		TER criterion: TER ≥ 1			
HR ₅ = 2.81 mL/ha					
1		0.41	0.81	1.62	4.06
5		1.97	3.94	7.89	19.7
10		3.88	7.75	15.5	38.8

MAF: Multiple application factor; PER: Predicted environmental rates; TER: toxicity to exposure ratio. Criteria values shown in bold breach the relevant trigger.

zRMS comments:

The probabilistic risk assessment presented in Table 9.10-4 above was struck through since lower HR₅ has been derived by the zRMS based on phytotoxicity endpoints derived in the vegetative vigour study (for details, please refer to point 9.10.2.3 above). The recalculated TER values based on the agreed endpoint are presented in table below. Since not all cMS accept the SSD approach for non-target terrestrial plants, separate calculations based on the lowest ER₅₀ derived from both, vegetative vigour and seedling emergence studies, are presented below.

Intended use		Winter oilseed rape			
Active substance/product		GF-4021			
Application rate (mL/ha)		1 × 250			
MAF		1			
Buffer strip (m)	Drift rate (%)	PER_{off-field} (mL/ha)	PER_{off-field} 50% drift red. (mL/ha)	PER_{off-field} 75% drift red. (mL/ha)	PER_{off-field} 90% drift red. (mL/ha)
1	2.77	6.93	3.46	1.73	0.69
5	0.57	1.43	0.71	0.36	0.14
10	0.29	0.73	0.36	0.18	0.07
Toxicity value		TER criterion: TER ≥ 5			
HR ₅₀ = 2.68 mL/ha (standard endpoint)					
1		0.39	0.77	1.55	3.88
5		1.87	3.77	7.44	19.1
10		3.67	7.44	14.9	38.3
Toxicity value		TER criterion: TER ≥ 1			
HR ₅ = 1.93 mL/ha (based on SSD)					
1		0.28	0.56	1.12	2.79
5		1.35	2.72	5.36	13.8
10		2.64	5.36	10.7	27.6

Based on the above calculations acceptable risk to non-target terrestrial plants may be concluded from the intended Central Zone uses of GF-4021, provided that following risk mitigation measures are respected:

1. Standard risk assessment:

- 10 m unsprayed buffer zone to non-agricultural land combined with 50% drift reduction,
- 5 m unsprayed buffer zone to non-agricultural land combined with 75% drift reduction

2. Probabilistic risk assessment:

- 5 m unsprayed buffer zone to non-agricultural land, or
- 75% drift reduction.

Concerned Member States must decide on applicability of proposed risk mitigation measures in their countries.

9.10.3 Overall conclusions

Regulatory testing has been conducted with the product according to EU requirements. Risk assessment was performed using standard and probabilistic approach. Overall, acceptable risk to non-target terrestrial plants could be concluded from the intended uses of GF-4021, provided that following risk mitigation measures are respected:

1. Standard risk assessment:

- 10 m unsprayed buffer zone to non-agricultural land combined with 50% drift reduction,
- 5 m unsprayed buffer zone to non-agricultural land combined with 75% drift reduction

2. Probabilistic risk assessment:

- 5 m unsprayed buffer zone to non-agricultural land, or
- 75% drift reduction.

Concerned Member States must decide on applicability of proposed risk mitigation measures in their countries.

~~The TER value calculated using the HR₅ value determined based on the vegetative vigour data for GF-4021 is greater than the relevant trigger of 1 and, therefore, an acceptable risk can be concluded, when considering any of the below mitigation measures:~~

- ~~• 1 m buffer zone with 75% drift reducing nozzles or~~
- ~~• 5 m buffer zone with without drift reducing nozzles.~~

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

No effects on other terrestrial organisms are anticipated if the previously proposed risk mitigations are implemented during applications of GF-4021 in winter oilseed rape.

9.12 Monitoring data (KCP 10.8)

Monitoring studies are not available for halauxifen-methyl, picloram, aminopyralid and GF-4021 and are not considered necessary in light of the acceptable risk concluded for all non-target organisms from uses of GF-4021 in winter oilseed rape at a single rate of 1×0.25 L./ha.

9.13 Classification and Labelling

Table 9.13-1: Justification for Classification and Labelling of GF-4021



Hazard symbols		
		
Triggered by H410		
Hazard statements		
Chronic aquatic Cat 1	H410	Triggered by study data (NOEC for <i>M. spicatum</i> <0.1 mg/L and substances in GF-4021 not readily biodegradable)
Precautionary statements		
P391		Mandatory phrase (H410)
P501		Mandatory Recommended phrase (H410)
EU specific statements		
EUH401		All plant protection products subject to 1107/2009/EC shall also include this phrase.

Table 9.13-2: Classification Proposal for GF-4021

Hazard symbols		
		
GHS09		
Hazard statements		
Chronic aquatic Cat 1	H410	Very toxic to aquatic life with long lasting effects.
Precautionary statements		
P391		Collect spillage
P501		Dispose of contents/container in accordance with applicable regulations.
EU specific statements		
EUH401		To avoid risks to human health and the environment, comply with the instructions for use.

zRMS comments:

CLP classification of GF-4021 provided by the Applicant above is agreed by the zRMS. Additional information has been added by the zRMS for completeness. It is also noted that in case the substance/mixture is classified as H410, precautionary statements P391 and P501 are mandatory.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.1/01	Banman, C.S.; Moore, S.	2015	Picloram: Toxicity to the Aquatic Macrophyte, <i>Myriophyllum spicatum</i> . DAS Report No.: 140737. SynTech Research Laboratory Services LLC. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2.1/02	Gonsior, G.	2015	Picloram metabolite 5,6-dichloropicloram: Growth Inhibition of <i>Myriophyllum spicatum</i> in a Water/Sediment System. DAS Report No.: 150390. Eurofins Agroscience Services EcoChem GmbH. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2.1/03	Eser, S.	2020	GF-4021: Growth Inhibition of <i>Myriophyllum spicatum</i> in a Water/Sediment System. DAS Report No.: 190151. Eurofins Agroscience Services Ecotox GmbH. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2.1/04	Goudie, O.	2020	GF-4021: A 72-Hour Toxicity Test with the Freshwater Alga, <i>Raphidocelis subcapitata</i> . DAS Report No.: 190111. Eurofins EAG Agroscience, LLC. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.3.1.1/01 KCP 10.3.1.2/01	Tomé, H.V.V.; Porch, J.R.	2020	GF-4021: An Acute Oral and Contact Toxicity Study with Honey Bee. DAS Report No.: 190458. Eurofins EAG Argoscience LLC GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.3.1.2/01	Wendling, K.	2021a	GF-4021 - Honey Bee (<i>Apis mellifera</i> L.) Chronic Oral Toxicity Test 10 Day Feeding Test in the Laboratory. DAS Report No.: 200622. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)

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 10.3.1.3/01	Wendling, K.	2021b	GF-4021: – Honey Bee (<i>Apis mellifera</i> L.) 22 Day Larval Toxicity Test (Repeated Exposure). DAS Report No.: 200623. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.3.2/01	Fallowfield, L.	2020	GF-4021: A Rate-Response Laboratory Study of the Effects of Fresh Residues on the Predatory Mite <i>Typhlodromus pyri</i> (Acari: Phytoseiidae). DAS Report No.: 190467. Mambo-Tox A Division of Cawood Scientific Ltd. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.3.2/02	Stevens, J.	2020	GF-4021: A Rate-Response Laboratory Study of the Effects of Fresh Residues on the Parasitic Wasp <i>Aphidius rhopalosiphii</i> (Hymenoptera, Braconidae). DAS Report No.: 190464. Mambo-Tox A Division of Cawood Scientific Ltd. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.4.1.1/01	McCormac, A.	2020	GF-4021: Determination of Chronic Toxicity to the Earthworm <i>Eisenia andrei</i> (Oligochaeta: Lumbricidae) in an artificial soil substrate. DAS Report No.: 190475. Mambo-Tox A Division of Cawood Scientific Ltd. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.5/01	Hammesfahr, U.	2020	GF-4021: Effects on the Activity of the Soil Microflora in the Laboratory. DAS Report No.: 190194. Ibacon GmbH GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.6.2/01	Bramby-Gunary, J.	2020a	GF-4021 Seedling Emergence and Seedling Growth Terrestrial Non Target Plants. DAS Report No.: 190546. AgroChemex Ltd. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)

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 10.6.2/02	Bramby-Gunary, J.	2020b	GF-4021 Vegetative Vigour Terrestrial Non Target Plants. DAS Report No.: 190545. AgroChemex Ltd. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)

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

zRMS comments:

As most endpoints for halauxifen-methyl, picloram and aminopyralid as well as their relevant metabolites were taken from the EU review, for the list of respective studies please refer to Volume 2 of the RAR for particular substances. The list below was not validated.

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 (KCA 8.1.1.1)	...	2011	XDE-729 Methyl: An Acute Oral Toxicity Study with the Northern Bobwhite. DAS Report No.: 090026, 379-211 Wildlife International, Ltd. GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.1 (KCA 8.1.1.1)	...	2011	XDE-729 Methyl: An Acute Oral Toxicity Study with the Zebra Finch (<i>Poephila guttata</i>) DAS Report No.:090027, 379-212 Wildlife International, Ltd. GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.1 (KCA 8.1.1.1)	1986	Picloram Potassium Salt: An Acute Oral Toxicity Study with the Mallard DAS Report No.: ES-DR-0049-3936-5, ES-835 Wildlife International GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.1 (KCA 8.1.1.1)	...	2001	XDE-750: An Acute Oral Toxicity Study with the Northern Bobwhite. DAS Report No.: 011046 GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.1 (KCA 8.1.1.2)	...	2011	XDE-729 Methyl: A dietary LC50 study with the Mallard DAS Report No.: 090029, 379-214. Wildlife International, Ltd. GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)

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 (KCA 8.1.1.2)	...	2011	XDE-729 Methyl: A dietary LC50 study with the Northern Bobwhite. DAS Report No.: 090028. Wildlife International, Ltd. GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.1 (KCA 8.1.1.2)	...	1986	Picloram Potassium Salt: A Dietary LC50 Study with the Bobwhite DAS Report No.: 103-244 ... GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.1 (KCA 8.1.1.2)	...	2001	XDE-750: A Dietary LC50 Study with the Northern Bobwhite. DAS Report No.: 011047. ... GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.1 (KCA 8.1.1.3)	...	2011a	XDE-729 Methyl: A reproduction study with the Northern Bobwhite DAS Report No.: 101137, 379-246 ... GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.1 (KCA 8.1.1.3)	...	2011b	XDE-729 Methyl: A reproduction study with the Mallard DAS Report No.: 101139, 379-247. GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.1 (KCA 8.1.1.3)	2002	Avian Reproduction Study with Picloram Acid in Northern Bobwhite (<i>Colinus virginianus</i>). DAS Report No.: K-038323-117, 011172, 01014. GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)

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 (KCA 8.1.1.4)	...	2003	Avian Reproduction Study with XDE-750 in Northern Bobwhite Quail (<i>Colinus virginianus</i>). DAS Report No.: 011271. GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.1.2 (KCA 5.2.1)	...	2011	XDE-729 Methyl Technical Grade Active Ingredient: Acute Oral Toxicity Up and Down Procedure in Rats. DAS Report No.: 110543. GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.1.2 (KCA 5.2.1)	...	1987	Picloram Acid (Picloram Technical): Acute Oral Toxicity Study in Fischer 344 Rats. DAS Report No.: K-038323-042A. GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.1.2 (KCA 5.6.11)	2012	XDE-729 Methyl: Developmental toxicity study in New Zealand white rabbits. DAS Report No.: 111137 ... GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.1.2 (KCA 5.6.2)	...	1992	Picloram Triisopropanolamine Salt: Oral Gavage Teratology Study in New Zealand White Rabbits. DAS Report No.: K-049877-015. GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.1.2 (KCA 5.2.1)	2001	XDE-750: Acute Oral Toxicity Study in Fischer 344 Rats. DAS Report No.: 011115. GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)

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 10.1.2 (KCA 5.10)	2004	Oral Gavage Developmental Toxicity Study in New Zealand White Rabbits. DAS Report No.: 031142. The Dow Chemical Company. GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.2.1.1)	2011a	XDE-729 Methyl: Acute toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions. DAS Report No.: 090187, 64605. GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.2.1)	...	2001	Picloram Acid: A 96-Hour Static Acute Toxicity Test with the Rainbow Trout (<i>Oncorhynchus mykiss</i>). DAS Report No.: K-038323-122, 379A-103. ... GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.2.1)	2001a	Picloram Acid: A 96-Hour Static Acute Toxicity Test with the Bluegill (<i>Lepomis macrochirus</i>) DAS Report No.: K-038323-123, 011195, 379A-102. GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.2.1.2)	...	2011a	XDE-729 Methyl: Acute Toxicity to the Fathead Minnow, <i>Pimephales promelas</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 090186, 64604. GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.2.1.2)	2011b	XDE-729 Methyl: Acute Toxicity to the Sheepshead Minnow, <i>Cyprinodon variegatus</i> , Determined Under Flow-Through Conditions DAS Report No.: 090188, 64606. GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)

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 (KCA 8.2.1.3)	...	2011b	XDE-729 Acid: Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions. DAS Report No.: 101152, 65970. GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.2.1.3)	2011a	X11449757: Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 101166, 66008. GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.2.1.3)	2012a	X11406790 (XDE-729 Metabolite): Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 120020, 68212. GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.2.1)	2001	XDE-750 Herbicide: An Acute Toxicity Study with the Rainbow Trout, <i>Oncorhynchus mykiss</i> Walbaum. DAS Report No.: 011078 GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.2.1)	2002	XDE-750: Acute Toxicity to Bluegill Sunfish, (<i>Lepomis macrochirus</i>) Under Static Conditions. DAS Report No.: 011225. GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.16)	2012	XDE-729 Methyl: Fish Short-Term Reproduction Assay with the Fathead Minnow(<i>Pimephales promelas</i>). DAS Report No.: 102125, 379A-153. GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)

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 (KCA 8.16)	2012	XDE-729 Acid: Fish Short-Term Reproduction Assay with the Fathead Minnow (<i>Pimephales promelas</i>). DAS Report No.: 120535, 379A-154 Wildlife International, Ltd. GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.2.4)	2012a	XDE-729 Methyl: Early Life-Stage Toxicity Test with the Sheepshead Minnow, <i>Cyprinodon variegatus</i> , Under Flow-Through Conditions DAS Report No.: 120017, 68313. GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.2.4)	2011d	XDE-729 Acid: An early Life-stage Toxicity Test with the Fathead minnow, <i>Pimephales promelas</i> , Under Flow Through Conditions. DAS Report No.: 101151, 65971. GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.2.4)	2011c	XDE-729 Methyl: Early Life-stage Toxicity Test with the Fathead minnow, <i>Pimephales promelas</i> , Under Flow Through Test Conditions. DAS Report No.:101134, 65896. GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.2.4)	2012b	X11449757: Early life stage toxicity test with the Fathead Minnow, <i>Pimephales promelas</i> , under flow through conditions. DAS Report No.: 101165, 66009. GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.2.4)	...	2011	Aminopyralid: Early Life-Stage Toxicity Test with the Sheepshead Minnow, <i>Cyprinodon variegatus</i> , Under Flow-Through Conditions. DAS Report No.: 101582. GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)

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 (KCA 8.2.2.2)	...	1984	The Toxicity of Technical Picloram to the Embryo, Larval, and Juvenile Stages of the Rainbow Trout (<i>Salmo gairdneri</i> Richardson). DAS Report No.: ES-DR-0114-1351-8, ES-703. GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.2.2.3)	2002	XDE-750: Toxicity to the Early Life-Stages of the Fathead Minnow, <i>Pimephales promelas</i> Rafinesque. DAS Report No.: 021029. GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.2.2.3)	2011	XDE-729 Methyl: Bioconcentration and Metabolism Study with Bluegill, <i>Lepomis macrochirus</i> DAS Report No.: 101135, 66001 GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.3.1.1)	Rebstock, M.	2011c	XDE-729 Methyl: Acute toxicity to the Water Flea, <i>Daphnia magna</i> , Determined Under Static Test Conditions DAS Report No.: 090185, 64603 ABC Laboratories, Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.3.1.1)	Bergfield, A.	2011b	XDE-729 Acid: Acute toxicity to the Water Flea, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions. DAS Report No.: 101149, 65969 ABC Laboratories, Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.3.1.1)	Bergfield, A.	2011c	X11449757: Acute toxicity to the Water Flea, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions. DAS Report No.: 101163, 66007. ABC Laboratories, Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)

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 (KCA 8.3.1.1)	Gaertner, K.	2012b	X11406790 (XDE-729 Metabolite): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static Test Conditions DAS Report No.: 120019, 68211 ABC Laboratories, Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.2.4)	Drottar, K.R. Kendall, T.Z. Krueger, H.O.	2001	Picloram (Acid): A 48: Hour Static Acute Toxicity Test with the Cladoceran (<i>Daphnia magna</i>). DAS Report No.: K-038323-124, 379A-101B; 011198. Wildlife International, Ltd. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.3.1.1)	Marino, T.S., C.A. Hales, E.L. McClymont and A.M. Yaroch	2001	XDE-750 Herbicide: An Acute Toxicity Study with the Daphnid, <i>Daphnia magna</i> . DAS Report No.: 011079. The Dow Chemical Company. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.3.1.4)	2002	XDE-750 – Acute Toxicity to Eastern Oysters (<i>Crassostrea virginica</i>) under Flow-Through Conditions. DAS Report No.: 011268. GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.2.5)	Boeri, R.L., Wyskiel, D.C., Ward, T.J.	2002	Picloram acid: life cycle study in the daphnid, <i>Daphnia magna</i> . DAS Report No.: K-038323-130, 021029, 2391-DO. Wilbury Laboratories Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.3.2.1)	Bergfield, A.	2011e	XDE-729 Methyl: Chronic Toxicity with the Water Flea, <i>Daphnia magna</i> , Exposed Under Static-Renewal Test Conditions DAS Report No.: 101133, 65897 ABC Laboratories, Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)

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 (KCA 8.3.2.1)	Bergfield, A.	2011f	XDE-729 Acid: Chronic Toxicity Test with the Water Flea, <i>Daphnia magna</i> , Exposed Under Static-Renewal Conditions DAS Report No.: 101150, 65972 ABC Laboratories, Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.3.1.2)	Henry, K.S.; Marino, T.A.; Staley J.L. and McClymont, E.L.	2003	XDE-750: 21-Day Chronic Toxicity with the Daphnid, <i>Daphnia magna</i> Straus. DAS Report No.: 021085. The Dow Chemical Company GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.3.2.2)	Gerke, A.	2011e	XDE-729 Methyl: Chronic Toxicity in Whole Sediment to Freshwater Midge, <i>Chironomus riparius</i> DAS Report No.: 101130, 65899. ABC Laboratories, Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.5.1)	Gerke, A.	2011	XDE-729 Methyl: Whole sediment 10 day Acute Toxicity test with Midge Larvae (<i>Chironomus dilutus</i>). DAS Report No.: 090183, 64607. ABC Laboratories, Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.2.7)	Putt, E.A.	2002	Picloram Acid – The Full Life-Cycle Toxicity to Midge (<i>Chironomus riparius</i>) Under Static Conditions. DAS Report No.:K-038323-121, 12550.6157. Springborn Laboratories, Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.5.2)	Putt, A.E.	2002	XDE-750 – Full Life-Cycle Toxicity to Midge (<i>Chironomus riparius</i>) under Static Conditions. DAS Report No.: 011277. Springborn Smithers Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)

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 (KCA 8.5.2)	Putt, A.E	2004	4-amino-5,6-dichloro-2 pyridinecarboxylic acid –Sediment-water chironomid (<i>Chironomus riparius</i>) test using spiked water. DAS Report No.: 040372. Springborn Smithers Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.3.1.3)	Bergfield, A.	2011	XDE-729 Methyl: Acute toxicity Test with the Mysid shrimp, <i>Americamysis bahia</i> , Determined Under Flow-Through Conditions. DAS Report No.: 090184 ABC Laboratories, Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.3.1.4)	Hicks, S.L	2011	XDE-729 Methyl: Effect on New Shell Growth of the Eastern Oyster (<i>Crassostrea virginica</i>). DAS Report No.: 090120. ABC Laboratories, Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.3.2.4)	Hicks, S.L.	2011b	XDE-729 Methyl: Life-Cycle Toxicity Test of the Saltwater Mysid, <i>Americamysis bahia</i> , Conducted Under Flow-Through Test Conditions. DAS Report No.: 101131, 65895. ABC Laboratories, Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.16)	Dinehart, S.A.	2012c	XDE-729 Methyl: Acute toxicity to the Tadpole (<i>Xenopus laevis</i>) determined under flow through test conditions DAS:090121, 64610. ABC Laboratories, Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.16)	2012	XDE-729 Methyl: Amphibian metamorphosis Assay for the Detection of Thyroid Active Substances. DAS Report No.:102126, 379A-152. GLP (Y/N): Y Published (Y/N): N	Y	Corteva Agriscience (Dow AgroSciences)

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 (KCA 8.11.1)	Gerke, A.	2011i	XDE-729 Methyl: Whole sediment acute toxicity to a marine amphipod (<i>Leptocheirus plumulosus</i>). DAS Report No.: 101132, 66366. ABC Laboratories, Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.6)	Drottar, K.R. Kendall, T.Z. Krueger, H.O.	2001c	Picloram Acid: A 14-day Toxicity Test with Duckweed (<i>Lemna Gibba</i> G3). DAS Report No.: K-038323-126, 379A-104. Wildlife International, Ltd. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.6)	Hoberg, J.R.	2002c	XDE-750 – Toxicity to Duckweed, <i>Lemna gibba</i> . DAS Report No.: 011223. Springborn Smithers Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.6)	Rebstock, M.	2011	XDE-729 Methyl: Growth Inhibition Test with the Freshwater Aquatic Plant, Duckweed, <i>Lemna gibba</i> DAS Report No.: 090182, 64595. ABC Laboratories, Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.6)	Rebstock, M.	2011l	XDE-729 Acid: Growth Inhibition Test with the Freshwater Aquatic Plant, Duckweed, <i>Lemna gibba</i> DAS Report No.: 101145, 65968. ABC Laboratories, Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.6)	Rebstock, M.	2011m	X11449757: Growth Inhibition Test with the Freshwater Aquatic Plant, Duckweed, <i>Lemna gibba</i> . DAS Report No.: 101159, 66011. ABC Laboratories, Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)

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 (KCA 8.6)	Rebstock, M.	2012b	X11406790: Growth Inhibition Test with the Freshwater Aquatic Plant, Duckweed, <i>Lemna gibba</i> . DAS Report No.: 120022, 68209. ABC Laboratories, Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.6)	Gonsior, G.	2012a	XDE-729 Methyl - Growth Inhibition of <i>Myriophyllum spicatum</i> in a Water/Sediment System. DAS Report No.: 102023, S11-02965. Eurofins Agrosience Services EcoChem GmbH. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.6)	Gonsior, G.	2012b	XDE-729 Acid - Growth Inhibition of <i>Myriophyllum spicatum</i> in a Water/Sediment System. DAS Report No.:120533, S12-00215 Eurofins Agrosience Services EcoChem GmbH GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.6)	Gonsior, G.	2012c	X11449757 - Growth Inhibition of <i>Myriophyllum spicatum</i> in a Water/Sediment System. DAS Report No.: 102015, S12-00216. Eurofins Agrosience Services EcoChem GmbH GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.6)	Gonsior, G.	2012	X11406790 - Growth Inhibition of <i>Myriophyllum spicatum</i> in a Water/Sediment System. DAS Report No.: 120534, S12-00217. Eurofins Agrosience Services EcoChem GmbH. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.2.6)	Desjardins, D. Drottar, K.R. Kendall, T.Z. Krueger, H.O.	2001	Picloram Acid: A 96-Hour Toxicity Test with the Freshwater Alga (<i>Selenastrum capricornutum</i>). DAS Report No.: K-038323-125, 379A-105. Wildlife International, Ltd. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)

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 (KCA 8.4)	Rebstock, M.	2011d	XDE-729 Methyl: Growth Inhibition Test with the Freshwater Diatom, <i>Navicula pelliculosa</i> DAS Report No.: 090174, 67182. ABC Laboratories, Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.4)	Weber, K.	2011a	Testing Effects of XDE-729 Methyl on the Single Cell Green Alga, <i>Pseudokirchneriella subcapitata</i> , in a 96 h Static Test. DAS Report No.: 090173, S09-00613 EurofinsAgroScience Services GmbH GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.4)	Weber, K.	2011b	Testing of Effects of XDE-729 Methyl on the Blue-Green Alga, <i>Anabaena flos-aquae</i> , in a 96 h Static Test. DAS Report No.: 090175, S09-00615. EurofinsAgroScience Services GmbH GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.4)	Rebstock, M.	2011	XDE-729 Methyl: Static Growth Inhibition Test with the Marine Diatom, <i>Skelotonema costatum</i> DAS Report No.: 090176, 64717 ABC Laboratories, Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.4)	Rebstock, M.	2011	XDE-729 Acid: Growth Inhibition Test with the Unicellular Green Alga, <i>Pseudokirchneriella subcapitata</i> . DAS Report No.: 102027, 66685. ABC Laboratories, Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.4)	Rebstock, M.	2011	XDE-729 Acid: Growth Inhibition Test with the Freshwater Diatom, <i>Navicula pelliculosa</i> DAS Report No.:102029, 66687. ABC Laboratories, Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)

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 (KCA 8.4)	Rebstock, M.	2011	XDE-729 Acid: Growth Inhibition Test with the Blue-Green Alga, <i>Anabaena flos-aquae</i> . DAS Report No.: 101144, 65967. ABC Laboratories, Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.4)	Rebstock, M.	2011	XDE-729 Acid: Static Growth Inhibition Test with the Marine Diatom, <i>Skeletonema costatum</i> . DAS Report No.: 102028, 66686. ABC Laboratories, Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.4)	Rebstock, M.	2011	Growth Inhibition Test with the Unicellular Green Alga, <i>Pseudokirchneriella subcapitata</i> - X11449757. DAS Report No.: 101158, 66006. ABC Laboratories, Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.4)	Rebstock, M.	2012	X11406790: Growth Inhibition Test with the Unicellular Green Alga, <i>Pseudokirchneriella subcapitata</i> DAS Report No.: 120021, 68210. ABC Laboratories, Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.2.6)	Kirk, H.D. Gilles, M.M. McClymont, E.L. McFadden, L.G.	2001	Picloram (Technical): Growth Inhibition Test with the Bluegreen Alga, <i>Anabaena flos-aquae</i> . DAS Report No.: K-038323-114; 001153. The Dow Chemical Company. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.4)	Hughes, J. S.	2002	The Toxicity of Picloram, Potassium Salt, to <i>Selenastrum capricornutum</i> . DAS Report No.: ES-DR-0049-3936-7, ES-2223 Malcolm Pirnie Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)

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 (KCA 8.4)	Hoberg, J.R	2002	XDE-750 – Toxicity to the Freshwater Green Alga, <i>Pseudokirchneriella subcapitata</i> . DAS Report No.: 011222. Springborn Smithers Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.2 (KCA 8.4)	Hoberg, J.R	2002	XDE-750 – Acute Toxicity to the Freshwater Diatom, <i>Navicula pelliculosa</i> . DAS Report No.: 011278. ABC Laboratories, Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.3.1 (KCA 8.7.1)	Schmitzer S.	2011	Effects of XDE-729 Methyl (Acute Contact and Oral) on Honey Bees (<i>Apis mellifera</i> L.) in the Laboratory. DAS Report No.: 101128/ 101129, 49528035 Institut für Biologische Analytik, und Consulting IBACON GmbH GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.3.1 (KCA 8.3.1.1)	Hoberg, J.	2001	Picloram Acid - Acute Contact and Oral Toxicity Tests with Honey Bees (<i>Apis mellifera</i>). DAS Report No.: 011173/ 011174. Springborn Laboratories, Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.3.1 (KCA 8.7.1)	Aufderheide, J.	2001	XDE-750: Acute Oral Toxicity Test with the Honeybee (<i>Apis mellifera</i>). DAS Report No.: 011045. ABC Laboratories Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.3.1 (KCA 8.7.2)	Aufderheide, J.	2001	XDE-750: Acute Contact Toxicity Test with the Honeybee, <i>Apis mellifera</i> . DAS Report No.: 011044. ABC Laboratories Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)

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 10.4 (KCA 8.9.1)	Witte, B.	2011	Acute Toxicity (14 Days) of XDE-729 Methyl to the Earthworm, <i>Eisenia fetida</i> , in Artificial Soil with 5% Peat. DAS Report No.: 090099, 49524021. Institut für Biologische Analytik, und Consulting IBACON GmbH. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.4 (KCA 8.9.1)	Witte, B.	2010b	XDE-729 Acid: Acute Toxicity (14 Days) of XDE-729 Acid to the Earthworm, <i>Eisenia fetida</i> , in Artificial Soil with 5% Peat. DAS Report No.: 101141, 56861021. Institut für Biologische Analytik, und Consulting IBACON GmbH GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.4 (KCA 8.9.1)	Witte, B.	2010c	Acute Toxicity (14 days) of X11449757 (metabolite of XDE-729) to the Earthworm, <i>Eisenia fetida</i> , in Artificial Soil with 5% Peat. DAS Report No.: 101155, 56872021. Institut für Biologische Analytik, und Consulting IBACON GmbH GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.4 (KCA 8.9.1)	Boeri, R.L., Ward, T.J.	2002	Picloram acid: 14-day soil exposure acute toxicity to the earthworm, <i>Eisenia foetida</i> . DAS Report No.: K-038323-120 , 011175, 2290-DO Wilbury Laboratories Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.4 (KCA 8.9.1)	Ward, T.J., Boeri, R.L.	2001	XDE-750: 14-Day Soil Exposure Acute Toxicity to the Earthworm, <i>Eisenia foetida</i> . DAS Report No.: 011049. Wilbury Laboratories, Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.4 (KCA 8.9.2)	Witte, B.	2011	Effects of XDE-729 Methyl on Reproduction and Growth of Earthworms, <i>Eisenia fetida</i> , in Artificial Soil with 5% Peat (Revised). DAS Report No.: 090100, 49525022. Institut für Biologische Analytik, und Consulting IBACON GmbH GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)

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 10.4 (KCA 8.9.2)	Witte, B.	2010	Effects of XDE-729 Acid on Reproduction and Growth of Earthworms, <i>Eisenia fetida</i> , in Artificial Soil with 5% Peat. DAS Report No.: 101142, 56862022. Institut für Biologische Analytik, und Consulting IBACON GmbH GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.4 (KCA 8.9.2)	Witte, B.	2010	Effects of X11449757 (metabolite of XDE-729) on Reproduction and Growth of Earthworms, <i>Eisenia fetida</i> , in Artificial Soil with 5% Peat. DAS Report No.: 101156, 56873022. Institut für Biologische Analytik, und Consulting IBACON GmbH GLP/GEP (Y/N):Y Published (Y/N):N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.4 (KCA 8.9.2)	Mallett, M.J.	2001	The Effects of Picloram on Reproduction and Growth in the Earthworm <i>Eisenia Foetida</i> . DAS Report No: GHE T-1148; CEMS-1639. CEM Analytical Services. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.4 (KCA 8.9.2)	McCormac, A.	2012	Determination of the chronic (sub-lethal) toxicity of aged residues of technical-grade XDE-729 Methyl to the earthworm <i>Eisenia fetida</i> in two natural soil substrates. DAS Report No.: 110605, DOW-11-38. Mambo-Tox Ltd. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.4 (KCA 8.9.2)	Davies, N.	2004	XDE-750: Effects on Reproduction and Growth in the Earthworm, <i>Eisenia foetida</i> . DAS Report No.: 040285. CEM Analytical Services Limited, UK. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.4 (KCA 8.9.2)	Gerke, A.	2011g	XDE-729 Methyl: Inhibition of Reproduction of Collembola, <i>Folsomia candida</i> , in Artificial Soil. DAS Report No.: 090181, 64611. ABC Laboratories, Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)

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 10.4 (KCA 8.9.2)	Witte, B.	2011a	Effects of XDE-729 Acid on Reproduction of the Predatory Mite <i>Hypoaspis aculeifer</i> in Artificial Soil with 5% Peat. DAS Report No.: 102025, DR-0402-7809-066. Institut für Biologische Analytik, und Consulting IBACON GmbH GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.4 (KCA 8.9.2)	Luhrs, U.	2011	Effects of XDE-729 Methyl on Reproduction of the Predatory Mite <i>Hypoaspis aculeifer</i> in Artificial Soil with 5% Peat. DAS Report No.: 110280, 64641089. Institut für Biologische Analytik, und Consulting IBACON GmbH GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.4 (KCA 8.9.2)	Witte, B.	2011b	Effects of XDE-729 Acid on Reproduction of the Collembola <i>Folsomia candida</i> in Artificial Soil with 5% Peat. DAS Report No.: 102024, DR-0402-7809-067 Institut für Biologische Analytik, und Consulting IBACON GmbH. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.4 (KCA 8.9.2)	Witte, B.	2011c	Effects of X11449757 (metabolite of XDE-729) on Reproduction of the Predatory Mite <i>Hypoaspis aculeifer</i> in Artificial Soil with 5% Peat. DAS Report No.: 101154, DR-0417-6492-005. Institut für Biologische Analytik, und Consulting IBACON GmbH GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.4 (KCA 8.9.2)	Gerke, A.	2011h	X11449757: Inhibition of Reproduction of Collembola, <i>Folsomia candida</i> , in Artificial Soil. DAS Report No.: 101153, DR-0417-6492-009. ABC Laboratories, Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.5 (KCA 8.10.1)	Feil, N.	2011a	Effects of XR-729 methyl on the activity of the soil microflora in the laboratory DAS Report No.: 101127, 49527080. Institut für Biologische Analytik, und Consulting IBACON GmbH GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)

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 10.5 (KCA 8.10.1)	Feil, N.	2010b	Effects of XDE-729 acid on the activity of the soil microflora in the laboratory. DAS Report No.: 101143, 56863080. Institut für Biologische Analytik, und Consulting IBACON GmbH GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.5 (KCA 8.10.1)	Feil, N.	2011	Effects of X11449757 on the activity of the soil microflora in the laboratory. DAS Report No.: 101157, 56874080. Institut für Biologische Analytik, und Consulting IBACON GmbH GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.5 (KCA 8.10.1)	Mallett, M.J.	2001b	The effects of picloram on soil microflora respiration and nitrogen transformations. DAS Report No.: GHE T-1158, CEMS-1630. CEM Analytical Services GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.5 (KCA 8.10.1)	McMurray, A.	2002	A Laboratory Assessment of the Effects of XDE-750 on Soil Microflora Respiration and Nitrogen Transformation According to OECD Guidelines. DAS Report No.: GHE-T-1180. Chemex Environmental International Ltd. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.6 (KCA 8.12)	Rockcliff, C.	2011c	Evaluation of the Phytotoxicity of the XDE-729 acid GLP Seedling Emergence and Seedling Growth Test Terrestrial Non Target Plants (Based on OECD Guideline 208) - Europe 2011. DAS Report No.: 101955, STC/11/E601. Stockbridge Technology Centre Ltd. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)
KCP 10.6 (KCA 8.12)	Rockcliff, C.	2011d	Evaluation of the Phytotoxicity of the XDE-729 M-757 metabolite GLP Seedling Emergence and Seedling Growth Test Terrestrial Non Target Plants (Based on OECD Guideline 208) - Europe 2011. DAS Report No.: 101956, STC/11/E602. Stockbridge Technology Centre Ltd. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)

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 10.6 (KCA 8.15)	Lee, B.	2010 (Amendment 2011)	XDE-729 Methyl: Activated Sludge, Respiration Inhibition Test. DAS Report No.: 101140, 65898. ABC Laboratories, Inc. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)

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	Reason for rejection
KCP 10.3.1.2/02	Oberrauch, S.	2018	XDE-729 Methyl: Assessment of the Effects on the Adult Honey Bee, <i>Apis mellifera</i> L., in a 10 day Chronic Feeding Test Under Laboratory Conditions. DAS Report No.: 170071. Eurofins Agrosience Services EcoChem GmbH/Eurofins Agrosience Services Ecotox GmbH. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)	New active substance data, not necessary to finalise the risk assessment at the zonal level
KCP 10.3.1.2/03	Lenard, J.; Moore, S.	2017	Picloram: A laboratory Study to Determine the Chronic Oral Toxicity to the Adult Worker Honey Bee <i>Apis mellifera</i> L. (Hemiptera: Apidae). DAS Report No.: 170090. SynTech Research Laboratory Services LLC. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)	New active substance data, not necessary to finalise the risk assessment at the zonal level
KCP 10.3.1.2/04	Lenard, J.; Moore, S.	2017	Aminopyralid: A laboratory Study to Determine the Chronic Oral Toxicity to the Adult Worker Honey Bee <i>Apis mellifera</i> L. (Hemiptera: Apidae). DAS Report No.: 170092. SynTech Research Laboratory Services LLC. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)	New active substance data, not necessary to finalise the risk assessment at the zonal level

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	Reason for rejection
KCP 10.3.1.3/02	Oberrauch, S.	2018	XDE-729 Methyl – Honey Bee (<i>Apis mellifera</i> L.) 22 Day Larval Toxicity Test (Repeated Exposure). DAS Report No.: 170073. Eurofins Agrosience Services EcoChem GmbH/Eurofins Agrosience Services Ecotox GmbH. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)	New active substance data, not necessary to finalise the risk assessment at the zonal level
KCP 10.3.1.3/03	Lenard, J.; Moore, S.	2017	Picloram: A Repeated-Exposure Laboratory Toxicity Study in Larvae, Pupae and Emergent Adults of the Honey Bee <i>Apis mellifera</i> Linnaeus. (Hymenoptera: Apidae). DAS Report No.: 170091. SynTech Research Laboratory Services LLC. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)	New active substance data, not necessary to finalise the risk assessment at the zonal level
KCP 10.3.1.3/04	Lenard, J.; Moore, S.	2017	Aminopyralid: A Repeated-Exposure Laboratory Toxicity Study in Larvae, Pupae and Emergent Adults of the Honey Bee <i>Apis mellifera</i> Linnaeus. (Hymenoptera: Apidae). DAS Report No.: 170413. SynTech Research Laboratory Services LLC. GLP (Y/N): Y Published (Y/N): N	N	Corteva Agriscience (Dow AgroSciences)	New active substance data, not necessary to finalise the risk assessment at the zonal level

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
There were no data relied on and not submitted by the Applicant.					

Appendix 2 Detailed evaluation of the new studies

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

Not required to characterise the product in the current submission. The acute oral toxicity is evaluated based on the active substances.

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

Not required to characterise the product in the current submission.

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

Not required to characterise the product in the current submission. The acute oral toxicity is evaluated based on the active substances.

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

Not required to characterise the product in the current submission.

A 2.1.3 KCP 10.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)

Comments of zRMS:	<p>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.</p> <p>Information provided by the Applicant below has been thus not validated by the zRMS and is struck through and shaded.</p>
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~~According to the data requirements under regulation 1107/2009 (Commission Regulations (EU) 283/2013⁹ and 284/2013¹⁰), the risk to amphibians and reptiles shall be addressed. However, there is no EU guidance or validated regulatory protocol yet available, neither on the type of the necessary regulatory testing nor on how to conduct a risk assessment for amphibians and reptiles. Accordingly, specific toxicity tests for amphibian and reptile species are not requested and therefore no data on reptiles and terrestrial amphibians are available for the halauxifen methyl, picloram and aminopyralid. In the EU, there is no guidance or validated regulatory protocols yet available either on the type of regulatory testing necessary or how to conduct a risk assessment for amphibian and reptiles. No overt toxicity has been observed in any of the avian and mammalian studies relevant for the ecotoxicological risk assessment. In addition, acceptable acute and long term risks were concluded for birds and mammals under the very conservative assumptions of the screening level approach with a high margin of safety. As such no adverse effects or risks are expected for reptiles and terrestrial amphibians exposed via applications of GF 4021 at rates up to and including 0.25 L prod/ha.~~

⁹ Commission Regulation (EU) No 283/2013 setting out data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. Official Journal of the European Union: 1st March 2013.

¹⁰ Commission Regulation (EU) No 284/2013: setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. Official Journal of the European Union: 1st March 2013.

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 140737: Picloram: Toxicity to the Aquatic Macrophyte, *Myriophyllum spicatum*.

Comments of zRMS:	<p>The study was performed in line with OECD TG 239 with no major deviations in terms of environmental conditions.</p> <p>It is noted that pH in some test solutions increased by more than 1.5 units (maximum increase by 1.8 units in 313 µg a.i./L test group). However, this deviation is considered to have no significant impact on the results of the study. since all validity criteria were met.</p> <p>It is noted that the fresh and dry weight were determined for shoots and roots combined which is significant deviation from indications of OECD TG 239, since the validity criteria are related to the shoot fresh weight and in line with indications of the guideline, endpoints for fresh and dry weight should be determined for shoots only. Although the total fresh and dry weight cover also effects on shoots, without differentiation to shoots and roots it cannot be confirmed if the validity criteria were met and if more pronounced effects were observed on shoots which would result with lower endpoints.</p> <p>Despite this significant deviation the study may be considered as the source of additional information that may be used for identification of the most toxic active substance in formulation GF-4021, as even with uncertainty over the derived endpoints it is obvious that picloram is not more toxic than halauxifen-methyl (see point 9.5.1.1 for details).</p> <p>The following lowest endpoints will be used for comparative purposes until the valid endpoints are available from the EU renewal of picloram: study is considered acceptable with following endpoints relevant for the risk assessment:</p> <p>lowest 14-d E_rC_{50} = 0.458 mg a.s./L (based on nominal concentrations corrected for the test item purity) lowest 14-d E_yC_{50} = 0.192 mg a.s./L (based on nominal concentrations corrected for the test item purity)</p>
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Reference:	KCP 10.2.1/01
Report	Banman C.S, Moore, S.; 2015; Picloram: Toxicity to the Aquatic Macrophyte, <i>Myriophyllum spicatum</i> ; SynTech Research Laboratory Services LLC; Lab Study No. 14SRLS14C3; DAS Study No. 140737; 17 March 2015; Unpublished.
Guideline(s):	OECD 237; OCSPP.SUPP (US EPA)
Deviations:	Major Minor (see zRMS comment above)
GLP:	Yes
Acceptability:	Due to significant deviations from the test guideline, results of the study may be considered only as additional information used for comparative purposes and informative risk assessment Acceptable
Duplication (if vertebrate study)	-

MATERIALS AND METHODS

Test Items

Test item (chemical/other name):	Picloram
Purity:	82.1%
Description (physical state):	Tan powder
Lot/batch no.:	2H16162952
CAS no.:	1918-02-1

Test System

Organism (<i>Species</i>):	Aquatic plant, <i>Myriophyllum spicatum</i> L
Study type:	Laboratory study - water/sediment system
Study duration:	14 days
Parameters measured:	Test solution pH (range): 9.8 to 12.7 Test solution temperature (range): 19.8 to 20.4°C Oxygen saturation (range): 10.0 to 12.4 mg/L
Environmental conditions:	Photoperiod: 16 hours light / 8 hours dark Light intensity (range): 11,170 to 12,690 lux Temperature (range): 19.8 to 20.4°C pH: 8.1 – 10.0 Oxygen concentration: 9.8 – 12.7 mg/L
Observation intervals:	Daily
Test concentrations:	Nominal: Control, 9.54, 30.5, 97.7, 313 and 1000 µg a.i./L Mean calculated concentrations: Control (<LOQ), 10.1, 29.8, 108, 311 and 935 µg a.i./L
Acclimation period/conditions:	16 hours light: 8 hours dark. 20.0 ± 5.0 °C.
Growth medium:	Name: Hard Processed Water (blended spring and R.O. water)
Method of test item added to the test medium:	Water stock prepared and stirred into treatment vessels (water spiked)
No. of control replicates:	10
No. of test concentration replicates:	5
No. of rooted apical shoots per vessel:	4 plants, thinned to 3 plants at the start of the exposure period
Analytical verification:	Method: measuring concentrations of picloram using LC-MS/MS Samples taken : 0 and 14days Limit of Detection: Not applicable Limit of Quantitation: 2.0 µg/L Recoveries from QC fortifications: 99 to 112%
Test substance renewal days:	None

Methodology

Following a seven day acclimation period, *Myriophyllum spicatum* shoots were exposed for 14 days under static conditions. Shoots within a replicate were planted in sediment within a 300-mL borosilicate glass crystallization dish housed in a 2-L glass beaker. Artificial sediment used for the culturing of *Myriophyllum* was a modification of the OECD 219 sediment for the testing of Chironomids.

The start of the exposure period was marked by the addition of stock solution to each exposure vessel, with the exception of the control vessels which received no stock solution. The stock was mixed into the test beakers using a glass pipette for approximately one minute.

Samples were analysed for concentration of picloram. Parameters measured included growth rate and yield (NOEC, LOEC and EC50) of total shoot lengths, total plant wet weight and total plant dry weight.

Effects on yield for total shoot length, total plant wet weight and total plant dry weight were determined on a per plant basis, based on the growth of each plant during the 14 day growth intervals. In order to calculate yield at the end of the exposure period for wet weight and dry weight and shoot length, 15 plants from the pre-exposure surrogate vessels were sacrificed at the beginning of the exposure phase (day 0). Measurements of wet weight, dry weight and total shoot length (main and side shoots) were recorded to establish the Day 0 values, which were used to calculate the growth yields from the control and treatment level plants. Based on the average calculated values for shoot length, wet weight and dry weight; growth rate values were calculated for each replicate test vessel.

Raw or transformed data from treatment groups were compared to controls for normality and homogeneity of variance using the Shapiro-Wilks test and Bartlett's equality of variance test, respectively. If normality and homogeneity of variance were demonstrated for the raw or transformed values, then parametric analyses were conducted using analysis of variance (ANOVA) followed by Dunnett's test. If normality and/or homogeneity of variance were not demonstrated on raw or transformed values, nonparametric procedures were used.

RESULTS AND DISCUSSION

Mean measured recoveries from day 0 and 14 ranged from 93 to 111% of the nominal concentrations. ~~Samples were analysed for picloram.~~

The toxicity values were calculated based on nominal concentrations in units of µg active ingredient/L. Plants in the control vessels and two lowest treatment levels (9.54 and 30.5 µg a.i./L) were observed to be normal throughout the study. Plants in the 97.7 and 313 µg a.i./L treatment group had roots emerging from the nodes above the sediment level. Plants in the highest treatment group (1000 µg a.i./L) were yellow in colour at the end of the study, and had very little root development. The lowest ErC50 for growth rate in the 14-day exposure of the rooted aquatic macrophyte *Myriophyllum spicatum* to picloram was obtained for shoot length. The statistical NOErC and LOErC for this endpoint were 9.54 and 30.5 µg a.i./L and 558 µg a.i./L, respectively.

Table 1: Mean total shoot length including side shoots (cm)

Nominal concentration (µg/L)	Days after application		Yield (cm)	Reduction in yield (%)	Growth rate (1/day)	Reduction in growth rate (%)
	0 ¹	14				
Control	7.9	43.2	35.2	NA	0.1210	NA
9.54		40.3	32.4	7.89	0.1160	4.14
30.5		33.5	25.6	27.4*	0.1021	15.6*
97.7		29.4	21.5	39.0*	0.0934	22.8*
313		23.2	15.3	56.4*	0.0759	37.3*
1000		12.6	4.7	86.8*	0.0329	72.8*

* significantly different reduction compared to the pooled control

1) based on 15 additional plants, representative of those used in the test

Table 2: Mean total plant fresh weight (g)

Nominal concentration (µg/L)	Days after application		Yield (cm)	Reduction in yield (%)	Growth rate (1/day)	Reduction in growth rate (%)
	0 ¹	14				
Control	0.3322	1.7923	1.4602	NA	0.1200	NA
9.54		1.6779	1.3457	7.84	0.1152	4.03
30.5		1.4862	1.1541	21.0*	0.1057	11.9
97.7		1.3461	1.0139	30.6*	0.0994	17.1*
313		1.1648	0.8326	43.0*	0.0884	26.3*
1000		0.7107	0.3785	74.1*	0.0530	55.8*

* significantly different reduction compared to the pooled control

1) based on 15 additional plants, representative of those used in the test

Table 3: Mean total plant dry weight (g)

Nominal concentration (µg/L)	Days after application		Yield (cm)	Reduction in yield (%)	Growth rate (1/day)	Reduction in growth rate (%)
	0 ¹	14				
Control	0.0384	0.2298	0.1914	NA	0.1272	NA
9.54		0.2271	0.1887	1.37	0.1268	0.35
30.5		0.1798	0.1413	26.1*	0.1095	13.9*
97.7		0.1631	0.1247	34.8*	0.1022	19.7*
313		0.1346	0.0961	49.8*	0.0881	30.8*
1000		0.1182	0.0798	58.3*	0.0800	37.1*

* significantly different reduction compared to the pooled control

1) based on 15 additional plants, representative of those used in the test

The calculated EC₅₀ values, NOEC and LOEC based on growth rate and yield for each of the measured parameters (total shoot length, fresh weight and dry weight) are presented below.

Table 4: Summary of biological results (based on nominal concentrations - µg /L)

Parameter (µg/L)	Total shoot length		Total wet weight ^{shoot-length}		Total dry weight ^{shoot-length}	
	Growth rate	Yield	Growth rate	Yield	Growth rate	Yield
14-day EC ₅₀	558	234	864	468	>1000	333
95% Conf. Limits	327 - 707	126 - 456	559 - NA	139 - 676	NA	95 - 1027
14-day NOEC	9.54	9.54	30.5	9.54	9.54	9.54
14-day LOEC	30.5	30.5	97.7	30.5	30.5	30.5

Validity criteria:

- The mean total shoot length and shoot fresh weight in control plants must at least double during the exposure phase of the test and control plants must not show any visual symptoms of chlorosis (observed 5.5 and 5.4 increase in total shoot length and wet weight, respectively; no chlorosis observed in control cultures).
- The mean coefficient of variation of yield based on measurements of shoot fresh weight in the control cultures must not exceed 35% (observed: 13.2%).

CONCLUSION

The lowest E_rC₅₀ for growth rate in the 14-day exposure of the rooted aquatic macrophyte *Myriophyllum spicatum* to picloram was obtained for shoot length. The statistical NOE_rC, LOE_rC and E_rC₅₀ for this endpoint were 9.54 and 30.5 µg a.i./L and 558 µg a.i./L, respectively.

The lowest E_yC₅₀ for yield in the 14-day exposure of the rooted aquatic macrophyte *Myriophyllum spicatum* to picloram was obtained for total shoot length. The statistical NOE_yC, LOE_yC and E_yC₅₀ for this endpoint were 9.54, 30.5 and 234 µg a.i./L, respectively.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Aquatic macrophyte	<i>Myriophyllum spicatum</i>	Picloram	14 day	ErC ₅₀	558	µg/L

A 2.2.1.2 Study 150390: Picloram metabolite 5,6-dichloropicloram: Growth Inhibition of *Myriophyllum spicatum* in a Water/Sediment System.

Comments of zRMS:	<p>The study was performed in line with OECD TG 239 with some deviations discussed below. All validity criteria were met.</p> <p>It is noted that pH in some test solutions increased by more than 1.5 units (maximum increase by 1.86 units). However, this deviation is considered to have no significant impact on the results of the study. since all validity criteria were met.</p> <p>It is noted that the number of shoots tested in control and test item groups was not in line with recommendations of OECD TG 239. According to the test guideline, 6 replicates per control and 4 replicates per test item group with 3 shoots each are recommended, resulting with 18 and 12 plants per control and test item group, respectively. In this study one shoot per replicate was used with 10 replicates per control and 5 replicates per test item group, resulting with 10 and 5 plants per control and test item group, respectively. In general, this deviation could reduce the statistical power of the study. However, this alternative test design with single shoot per replicate and 5 and 10 replicates per test groups and control, respectively, has been agreed during the general ecotox meeting (see EFSA Supporting publication 2019:EN-1673). For this reason this deviation is considered acceptable.</p> <p>The study author expressed the endpoints in terms of the nominal concentrations, justifying that the initial mean measured concentrations were within 80-120% of nominal (exact value: 91%). It is, however, noted that in line with indications of OECD TG 239, endpoints may be expressed in terms of the nominal concentrations only when the measured concentrations are maintained at 80-120% of nominal over the whole study period and not only at the test initiation. The overall mean measured concentrations of 5,6-dichloropicloram were at 79.1% of nominal due to the measured concentrations at 59-80% of nominal at test termination. For this reason the endpoints cannot be expressed as nominal concentrations. It would be possible to express the endpoints as initially measured concentrations provided that at test termination the measured concentrations were at 80-120% of initially measured concentrations. However, in this study the measured concentration at 14 d were at 70-85% of initially measured concentrations. Therefore, the endpoints from this study must be expressed in terms of the mean measured concentrations. Taking this into account, the endpoints derived by the study author were corrected for the overall mean measured concentration representing 79.1% of nominal.</p> <p>It is noted that the fresh and dry weight were determined for shoots and roots combined which is significant deviation from indications of OECD TG 239, since the validity criteria are related to the shoot fresh weight and in line with indications of the guideline, endpoints for fresh and dry weight should be determined for shoots only. Although the total fresh and dry weight cover also effects on shoots, without differentiation to shoots and roots it cannot be confirmed if the validity criteria were met and if more pronounced effects were observed on shoots which would result with lower endpoints.</p> <p>Despite this significant deviation the study may be considered as the source of additional information as it shows that the metabolite is clearly less toxic to rooted aquatic macrophytes than the parent compound.</p> <p>The following endpoints will be used for comparative purposes until the valid endpoints are available from the EU renewal of picloram: Overall, the study is considered acceptable with following endpoints relevant for the risk assessment:</p>
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	lowest 14-d E_rC_{50} = 61.9 mg test item/L (based on mean measured concentrations) lowest 14-d E_yC_{50} = 32.0 mg test item/L (based on mean measured concentrations)
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Reference:	KCP 10.2.1/02
Report:	Gonsior, G; 2015; Picloram metabolite 5,6-dichloropicloram: Growth Inhibition of <i>Myriophyllum spicatum</i> in a Water/Sediment System; Eurofins Agroscience Services EcoChem GmbH, Eutinger Str. 24, D-75223 Niefern-Öschelbronn, Germany; S15-02583; 150390; 26 October 2015; Unpublished.
Guideline(s):	OECD 239
Deviations:	In deviation to the guideline recommendation which only evaluates the shoot biomass, the plant biomass was assessed by measuring plant fresh and dry weight. This avoids underestimating effects on rooted aquatic macrophytes, especially for test items which may affect root development. For deviations regarding the test design, see zRMS comments above
GLP:	Yes
Acceptability:	Due to significant deviations from the test guideline, results of the study may be considered only as additional information used for comparative purposes and informative risk assessment Acceptable
Duplication (if vertebrate study):	-

MATERIALS AND METHODS

Test Item(s)

ISO Common name:	Not applicable
Test item (chemical/other name):	Picloram metabolite 5,6-dichloropicloram
Purity:	94 % w/w
Description (physical state):	Solid off white
Lot/batch no.:	200201825-59, TSN103891
CAS no.:	150114-71-9

Test System

Organism (<i>Species</i>):	Aquatic plant, <i>Myriophyllum spicatum</i> L
Study type:	Laboratory study - water/sediment system
Study duration:	14 days
Parameters measured:	Test solution pH (range): 8.16 ± 0.63 Test solution temperature (range): 20.2 ± 0.5 °C Oxygen saturation (range): 122 ± 29 %
Environmental conditions:	Photoperiod: 16-h day-length Light intensity (range): 120 – 160 $\mu\text{Em}^{-2}\text{s}^{-1}$ Temperature (range): 20.2 ± 0.5 °C pH: 6.89 – 9.54
Observation intervals:	0, 7 and 14 days
Test concentrations:	Nominal: 0.954, 3.05, 9.77, 31.3 and 100 mg test item /L Mean calculated concentrations: 83-96 % of nominal for 5,6-dichloropicloram at test start
Acclimation period/conditions:	14 days
Growth medium:	Name: SMART AND BARKO medium
Method of test item added to the test medium:	Spiked water
No. of control replicates:	10

No. of test concentration replicates:	5
No. of rooted apical shoots per vessel:	1
Analytical verification:	Method: measuring concentrations of 5,6-dichloropicloram using HPLC-MS/MS Samples taken : 0 days and 14 days Limit of Detection: The limit of detection (LOD) was defined as 30 % of the limit of quantification Limit of Quantitation: 0.0954 mg/L in test medium, respectively 0.01 mg/kg in sediment for the test item. Recoveries from QC fortifications: (70 ± 110 % mean recovery, ≤ 20 % RSD)
Test substance renewal days:	-

Methodology

Plants were grown in a static water-sediment system using artificial sterilised sediment overlaid with Smart and Barko medium under the same conditions as used in the pre-culture. The study was conducted in 2 L glass-beakers measuring approx. 12 cm in diameter and 24 cm height. Only one shoot per test vessel was planted.

The volume of added water was recorded, and the level marked on the outside of the test vessels. Each vessel contained approx. 350 g of moist sediment containing growth nutrients (ammonium chloride and sodium phosphate), with the sediment surface overlaid with moist sediment without nutrients, and a thin layer of washed quartz sand, to minimise displacement of the sediment when the growth medium was added. Afterwards the test vessels were filled carefully with growth medium (1.5 L).

Two days after preparation of the test vessels and before application of the test item, one rooted apical shoot per vessel was planted carefully, ensuring the plant was rooted into the sediment. Shortly afterwards, application of the test item was performed and mixed in with gentle stirring. The test item was spiked to the water at nominal concentrations of 0.954, 3.05, 9.77, 31.3 and 100 mg Picloram metabolite 5,6-dichloropicloram /L. Ten replicates were used for the control and five for each test item group.

On day 0 fifteen additional plants, representative of those used in the test, were selected from the available plant material. The plants were blotted dry prior to assessment of plant fresh weight and shoot length. The plants were placed separately in labelled glass beakers and dried at 60 °C for > 48 hours. The weight of the dry plant samples was recorded. On day 14 plants were harvested from each treatment group for assessment of total plant fresh weight, total plant dry weight, shoot length and number and length of side shoots. In addition observations on shoot and root development (e.g. necrosis, deformation) were documented.

Table 5. Data were used to calculate the following parameters for each plant:

Parameter	Day 14
growth rate for total shoot length	X
yield for total shoot length	X
growth rate for total plant fresh weight	X
yield for total plant fresh weight	X
growth rate for total plant dry weight	X
yield for total plant dry weight	X

For each of these parameters EC₁₀, 20, 50 values were calculated where reliable and in addition the NOEC and LOEC were determined where possible.

All data were subjected to ANOVA. A test for normality of the data was carried out by calculating the Shapiro-Wilk's statistic. For homogeneity of variances across treatment groups a Bartlett's or

Levene's test was performed. If data were normally distributed and variance was homogeneous a Dunnett's t-test was performed. If Shapiro Wilks test indicated a non-normal distribution of residuals a Bonferroni-U Exact Test was performed to determine significant differences from controls (SAS® Proprietary Software 9.3). The EC50 (yield and growth rate) was calculated where possible using Probit analysis. Only concentrations within a clear dose response were used for calculations.

RESULTS AND DISCUSSION

The average pH-value was determined to be 8.16 ± 0.63 , the average temperature was measured to be 20.2 ± 0.5 °C and the oxygen saturation was determined to be 122 ± 29 %. The test item had no influence on the pH-value of the test solutions. All parameters were within the range recommended in the OECD Guideline (26-Sep-2014).

The measured concentration of the test item based on the 5,6-dichloropicloram content in the test vessels at test start ranged between 83 and 96 % of nominal in the overlaying water (see table below). The concentrations of 5,6-dichloropicloram in the water phase were between 59 – 80 % of nominal at test end. In pore water 1% of the applied amount was measured at 100 mg/L. As the mean concentrations of 5,6-dichloropicloram at test start were between 80 and 120 % of nominal all toxicological endpoints were evaluated using nominal concentrations of the test item.

Time	Nominal concentration	Overlaying water (measured concentrations)	
	Test item	5,6-dichloropicloram	
[d]	[mg/L]	[mg/L]	[% of nominal]
0	control	n.d.	-
14		n.d.	-
0	0.954	0.846	89
14		0.634	66
0	3.05	2.54	83
14		1.79	59
0	9.77	9.15	94
14		6.59	67
0	31.3	30.1	96
14		22.7	73
0	100	94.2	94
14		79.8	80
14 ¹⁾	100	40.0	1
Mean test start (0d)			91

LOQ = 0.0954 mg/L 5,6-dichloropicloram in water; ¹⁾ pore water (based on 1.5 L test medium and 32.3 mL pore water); n.d. = not detectable

At test end in the sediment, concentrations of 5,6-dichloropicloram were detectable at 0.954, 3.05, 9.77, 31.3 and 100 mg/L, with recoveries ranging between 10 -13 % of the amount applied.

The mean control growth rate based on shoot length, fresh weight and dry weight was 0.1336, 0.1467 and 0.1349 /day respectively, which is equivalent to a mean doubling time of 5.2, 4.7 and 5.1 days respectively.

The coefficient of variation (C.V.) for control growth based on shoot length, fresh weight and dry weight was 16.5 %, 12.5 % and 16.3 % respectively.

The mean control yield (and C.V.) based on shoot length was 47.6 cm (C.V. = 29.8 %), for fresh weight yield was 2.0404 g (C.V. = 26.2 %), and for dry weight yield was 0.2218 g (C.V. = 30.7 %).

Since the CV for fresh weight and shoot length yield was below 35 % and a doubling of shoot biomass and length was reached within the test duration the mean control growth rates and variability were considered acceptable.

Table 6: Mean total shoot length including side shoots (cm)

Nominal concentration (mg/L)	Days after application		Yield (cm)	Reduction in yield (%)	Growth rate (1/day)	Reduction in growth rate (%)
	0 ¹	14				
Control	8.3	55.9	47.6	-	0.1336	-
0.954	8.3	58.6	50.3	-5.7	0.1387	-3.8
3.05	8.3	61.9	53.6	-12.6	0.1432	-7.2
9.77	8.3	53.6	45.3	4.8	0.1329	0.5
31.3	8.3	31.3	23.0*	51.7*	0.0943*	29.4*
100	8.3	19.9	11.6*	75.6*	0.0617*	53.8*

* significantly different reduction compared to the control

1) based on 15 additional plants, representative of those used in the test

Table 7: Mean total plant fresh weight (g)

Nominal concentration (mg/L)	Days after application		Yield (cm)	Reduction in yield (%)	Growth rate (1/day)	Reduction in growth rate (%)
	0 ¹	14				
Control	0.2908	2.3312	2.0404	-	0.1467	-
0.954	0.2908	2.1889	1.8981	7.0	0.1432	2.4
3.05	0.2908	2.2078	1.9170	6.0	0.1438	2.0
9.77	0.2908	2.5127	2.2219	-8.9	0.1533	-4.5
31.3	0.2908	1.5580	1.2672*	37.9*	0.1192*	18.7*
100	0.2908	0.8080	0.5172*	74.7*	0.0727*	50.4*

* significantly different reduction compared to the control

1) based on 15 additional plants, representative of those used in the test

Table 8: Mean total plant dry weight (g)

Nominal concentration (mg/L)	Days after application		Yield (cm)	Reduction in yield (%)	Growth rate (1/day)	Reduction in growth rate (%)
	0 ¹	14				
Control	0.0378	0.2596	0.2218	-	0.1349	-
0.954	0.0378	0.2388	0.2010	9.4	0.1305	3.3
3.05	0.0378	0.2475	0.2097	5.5	0.1330	1.4
9.77	0.0378	0.3014	0.2636	-18.8	0.1476	-9.4
31.3	0.0378	0.1975	0.1597	28.0	0.1165	13.6
100	0.0378	0.1037	0.0659*	70.3*	0.0717*	46.8*

* significantly different reduction compared to the control

1) based on 15 additional plants, representative of those used in the test

The calculated EC₅₀ values, NOEC and LOEC based on growth rate and yield for each of the measured parameters (total shoot length, fresh weight and dry weight) are presented below.

Table 9: Summary of biological results based on nominal concentrations of picloram metabolite 5,6-dichloropicloram

Parameter (mg/L)	Total shoot length		Fresh weight		Dry weight	
	Growth rate	Yield	Growth rate	Yield	Growth rate	Yield
14-day EC ₅₀	78.2	40.4	93.1	50.0	>100 ¹⁾	59.2
95% Conf. Limits	63.7 - 102	34.2 – 48.1	75.7 – 124	43.0 – 58.8	-	50.8 – 70.3
14-day EC ₂₀	18.4	10.9	24.7	16.4	29.3	19.8
95% Conf. Limits	13.0 – 23.5	8.03 – 13.8	17.4 – 31.3	12.2 – 20.5	20.9 – 36.8	14.7 – 24.4
14-day EC ₁₀	30.2	17.1	39.0	24.1	45.4	28.8
95% Conf. Limits	23.7 – 36.9	13.5 – 20.7	30.7 – 47.2	19.2 – 28.7	36.0 – 54.8	23.1 – 34.2
14-day NOEC	9.77	9.77	9.77	9.77	31.3	31.3
14-day LOEC	31.3	31.3	31.3	31.3	100	100

(-) Values not reliable, control CV exceeded the effect level

CONCLUSION

Following exposure of the aquatic macrophyte *Myriophyllum spicatum* to 5,6-dichloropicloram for 14 days, the E_rC₅₀ and E_yC₅₀ values based on total shoot length were 78.2 mg/L and 40.4 mg/L respectively. The NOEC for growth rate and yield based on total shoot length was 9.77 mg/L. The E_rC₅₀ and E_yC₅₀ values based on biomass (fresh weight) were 93.1 mg/L and 50.0 mg/L respectively. The NOEC for growth rate and yield based on biomass (fresh weight) was 9.77 mg/L. The E_rC₅₀ and E_yC₅₀ values based on biomass (dry weight) were >100 mg/L and 59.2 mg/L respectively. The NOEC for growth rate and yield based on biomass (dry weight) was 31.3 mg/L.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Water Milfoil	<i>Myriophyllum spicatum</i>	5,6-dichloropicloram	14 day	E _r C ₅₀ (nominal)	78.2	mg/L
Water Milfoil	<i>Myriophyllum spicatum</i>	5,6-dichloropicloram	14 day	E _y C ₅₀ (nominal)	40.4	mg/L

A 2.2.1.3 Study 190151: GF-4021: Growth Inhibition of *Myriophyllum spicatum* in a Water/Sediment System.

Comments of zRMS:	<p>In terms of test conditions and experimental treatment the study design was in line with recommendations of OECD 239. No deviations regarding environmental conditions were observed and all validity criteria were met.</p> <p>However, it was noted that the number of shoots tested in the control and the test item groups was not in line with recommendations of OECD 239. According to the test guideline, 6 replicates per control and 4 replicates per test item group with 3 shoots each are recommended, resulting in 18 and 12 plants per control and test item group, respectively. In this study one shoot per replicate was used with 10 replicates per control and 5 replicates per test item group, resulting in 10 and 5 plants per control and test item group, respectively. In general, this deviation could reduce the statistical power of the study. However, this alternative test design with single shoot per replicate and 5 and 10 replicates per test groups and control, respectively, has been agreed during the general ecotox meeting (see EFSA Supporting publication 2019:EN-1673). For this reason this deviation is considered acceptable.</p> <p>The measured concentrations of the test item in the test vessels were analysed based on the content of halauxifen-methyl, aminopyralid and picloram in fresh and aged medium at each renewal. The mean measured concentrations of aminopyralid and picloram during the renewal period were within the range of 80-120 % of the nominal. However, degradation of halauxifen-methyl was observed between renewals of the test solutions. Therefore, the toxicological endpoints were evaluated using nominal and geometric mean measured concentrations of the test item, based on the recoveries of halauxifen-</p>
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	<p>methyl as this was the least stable active ingredient under the test conditions.</p> <p>Overall, the study is considered acceptable with the following endpoints:</p> <p>lowest 14-d E_rC_{50} = 0.00817 mg product/L (based on geometric mean measured concentrations)</p> <p>lowest 14-d E_yC_{50} = 0.00568 mg product/L (based on geometric mean measured concentrations)</p> <p>NOE_rC = 0.00141 mg product/L (based on geometric mean measured concentrations)</p>
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Reference:	KCP 10.2.1/03
Report:	Eser, S.; 2020; GF-4021: Growth Inhibition of <i>Myriophyllum spicatum</i> in a Water/Sediment System; Eurofins Agroscience Services Ecotox GmbH, D-75223 Niefern-Öschelbronn, Germany.; Lab Study No. S19-00162; DAS Study No. 190151 ; 06 October 2020; Unpublished.
Guideline(s):	OECD 239
Deviations:	Yes (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study):	-

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-4021
Purity:	aminopyralid; content of a.i. (analysed): 3.3 % w/w; halauxifen-methyl; content of a.i. (analysed): 1.08 % w/w; picloram; content of a.i. (analysed): 5.1 % w/w.
Description (physical state):	liquid / amber
Lot/batch no.:	ENBK-170903-012, TSN401447

Test System

Organism (<i>Species</i>):	Aquatic plant, <i>Myriophyllum spicatum</i> L
Study type:	Laboratory study - water/sediment system
Study duration:	14 days
Parameters measured:	Test solution pH (mean + SD): 7.70 ± 0.28 Test solution temperature (mean + SD): $19.8 \pm 0.5^\circ\text{C}$ Oxygen saturation (mean + SD): $106 \pm 5 \%$
Environmental conditions:	Photoperiod: 16-h day-length Light intensity (range): $120 - 160 \mu\text{Em}^{-2}\text{s}^{-1}$
Observation intervals:	0, 7 and 14 days
Test concentrations:	Nominal: 0 (control), 0.191, 0.610, 1.95, 6.25 and 20.0 $\mu\text{g/L}$, corresponding to - (control), 0.135, 0.431, 1.41, 4.52 and 15.1 μg test item/L (geometric mean measured concentrations)
Acclimation period/conditions:	>14 days
Growth medium:	Smart and Barko medium
Method of test item added to the test medium:	Spiked water
No. of control replicates:	10

No. of test concentration replicates:	5
No. of rooted apical shoots per vessel:	1
Analytical verification:	<p>Method: measuring concentrations of halauxifen-methyl, aminopyralid and picloram using HPLC-MS/MS.</p> <p>Samples taken: daily from fresh and aged solutions</p> <p>Samples analysed:</p> <p>The overlying water from the test vessels of all sampled treatment groups and control taken from day 0, 3, 6, 9 and 13 (fresh) and from day 1, 4, 7, 10 and 14 (aged) was analysed for halauxifen-methyl.</p> <p>The overlying water from the test vessels of all sampled treatment groups taken from day 0 (fresh) and from day 1 (aged) was analysed for aminopyralid and picloram.</p> <p>Additionally, the overlying water of the highest test concentration taken on every day (fresh) was analysed for halauxifen-methyl.</p> <p>Samples of the wet sediment taken at test termination on day 14 from all treatment groups and control were analysed for halauxifen-methyl, aminopyralid and picloram.</p> <p>Pore water samples were not analysed because no quantifiable residues of the analyte were detected in the sediment at test end for all active ingredients.</p> <p><i>Water:</i></p> <p>Halauxifen-methyl: LOD = 0.0000636 µg/L; LOQ = 0.000212 µg/L</p> <p>Aminopyralid: LOD = 0.000197 µg/L; LOQ = 0.000656 µg/L</p> <p>Picloram: LOD = 0.000306 µg/L; LOQ = 0.00102 µg/L</p> <p><i>Sediment:</i></p> <p>Halauxifen-methyl: LOD = 0.000210 mg/kg; LOQ = 0.000700 mg/kg</p> <p>Aminopyralid: LOD = 0.0021 mg/kg; LOQ = 0.00700 mg/kg</p> <p>Picloram: LOD = 0.0021 mg/kg; LOQ = 0.00700 mg/kg</p> <p>Recoveries from QC fortifications: (70-110% mean recovery, ≤20% RSD)</p>
Test substance renewal days:	daily renewal

Methodology

Plants were grown in a semi-static water-sediment system with daily renewal of test solutions using artificial sterilised sediment overlaid with Smart and Barko medium under the same conditions as used in the pre-culture.

On the day of test start, one rooted apical shoot per vessel was planted carefully, ensuring the plant was rooted into the sediment. Shortly afterwards, application of the test item was performed and mixed in with gentle stirring. The test item was spiked to the water at nominal concentrations of 0 (control), 0.191, 0.610, 1.95, 6.25 and 20.0 µg test item/L.

Ten replicates were used for the control and five for each test item group. On day 0 fifteen additional plants, representative of those used in the test, were selected from the available plant material. The plants were blotted dry prior to assessment of shoot fresh weight and shoot length. The plants were

placed separately in labelled glass beakers and dried at 60°C for >48 hours. The weight of the dry shoot samples was recorded.

On day 14 plants were harvested from each treatment group for assessment of shoot fresh weight, shoot dry weight, shoot length and number and length of side shoots.

Data were used to calculate the EC₁₀, 20, 50 values, and NOEC/LOEC values where possible for: growth rate and yield for total shoot length; growth rate and yield for shoot fresh weight; and growth rate and yield for shoot dry weight. In addition, observations on shoot and root development (e.g. necrosis, deformation) were documented.

The EC_x (yield and growth rate) values were calculated using Probit analysis following Gompertz distribution for all nominal and geometric mean measured concentration endpoints

A test for normality of the data was carried out by calculating the Shapiro-Wilk's statistic. For homogeneity of variances across treatment groups a Bartlett's or Levene's test was performed. If data were normally distributed and variance was homogeneous a Dunnett's test was performed. If data were normally distributed, but the variance was not homogeneous a Bonferroni-Holms corrected Welch's test was performed. If Shapiro Wilks test indicated a non-normal distribution of residuals a Bonferroni-U Exact Test was performed to determine significant differences from controls (SAS® Proprietary Software 9.4).

RESULTS AND DISCUSSION

The measured concentration of the test item in the test vessels based on the halauxifen-methyl content in the freshly prepared test solution ranged between 77 and 112 % of nominal in the overlaying water. The mean measured content of halauxifen-methyl for all concentrations in the freshly prepared test solutions was 92 % of nominal. In the aged test solutions, the measured concentration of the test item based on the halauxifen-methyl content in the test vessels ranged from 38 to 85 % of nominal in the overlaying water. The mean measured content of halauxifen-methyl for all concentrations in the aged test solutions was 56 % of nominal. In the sediment, no concentrations of halauxifen-methyl above the LOQ were detectable at all nominal concentration levels at test end after 14 days. Therefore, no pore water samples were analysed.

The measured concentration of the test item in the test vessels based on the aminopyralid content in the freshly prepared test solutions at test start ranged between 91 and 105 % of nominal in the overlaying water. The mean measured content of aminopyralid for all concentrations in the freshly prepared test solutions was 98 % of nominal. In the aged test solutions on day 1 the measured concentration of the test item based on the aminopyralid content in the test vessels ranged from 89 to 103 % of nominal in the overlaying water. The mean measured content of aminopyralid for all concentrations in the aged test solutions was 96 % of nominal. In the sediment, no concentrations of aminopyralid above the LOQ were detectable at all nominal concentration levels at test end after 14 days. Therefore, no pore water samples were analysed.

The measured concentration of the test item in the test vessels based on the picloram content in the freshly prepared test solutions at test start ranged between 96 and 104 % of nominal in the overlaying water. The mean measured content of picloram for all concentrations in the freshly prepared test solutions was 100 % of nominal. In the aged test solutions on day 1 the measured concentration of the test item based on the aminopyralid content in the test vessels ranged from 78 to 105 % of nominal in the overlaying water. The mean measured content of picloram for all concentrations in the aged test solutions was 95 % of nominal. In the sediment, no concentrations of picloram above the LOQ were detectable at all nominal concentration levels at test end after 14 days. Therefore, no pore water samples were analysed.

The mean measured concentrations of aminopyralid and picloram during the renewal period were within the range of 80-120 % of the nominal. However, degradation of halauxifen-methyl was observed between renewals of test solutions. Therefore, the toxicological endpoints were evaluated using nominal and geometric mean measured concentrations of the test item, based on the recoveries of halauxifen-methyl as this was the least stable active ingredient under these test conditions. The corresponding to geometric mean measured concentrations were 0.135, 0.431, 1.41, 4.52 and 15.1 µg test item/L.

The mean control growth rate based on shoot length, shoot fresh weight and shoot dry weight was 0.0976, 0.1101 and 0.1119 /day respectively, which is equivalent to a mean doubling time of 7.1, 6.3 and 6.2 days respectively. The coefficient of variation (C.V.) for control growth based on shoot length, shoot fresh weight and shoot dry weight was 10.6, 8.8 and 10.9 %, respectively.

The mean control yield (and C.V.) based on shoot length was 18.9 cm (C.V. = 18.8 %), for shoot fresh weight yield was 0.561 g (C.V. = 16.2 %), and for shoot dry weight yield was 0.0504 g (C.V. = 20.5 %).

The coefficient of variation for yield shoot fresh weight for the control was below 35 % (actual 16.2 %) and a doubling of shoot biomass and length was reached within the test duration (actual 7.1, 6.3 and 6.2 days for shoot length, shoot fresh weight and shoot dry weight, respectively). The control growth rates and variability were therefore considered acceptable. Results are summarised in the following tables.

Table 10: Mean total shoot length including side shoots (cm)

Nominal concentration (µg GF-4021/L)	Geometric mean measured concentration (µg GF-4021/L)	Days after application		Yield (cm)	Reduction in yield (%)	Growth rate (1/day)	Reduction in growth rate (%)
		0 ¹⁾	14				
Control	-	6.4	25.3	18.9	-	0.0976	-
0.191	0.135	6.4	27.7	21.3	-12.7	0.1042	-6.8
0.610	0.431	6.4	29.7	23.3	-23.3	0.1094	-12.1
1.95	1.41	6.4	22.7	16.3	13.8	0.0877	10.1
6.25	4.52	6.4	15.8	9.4*	50.3	0.0631*	35.3
20.0	15.1	6.4	9.4	3.0*	84.1	0.027*	72.3

* significantly different reduction compared to the control

¹⁾ based on 15 additional plants, representative of those used in the test

Table 11: Mean shoot fresh weight (g)

Nominal concentration (µg GF-4021/L)	Geometric mean measured concentration (µg GF-4021/L)	Days after application		Yield (g)	Reduction in yield (%)	Growth rate (1/day)	Reduction in growth rate (%)
		0 ¹⁾	14				
Control	-	0.1512	0.7122	0.561	-	0.1101	-
0.191	0.135	0.1512	0.7663	0.6151	-9.6	0.1149	-4.4
0.610	0.431	0.1512	0.7565	0.6053	-7.9	0.1146	-4.1
1.95	1.41	0.1512	0.5931	0.4419	21.2	0.0951	13.6
6.25	4.52	0.1512	0.4466	0.2954*	47.3	0.0737*	33.1
20.0	15.1	0.1512	0.2676	0.1164*	79.3	0.0386*	64.9

* significantly different reduction compared to the control

¹⁾ based on 15 additional plants, representative of those used in the test

Table 12: Mean shoot dry weight (g)

Nominal concentration (µg GF-4021/L)	Geometric mean measured concentration (µg GF-4021/L)	Days after application		Yield (g)	Reduction in yield (%)	Growth rate (1/day)	Reduction in growth rate (%)
		0 ¹⁾	14				
Control	-	0.0131	0.0635	0.0504	-	0.1119	-
0.191	0.135	0.0131	0.0685	0.0554	-9.9	0.117	-4.6
0.610	0.431	0.0131	0.0707	0.0576	-14.3	0.1201	-7.3
1.95	1.41	0.0131	0.0592	0.0461	8.5	0.1061	5.2
6.25	4.52	0.0131	0.0459	0.0328*	34.9	0.0866*	22.6
20.0	15.1	0.0131	0.0332	0.0201*	60.1	0.0644*	42.4

* significantly different reduction compared to the control

¹⁾ based on 15 additional plants, representative of those used in the test

The calculated endpoints based on growth rate and yield for each of the measured parameters (total shoot length, fresh weight and dry weight) are presented below.

Table 13: Summary of biological results based on nominal and geometric mean measured concentrations of GF-4021

Parameter (µg test item/L)	Total shoot length		Fresh weight		Dry weight	
	Growth rate	Yield	Growth rate	Yield	Growth rate	Yield
Nominal concentrations						
14-day EC ₅₀	11.0	7.71	12.4	7.87	> 20.0	13.7
95% Conf. Limits	9.30 – 13.0	6.52 – 9.01	10.2 – 15.3	6.54 – 9.39	n.d.	11.3 – 17.0
14-day EC ₂₀	4.07	2.86	3.94	2.47	7.46	-
95% Conf. Limits	3.03 – 5.08	2.12 – 3.58	2.83 – 5.03	1.75 – 3.19	5.50 – 9.48	-
14-day EC ₁₀	-	-	1.85	-	-	-
95% Conf. Limits	-	-	1.12 – 2.61	-	-	-
14-day NOEC	1.95	1.95	1.95	1.95	1.95	1.95
14-day LOEC	6.25	6.25	6.25	6.25	6.25	6.25
Geometric mean measured concentrations						
14-day EC ₅₀	8.17	5.68	9.22	5.80	> 15.1	10.2
95% Conf. Limits	6.87 – 9.72	4.78 – 6.66	7.57 – 11.4	4.80 – 6.95	n.d.	8.39 – 12.8
14-day EC ₂₀	2.95	2.05	2.86	1.77	5.49	-
95% Conf. Limits	2.18 – 3.70	1.52 – 2.59	2.04 – 3.67	1.25 – 2.30	4.02 – 7.01	-
14-day EC ₁₀	-	-	1.32	-	-	-
95% Conf. Limits	-	-	0.792 – 1.87	-	-	-
14-day NOEC	1.41	1.41	1.41	1.41	1.41	1.41
14-day LOEC	4.52	4.52	4.52	4.52	4.52	4.52

(-) Values not reliable, control CV exceeded the effect level; n.d. not determined

CONCLUSION

Following a daily renewal exposure of the aquatic rooted macrophyte *Myriophyllum spicatum* to GF-4021 for 14 days the most sensitive parameters for yield was shoot length with an E₅₀ of 7.71 µg test item/L (nominal concentrations) or 5.68 µg test item/L (geometric mean measured concentrations). For growth rate, the most sensitive parameter was shoot length with an E₅₀ of 11.0 µg test item/L (nominal concentrations) or 8.17 µg test item/L (geometric mean measured concentrations).

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Eurasian milfoil	<i>Myriophyllum spicatum</i>	GF-4021	14 day	ErC ₅₀ , mm	8.17	µg/L
Eurasian milfoil	<i>Myriophyllum spicatum</i>	GF-4021	14 day	E _y C ₅₀ , mm	5.68	µg/L

A 2.2.1.4 Study 190111: GF-4021: A 72-Hour Toxicity Test with the Freshwater Alga, *Raphidocelis subcapitata*.

Comments of zRMS:	<p>The study was performed fully in line with OECD 201 with no deviations.</p> <p>The measured concentrations of the test item were analysed based on the content of halauxifen-methyl, aminopyralid and picloram in fresh and spent test substance treatment solutions. The mean measured concentrations of aminopyralid and picloram at test initiation and termination were within the range of 80-120 % of the nominal concentrations. However, measured concentrations of halauxifen-methyl dropped below 80% of nominal concentrations. Therefore, the toxicological endpoints were evaluated using nominal and geometric mean measured concentrations of the test item, based on the recoveries of halauxifen-methyl as this was the least stable active ingredient under the test conditions.</p> <p>All validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>72 h ErC₅₀ = 0.15 mg product/L (based on geometric mean measured concentrations) 72 h E_yC₅₀ = 0.081 mg product/L (based on geometric mean measured concentrations) 72 h NOErC = 0.038 mg product/L (based on geometric mean measured concentrations)</p>
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Reference:	KCP 10.2.1
Report:	Goudie, O.; 2020; GF-4021: A 72-Hour Toxicity Test with the Freshwater Alga, <i>Raphidocelis subcapitata</i> ; Eurofins EAG Agrosience, LLC, Easton, Maryland, USA; Lab Study No. 379P-159; DAS Study No. 190111 ; 02 October 2020; Unpublished.
Guideline(s):	OECD 201 (2011)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study):	-

MATERIALS AND METHODS

Test Item(s)

Test item (Common name): GF-4021

Purity: 3.3 wt% aminopyralid, 1.08 wt% halauxifen-methyl, 5.1 wt% picloram; test item density 0.9457 g/mL

Description (physical state): Tan granules with a mild odor

Lot/batch no.: ENBK-170903-012 [TSN401447]

Test System

Organism (*Species*): unicellular green alga (*Raphidocelis subcapitata*)

Study type: Laboratory study assessing algal growth

Study design: Static

Test concentrations:	Nominal: 0 (control), 0.026, 0.064, 0.16, 0.40, and 1.0 mg GF-4021/L Geometric mean measured GF-4021 concentrations (based on halauxifen-methyl analysis, the least stable active ingredient in the study. The limit of detection (LOD) for the analysis of halauxifen-methyl in AAP medium was 0.0081 µg a.i./L (0.000750 mg GF-4021/L), defined as 30% of the LOQ): <LOD (control), 0.016, 0.038, 0.084, 0.25 and 0.63 mg GF-4021/L
Duration:	72 hrs
Parameters measured:	Cell Density, Growth Rate, Yield
Environmental conditions:	Test solution pH (range): 7.2 to 8.0 Temperature (range): 24.6 to 24.9°C Photoperiod: Continuous light Light intensity (range): 5,550 to 6,330 lux
Observation intervals:	0, 24, 48, 72 hours
Age of inoculum:	4 days
Acclimation period/conditions:	The prepared cultures were maintained in a temperature-controlled environmental chamber under continuous light. Periodically, new cultures were cloned from an existing culture derived from the parent stock. All cultures were maintained under the same conditions as those used for testing.
Initial cell density:	1.0×10^4 cells/mL
Growth medium:	Name: Freshwater AAP medium pH at test initiation: 7.2 to 7.3 pH at test termination: 7.3 to 8.0 Constant stirring ² : swirled on an orbital shaker table at 100 rpm
Method of test item added to the test medium:	A 10 mg GF-4021/L primary stock solution was prepared by transferring 0.0100 g of GF-4021 to a 1-L volumetric flask and the flask brought to volume with test medium. A secondary stock solution was prepared at a nominal concentration of 1.0 mg GF-4021/L by diluting a 200 mL aliquot of the primary stock to 2,000 mL with test medium. Appropriate aliquots of the secondary stock solution were used to prepare the test substance treatments at concentrations of 0.026, 0.064, 0.16, 0.40, and 1.0 mg GF-4021/L. The control consisted of test medium only.
No. of control replicates:	6
No. of test concentration replicates:	3
Analytical verification:	Method: Analysed for the concentration of the active ingredients aminopyralid, picloram, and halauxifen-methyl, using a liquid chromatography system with tandem mass spectrometry LC-MS/MS. Samples taken: 0, 24, 48, and 72 hrs (24 and 48 hours for halauxifen-methyl only) Limit of Detection (LOD): 0.00075 mg GF-4021/L 0.025 µg a.i./L (aminopyralid) 0.038 µg a.i./L (picloram) 0.0081 µg a.i./L (halauxifen-methyl)

Limit of Quantitation (LOQ):
0.0025 mg GF-4021/L
0.083 µg a.i./L (aminopyralid)
0.13 µg a.i./L (picloram)
0.027 µg a.i./L (halauxifen-methyl)

Recoveries from QC fortifications:
92 to 106% (halauxifen-methyl)
100 to 114% (aminopyralid)
91 to 118% (picloram)

Reference substance: Zinc Chloride (conducted as a separate non-GLP study).

Methodology

The in-life phase of the definitive test was conducted at a nominal concentration range of 0 (control), 0.026, 0.064, 0.16, 0.40, and 1.0 mg GF-4021/L.

Test chambers were sterile, 250-mL glass Erlenmeyer flasks plugged with sterile foam stoppers and contained 100 mL of test or control medium. The test chambers were labelled with the project number, test concentration and replicate, and were indiscriminately positioned daily on each of two mechanical shakers in an environmental chamber designed to maintain the desired test temperature throughout the test. The test flasks were continuously shaken at 100 rpm.

Three replicate test chambers were maintained in each treatment group, while six replicate test chambers were maintained in the negative control throughout the exposure period. An additional replicate for each of the control and test substance treatments was included for the purposes of providing solutions for analytical verification at 24 and 48 hours of the exposure.

At test initiation, an inoculum of the algal cells was added to each test chamber to achieve a nominal initial cell density of approximately 10,000 cells/mL.

Samples were collected from each replicate test chamber at approximately 24-hour intervals during the test to determine cell densities. Cell densities were used to calculate growth rates and yields which were subsequently used to calculate percent inhibition values relative to the negative control over the 72-hour exposure period. Test solutions were analysed for the concentration of the active ingredients aminopyralid, picloram, and halauxifen-methyl, using a liquid chromatography system with tandem mass spectrometry (LC-MS/MS).

The calculation of cell densities, yield, growth rates and percent inhibition values, evaluation of homogeneity of variance and normality, and regression analyses, were conducted using “The SAS System for Windows, Version 9.4”. Comparison tests were conducted using “CETIS Version 1.3.9.0”. The 72-hour growth rate and yield data were evaluated for normality and homogeneity of variance ($\alpha = 0.01$) using Shapiro-Wilk’s and Levene’s tests, respectively. The 72-hour growth rate and yield data met assumptions of normality and homogeneity of variance. Visual inspection of the data concluded that the response in test substance treatments was monotonic, with a reduction in calculated values correlating to an increase in concentration; therefore, the test substance treatments were compared to the negative control response using William’s multiple comparisons test ($p = 0.05$). The results of the statistical analyses, as well as an evaluation of the concentration response pattern, were used to determine the presence of a NOEC relative to each parameter at 72 hours.

RESULTS AND DISCUSSION

Measurements of temperature, pH and light intensity 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 at the time of preparation.

No residues of aminopyralid, picloram or halauxifen-methyl active ingredients in GF-4021, were detected in the control solutions above the LOD.

The overall geometric mean measured concentrations (based on aminopyralid analysis) were 0.027, 0.068, 0.17, 0.43, and 0.98 ~~32~~ mg GF-4021/L (98% to 108% of nominal). Recoveries ranged from 102% to 109% of the nominal concentrations at initiation (0-h) and from 92% to 111% of the nominal concentrations at termination (72-h).

The overall geometric mean measured concentrations (based on picloram analysis) were 0.028, 0.071, 0.18, 0.45, and 1.1 mg GF-4021/L (106% to 112% of nominal). Recoveries ranged from 104% to 111% of the nominal concentrations at initiation (0-h) and from 110% to 118% of the nominal concentrations at termination (72-h).

The overall geometric mean measured concentrations (based on of halauxifen-methyl analysis) were 0.016, 0.038, 0.084, 0.25 and 0.63 mg GF-4021/L (52% to 63% of nominal). Recoveries ranged from 94% to 102% of the nominal concentrations at initiation (0-h); from 61% to 81% of the nominal concentrations at 24-h; from 42% to 62% of the nominal concentrations at 48-h; and from 30% to 38% of the nominal concentrations at termination (72-h).

The biological response results are reported based upon both the nominal GF-4021 concentrations and geometric mean measured concentrations of GF-4021, based on halauxifen-methyl analysis, the least stable active ingredient in the study.

A 96-hour reference toxicant test was conducted as part of facility records. Testing was conducted with a nominal concentration range of: 0 (control), 0.060, 0.13, 0.25, 0.50, and 1.0 mg/L. Eight replicates were used for the control, and four replicates for the reference toxicant treatments. The target initial cell density was 10,000 cells/mL. The estimated 72-hour EC₅₀ value on cell density derived from this test was 0.17 mg/L with 95% confidence limits of 0.13 and 0.22 mg/L.

All study validity criteria for the study were met: 1) Mean cell density in the control at test termination should increase by a factor ≥ 16 to verify logarithmic phase growth (factor of 223 in this study); 2) Mean percent coefficient of variation for section-by-section specific growth rates should be $\leq 35\%$ (9% in this study); and 3) Coefficient of variation of average specific growth rates during the whole test period in control replicates should not exceed 7% (2% in this study).

Table 14: Mean cell density

Nominal concentration [mg GF-4021/L]	Geometric Mean Measured Concentration [mg GF-4021/L] ¹	Mean cell density [cells/mL]
		72 h
0 (control)	0 (control)	2,226,913
0.026	0.016	2,291,000
0.064	0.038	1,918,732
0.16	0.084	1,112,882*
0.40	0.25	32,359*
1.0	0.63	14,184*

¹ Based on halauxifen-methyl analysis.

* Statistically significant compared to the control.

Table 15: Mean growth rate and yield

Nominal concentration [mg GF-4021/L]	Geometric Mean Measured Concentration [mg GF-4021/L] ¹	Mean growth rate [cell/ml/h]	% inhibition ²	Mean yield [cell/ml]	% inhibition ²
		0-72 h	72 h	72 h	72 h
control	control	0.0750	--	2,216,913	--
0.026	0.016	0.0754	-1	2,281,000	-3
0.064	0.038	0.0728	3	1,908,732	14
0.16	0.084	0.0649*	13	1,102,882*	50
0.40	0.25	0.0163*	78	22,359*	99
1.0	0.63	0.0045*	94	4,184*	100

¹ Based on halauxifen-methyl analysis.

² Calculations were performed using SAS Version 9.4. Manual calculations may differ slightly.

* Statistically significant compared to the control.

Table 16: Effects of GF-4021 on algal growth based on nominal concentrations

Hour	EC Type	EC Value [mg GF-4021/L]	95% Confidence Limits [mg GF-4021/L]	NOEC [mg GF-4021/L]
72	ErC ₁₀	0.10	0.068 – 0.15	0.064
	ErC ₂₀	0.14	0.10 – 0.19	
	ErC ₅₀	0.26	0.22 – 0.32	
	EyC ₁₀	0.088	0.053 – 0.14	0.064
	EyC ₂₀	0.11	0.071 – 0.16	
	EyC ₅₀	0.16	0.12 – 0.20	

ECx values were calculated using non-linear regression with replicate data (growth rate and yield) and nominal GF-4021 concentrations.

Table 17: Effects of GF-4021 on algal growth based on geometric mean measured concentrations (based on halauxifen-methyl analysis)

Hour	EC Type	EC Value [mg GF-4021/L]	95% Confidence Limits [mg GF-4021/L]	NOEC [mg GF-4021/L]
72	ErC ₁₀	0.054	0.037 – 0.078	0.038
	ErC ₂₀	0.077	0.056 – 0.10	
	ErC ₅₀	0.15	0.13 – 0.19	
	EyC ₁₀	0.041	0.028 – 0.060	0.038
	EyC ₂₀	0.051	0.037 – 0.071	
	EyC ₅₀	0.081	0.065 – 0.10	

ECx values were calculated using non-linear regression with replicate data (growth rate and yield) and geometric mean measured GF-4021 exposure concentrations.

CONCLUSION

Based on nominal GF-4021 concentrations, the 72-hour ErC₁₀, ErC₂₀ and ErC₅₀ values were determined to be 0.10, 0.14 and 0.26 GF-4021/L, respectively; the 72-hour EyC₁₀, EyC₂₀ and EyC₅₀ values were determined to be 0.088, 0.11 and 0.16 mg GF-4021/L, respectively; and the 72-hour NOEC was determined to be 0.064 mg GF-4021/L for both growth rate and yield.

Based on geometric mean measured GF-4021 concentrations, based on halauxifen-methyl analyses, the least stable active ingredient in the study, the 72-hour ErC₁₀, ErC₂₀ and ErC₅₀ values were determined to be 0.054, 0.077 and 0.15 mg GF-4021/L, respectively; the 72-hour EyC₁₀, EyC₂₀ and EyC₅₀ values were determined to be 0.041, 0.051 and 0.081 mg GF-4021/L, respectively; and the 72-hour NOEC was determined to be 0.038 mg GF-4021/L for both growth rate and yield.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Freshwater green algae	<i>Raphidocelis subcapitata</i>	GF-4021	72-hr	ErC ₅₀ (nominal)	0.26	mg/L
Freshwater green algae	<i>Raphidocelis subcapitata</i>	GF-4021	72-hr	EyC ₅₀ (nominal)	0.16	mg/L
Freshwater green algae	<i>Raphidocelis subcapitata</i>	GF-4021	72-hr	ErC ₅₀ (geomean)	0.15	mg/L
Freshwater green algae	<i>Raphidocelis subcapitata</i>	GF-4021	72-hr	EyC ₅₀ (geomean)	0.081	mg/L

A 2.2.2 KCP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

Not required to characterise the product in the current submission.

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

Not required to characterise the product in the current submission.

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

Please refer to Point A 2.3.1.1.2.

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

Comments of zRMS:	<p>The study was performed fully in line with OECD 213 and OECD 214 with minor deviations.</p> <p>It was noted that only 9 instead of 10 bees from one of the replicates in the 0.30 µg a.s./bee toxic standard group were observed on day. As a reason a biologist oversight during observation was given. Since day 0 observations are not used for the calculation of endpoints, this deviation is considered to have no impact on the integrity of the study.</p> <p>It was also noted that the highest recorded relative humidity during the test was 78 % which is slightly higher than the recommended maximum of 70 %. However, this deviation is considered to have no effect on the study outcome since all validity criteria were met.</p> <p>Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>48h oral LD₅₀ > 87.50 µg product/bee 48h contact LD₅₀ > 250 µg product/bee</p>
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Reference:	KCP 10.3.1.1/01 and KCP 10.3.1.2/01
Report:	Tomé, H.V.V, Porch, J.R.; 2020; GF-4021: An Acute Oral and Contact Toxicity Study with the Honey Bee; Eurofins EAG Agrosience, LLC, Easton, Maryland, USA; Lab Study No. 379H-140; DAS Study No. 190458 ; 17 April 2020; Unpublished.
Guideline(s):	OECD 213 (1998) and OECD 214 (1998)
Deviations:	Minor (see commenting box above for details)
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study):	-

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-4021
Purity:	3.3 wt% aminopyralid, 1.08 wt% halauxifen-methyl, 5.1 wt% picloram
Description (physical state):	Liquid, Emulsifiable concentrate (EC)
Lot/batch no.:	ENBK-170903-012 (TSN401447)

Test System

Organism (<i>Species</i>):	Honey bee (<i>Apis mellifera</i>)
Study type:	Adult acute, oral and contact exposure
Study design:	Dose-response; acute oral and 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 doses:	Oral: 15.6, 31.3, 62.5, 125 and 250 µg GF-4021/bee (nominal dose) 15.6, 31.3, 62.5, 56.3 and 87.5 µg GF-4021/bee (consumed dose) Contact: 15.6, 31.3, 62.5, 125 and 250 µg GF-4021/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 within four weeks of test initiation. The bees were maintained in a clean holding cage.
Amount of treated diet consumed:	Consumption of the treated diets resulted in calculated dosages ranging from 15.6 to 106 µg GF-4021/bee.
Feeding method:	50% w/v sucrose solution <i>ad libitum</i> ; was given directly after treatment using syringes; no replacements of the food was necessary during the experimental time of the experiments (48 h).
Environmental conditions:	Temperature: 25-26°C oral 25-26°C contact Relative Humidity: 55-78% oral 54-78% contact Photoperiod: The environmental chambers were kept dark except when room lighting was used during observation periods.
Reference substance:	0.05, 0.10 and 0.30 µg dimethoate per bee (oral test) 0.05, 0.10 and 0.29 µg a.i./bee (actual consumed) 0.05, 0.10 and 0.30 µg dimethoate per bee (contact test)
Solvent substance (if applicable):	1% Tween 80 (surfactant for contact test only)

Methodology

Nominal doses ranged from 15.6 to 250 µg GF-4021/bee. Three replicate test chambers (10 bees per chamber) were maintained in each of the control and treatment groups.

Oral: The test item was administered orally in 50% (w/v) sucrose solution via feeding vials. The mean amount of test item solution consumed per bee was determined by weighing the feeding vials before and after 6 hours exposure. A negative (sucrose solution) control group was maintained concurrently.

Contact: The test item was administered topically to the dorsal side of the thorax of each bee in a 1.0 µL droplet of 1% Tween 80 surfactant solution in purified water. Negative (untreated) and surfactant control groups were maintained concurrently.

Additional groups of bees from the same source were concurrently dosed with dimethoate, at 0.05, 0.10, and 0.30 µg a.i./bee. The oral and contact 24-h LD₅₀ values (0.17 µg a.i./bee with a 95% confidence interval of 0.14 to 0.20 µg a.i./bee and 0.15 µg a.i./bee with a 95% confidence interval of 0.13 to 0.19 µg a.i./bee, respectively) were within the OECD recommended values, demonstrating the sensitivity of the test bees.

For all tests, observations of mortality and other signs of toxicity were made at 4, 24 and 48 hours after dosing.

Due to the low mortality in the GF-4021 treatment levels, the LD₅₀ values were determined by visual observation of the test data, and no statistical calculation of the LD₅₀ was required. Dimethoate oral and contact 24-h LD₅₀ values were calculated by Untrimmed Spearman-Kärber Test using CETIS.

RESULTS AND DISCUSSION

Results of the oral and contact exposure test are summarized in Tables 18 and 19. No mortality was observed at any control group. Mortality in the test item treatment groups ranged from 0% to 13% in the oral test and from 0% to 10% in the contact test. Dose consumption in the oral test was largely incomplete in the two highest test item treatment groups (45% and 35% of nominal dose, respectively) which may represent an avoidance effect. No sub-lethal effects were observed in the test item treatment groups at test termination.

The 24-h and 48-h oral LD₅₀ values were determined to be > 87.5 µg GF-4021/bee (actual dose consumed). The 24-h and 48-h contact LD₅₀ values were determined to be > 250 µg GF-4021/bee. The validity criterion of the study was met, i.e. mortality in the control treatments after 48 hours should not exceed 10% (mortality was 0% in all control treatments).

Table 18: Toxicity of GF-4021 to honeybees in oral and contact toxicity test

Treatment µg GF-4021/bee		Oral	Contact
Nominal	Mean consumed dose	Mortality (%)	
		48-hr	48-hr
Negative Control (0)	-	0	0
Surfactant Control (0)	-	-	0
15.6	15.6	0	0
31.3	31.3	0	3
62.5	62.5	0	0
125	56.3	13	3
250	87.5	10	10
Contact 48-hr LD ₅₀		> 250 µg GF-4021/bee	
Oral 48-hr LD ₅₀		> 87.5 µg GF-4021/bee	
Contact LD ₅₀ (24-hr) value of the reference item: 0.15 µg dimethoate/bee			
Oral LD ₅₀ (24-hr) value of the reference item: 0.17 µg dimethoate/bee			

Table 19: Sublethal effects of GF-4021 to honey bees oral and contact toxicity test

Treatment µg GF-4021/bee		Sublethal effects after 48 hrs (number of bees)		
Nominal	Consumed	On-back	Lethargic	Other
Contact:				
Negative Control (0)	-	0	0	0
Surfactant Control (0)	-	0	0	0
15.6	-	0	0	0
31.3	-	0	0	0
62.5	-	0	0	0
125	-	0	0	0
250	-	0	0	0
Oral:				
Control (0)	0	0	0	0
15.6	15.6	0	0	0
31.3	31.3	0	0	0
62.5	62.5	0	0	0
125	56.3	0	0	0
250	87.5	0	0	0

CONCLUSION

The 24-h and 48-h oral LD₅₀ values were determined to be >87.5 µg GF-4021/bee (actual dose consumed). The 24-h and 48-h contact LD₅₀ values were determined to be >250 µg GF-4021/bee.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Honey bee	<i>Apis mellifera</i>	GF-4021	48-hr – oral	LD ₅₀	>87.5	µg/bee
Honey bee	<i>Apis mellifera</i>	GF-4021	48-hr – contact	LD ₅₀	>250	µg/bee

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

A 2.3.1.2.1 Study 200622: GF-4021 - Honey Bee (*Apis mellifera* L.) Chronic Oral Toxicity Test 10 Day Feeding Test in the Laboratory

Comments of zRMS:	<p>The study was performed in line with OECD 245 with no major deviations.</p> <p>The analytical dose verification of the test item concentrations in the feeding solution from day 1 to 10 resulted in concentrations equivalent to mean recoveries of 94 to 106 % of nominal for aminopyralid acid, 88 to 95 % of nominal for halauxifen-methyl and 95 to 104 % of nominal for picloram, respectively.</p> <p>The analytical verification of the homogeneity samples T1 (lowest concentration of feeding solution) and T5 (highest concentration of feeding solution) resulted in concentrations equivalent to recoveries of 91 to 109 % of nominal for aminopyralid, 86 to 99 % of nominal for halauxifen-methyl and 92 to 108 % of nominal for picloram, respectively.</p> <p>The analytical verification for test item stability in the feeding solution T1 and T5 resulted in concentrations equivalent to recoveries of 93 to 111 % of nominal for aminopyralid, 75 to 100 % of nominal for halauxifen-methyl and 89 to 106 % of nominal for picloram, respectively.</p> <p>The measured concentrations were within ± 20 % of nominal (with the exception of the analyte halauxifen-methyl with a measured recovery of 79 and 75 % in the spent diet samples of T1 at 1DAA3 and 1DAA7, respectively). Therefore, the endpoints can be based on nominal concentrations.</p> <p>It was noted that the lowest recorded relative humidity during the test was 45.7 % which is slightly lower than the recommended minimum of 50 %. However, this deviation lasted less than 2 hours and is considered to have no effect on the outcome of the study since all validity criteria were met:</p> <ul style="list-style-type: none"> the average mortality across replicates for the untreated control group was ≤ 15 % at the end of the test (observed 0 % mortality), the average mortality in the reference substance treated group was ≥ 50 % at the end of the test (observed 100 %). <p>Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>LDD₅₀ = 56.1 µg product/bee/day NOEDD = 13.8 µg product/bee/day</p>
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Reference:	KCP 10.3.1.2/01
Report:	Wendling, K.; 2021a; GF-4021 - Honey Bee (<i>Apis mellifera</i> L.) Chronic Oral Toxicity Test 10 Day Feeding Test in the Laboratory; Eurofins Agrosience Services Ecotox

	Gmbh, Niefern-Öschelbronn, Germany; Lab Study No. S20-00657; Das Study No. 200622 ; 21 January 2021; Unpublished.
Guideline(s):	OECD 245 (2017)
Deviations:	Yes (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study):	-

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-4021
Purity:	aminopyralid: 31 g/L, 3.3 % w/w; halauxifen-methyl: 10 g/L, 1.08 % w/w; picloram: 48 g/L, 5.1 % w/w
Description (physical state):	liquid / amber; EC
Lot/batch no.:	ENBK-170903-012 [TSN401447]

Test System

Organism (Species):	Honey bee (<i>Apis mellifera</i>)
Study type:	Chronic oral
Study design:	Dose-response test; duration 10 days; minimum 3 replicates, each consisting of 10 bees in one cage per test concentration; assessment of mortality, food consumption and behavioural effects daily.
Test concentrations:	Oral: 0 (control); 500, 1000, 2000, 4000 and 8000 mg product/kg feeding solution
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 in the last 4 weeks. The bees were maintained in a clean holding cage at a temperature of approximately $33 \pm 2^\circ\text{C}$ and 50 to 70% humidity.
Amount of treated diet consumed:	Mean daily consumption of the treated diets ranged from 8.6 to 27.6 mg/bee/day of diet. Mean calculated daily dosages ranged from 13.8 to 68.4 μg /bee/day.
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. (The second application of reference item(R) took place 30 hours 10 minutes after the first application; the following applications of R were done in a $24 \text{ h} \pm 2 \text{ h}$ interval based on the second application). Consumption of the feeding solutions was monitored by weighing the syringe before and after feeding, correcting for evaporation.
Environmental conditions:	Temperature: 32.0 to 32.8°C Relative humidity: 45.7* to 60.7% *with short term deviation(s) < 2 hours

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 feeding solution

Solvent substance (if applicable): 50% w/v aqueous sucrose solution

Methodology

Honey bees were exposed to a 50% (w/v) aqueous sucrose solution containing five concentrations of GF-4021 by continuous and *ad libitum* feeding over a period of 10 days. The control group was fed with 50% (w/v) untreated aqueous sucrose solution. Mortality and behavioural abnormalities were assessed daily during the 10 day exposure period. The weight of surviving bees was determined after the 10 day exposure period. The chronic effects of GF-4021 were evaluated by comparing the results of the test item groups to those of the control group.

8 test units without bees but with full food syringes containing 50% (w/v) aqueous sucrose solution (4 units for the time interval of the control and test item groups and 4 units for the time interval of the reference item group) were additionally placed in the climatic chamber for the evaluation of the evaporation.

RESULTS AND DISCUSSION

In the control group, after 10 days of continuous feeding no mortality was observed.

After 10 days, mortality was statistically significantly increased compared to the control group at the test item groups of 4000 and 8000 mg product/kg feeding solution (a cumulative mortality of 17.5 % and 85.0 %, respectively) (Cochran-Armitage test, one-sided greater, $\alpha = 0.05$). Weight of surviving bees was statistically significantly different compared to the control group at the concentrations of 1000, 2000, 4000 and 8000 mg product/kg feeding solution (Dunnett's t-test, two-sided, $\alpha = 0.05$).

Affected and moribund bees were observed at concentrations of 2000, 4000 and 8000 mg product/kg feeding solution. Apathetic bees were observed at concentrations of 2000 and 8000 mg product/kg feeding solution.

The overall mean daily consumption of feeding solution over the entire test period was 32.0 mg/bee/day in the control group. At the concentrations of 500, 1000, 2000, 4000 and 8000 mg product/kg feeding solution the overall mean daily consumption of feeding solution was 27.6, 24.3, 15.2, 11.7 and 8.6 mg/bee/day, respectively. In the toxic reference item group, the overall mean daily consumption of feeding solution was 22.4 mg/bee/day.

At the end of the 10-day test period, the accumulated mean uptake of the test item at the concentrations of 500, 1000, 2000, 4000 and 8000 mg product/kg feeding solution was 139, 244, 305, 468 and 648 mg product/bee, respectively. The corresponding daily mean uptake was therefore 13.8, 24.3, 30.5, 46.8 and 68.4 mg product/bee/day, respectively.

Table 20: Toxicity of GF-4021 to honey bees in the chronic oral toxicity test

Treatment		Oral 10 day test									
Nominal concentration (mg product/kg feeding solution)	Daily dose (µg product/bee/day)	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	0.0	0.0	0.0	0.0	0.0	0.0
500	13.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1000	24.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2000	30.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4000	46.8	0.0	2.5	2.5	2.5	2.5	2.5	5.0	7.5	7.5	17.5 ^a
8000	68.4	0.0	5.0	25.0	32.5	40.0	45.0	55.0	62.5	75.0	85.0 ^a
Reference Item (0.9 mg a.i./kg feeding solution)		0.0	0.0	2.5	40.0	77.5	95.0	97.5	100.0	100.0	100.0
10 day LDD ₁₀		43.9 (95 % CL: 38.4 / 47.6) µg product/bee/day									
10 day LDD ₂₀		47.7 (95 % CL: 43.0 / 51.1) µg product/bee/day									
10 day LDD ₅₀		56.1 (95 % CL: 52.5 / 59.9) µg product/bee/day									
10 day NOEDD _{mortality}		30.5									
10 day LC ₁₀		3580 (95 % CL: 2830 / 4130) mg product/kg feeding solution									
10 day LC ₂₀		4160 (95 % CL: 3480 / 4710) mg product/kg feeding solution									
10 day LC ₅₀		5570 (95 % CL: 4950 / 6280) mg product/kg feeding solution									
10 day NOEC _{mortality}		2000									

^a statistically significantly different compared to the control group (Cochran-Armitage test, one-sided greater, $\alpha = 0.05$)

Table 21: Effect of GF-4021 on weight of surviving bees in honey bees in the chronic oral toxicity test

Treatment		Oral 10 day test				
Nominal concentration (mg product/kg feeding solution)	Daily dose (µg product/bee/day)	Mean weight surviving bees (g)				
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mean per Treatment Group ^a
Control (0)	0	0.1212	0.1066	0.1155	0.1273	0.1176
500	13.8	0.1014	0.1111	0.1175	0.1008	0.1077
1000	24.3	0.0831	0.0923	0.0993	0.0957	0.0926 ^b
2000	30.5	0.1013	0.1051	0.1054	0.0981	0.1025 ^b
4000	46.8	0.0767	0.0919	0.0804	0.0834	0.0837 ^b
8000	68.4	0.0784	0.0756	-	0.0697	0.0760 ^b
10 day NOEDD _{bee weight}		13.8 µg product/bee/day				
10 day NOEC _{bee weight}		500 mg product/kg feeding solution				

^a calculation based on the individual weight of bees per treatment group

^b statistically significantly different compared to control group (Dunnett's t-test, two-sided, $\alpha = 0.05$); statistical analysis was performed using means per replicate

Table 22: Sublethal effects of GF-4021 to honey bees in the chronic oral toxicity test

Treatment		Oral 10 day test									
Nominal concentration (mg product/kg feeding solution)	Daily dose (µg product/bee/day)	Behavioural abnormalities									
		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	0	0
500	13.8	0	0	0	0	0	0	0	0	0	0
1000	24.3	0	0	0	0	0	0	0	0	0	0
2000	30.5	0	0	2ap	0	1a, 1ap	0	1a	3ap, 1m	0	0
4000	46.8	0	0	2m	2a, 1m	2a, 1m	0	3a	0	0	0
8000	68.4	0	1a, 1m	9a, 5m	10a	6a	7a, 1m	5a, 1ap	1ap	0	0
Reference Item (0.9 mg a.i./kg feeding solution)		0	0	1ap	1a	0	1a, 1ap	1ap	-	-	-

a: affected
ap: apathy
m: moribund
-: all bees were dead

CONCLUSION

All validity criteria were met, therefore, the study is considered valid.

The LC₁₀ and LDD₁₀ after 10 days of continuous exposure were calculated as 3580 (95% CL: 2830 / 4130) mg product/kg feeding solution and 43.9 (95% CL: 38.4 / 47.6) µg product/bee/day, respectively.

The LC₂₀ and LDD₂₀ after 10 days of continuous exposure were calculated as 4160 (95% CL: 3480 / 4710) mg product/kg feeding solution and 47.7 (95% CL: 43.0 / 51.1) µg product/bee/day, respectively.

The LC₅₀ and LDD₅₀ after 10 days of continuous exposure were calculated as 5570 (95% CL: 4950 / 6280) mg product/kg feeding solution and 56.1 (95% CL: 52.5 / 59.9) µg product/bee/day, respectively.

The NOEC_{mortality} after 10 days of continuous exposure was determined to be 2000 mg product/kg feeding solution. Accordingly the corresponding NOEDD_{mortality}, based on the actual consumption of the respective feeding solutions, was determined to be 30.5 µg product/bee/day.

The NOEC_{bee weight} was determined to be 500 mg product/kg feeding solution. The corresponding NOEDD_{bee weight} was determined as 13.8 µg product/bee/day.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Honey bee	<i>Apis mellifera</i>	GF-4021	10 days	LDD ₅₀	56.1	µg/bee/day
Honey bee	<i>Apis mellifera</i>	GF-4021	10 days	NOEDD	13.8	µg/bee/day

A 2.3.1.2.1 Study 170071: XDE-729 Methyl - 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 submitted by the Applicant in order to address the chronic toxicity of halauxifen-methyl to bees. However, the study was not validated by the zRMS since GF-4021 contains more than one active substance and for this reason respective study with the formulated product was provided in order to fulfil the data requirements, while the active substance endpoints should be generated in the course of the EU renewal process.</p> <p>The study summary below has been struck through and shaded as being not necessary to finalise the risk assessment at the zonal level.</p>
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Reference:	KCP 10.3.1.2/02
Report:	Oberrauch, S.; 2018; XDE-729 Methyl - 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, Eutinger Straße 24, D - 75223 Niefern-Öschelbronn, Germany; Lab Study No. S17-00191; DAS Study No. 170071 ; 22 January 2018; Unpublished.
Guideline(s):	OECD guideline proposal (2016)
Deviations:	Not evaluated
GLP:	Yes
Acceptability:	Not evaluated, not required for the zonal assessment of GF-4021
Duplication (if vertebrate study):	-

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	XDE-729 Methyl
Purity:	99.7 % w/w
Description (physical state):	solid / white
Lot/batch no.:	YC2-106153-95 (TSN301574)

Test System

Organism (Species):	Honey bee (<i>Apis mellifera</i>)
Study type:	chronic-oral
Study design:	Dose-response test; duration 10 days; 4 replicates, each consisting of 10 bees in one cage per test concentration; assessment of mortality, diet consumption and behavioural effects daily.
Test concentrations:	0 (control, solvent control), 12.5, 25.0, 50.0, 100 and 200 mg/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 20.5 to 25.3 mg of diet. Calculated daily dosages ranged from 0.29 to 5.07 µg/bee.

Feeding method:	During holding/acclimation and after administration of the test dosages, bees were provided ad libitum a 50 % (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: 32.3–33.0 °C Relative humidity: 50.4–63.1 % Photoperiod: The environmental chamber was kept dark except when room lighting was used during observation periods.
Reference substance:	Dimethoate: 0.9 mg/kg food
Solvent substance (if applicable):	5 % acetone and 0.1 % xanthan

Methodology

~~Honey bees were exposed to a 50 % (w/v) aqueous sucrose solution containing five concentrations of XDE 729 Methyl and 5 % acetone with 0.1 % xanthan by continuous and ad libitum feeding over a period of 10 days. The control group was fed with pure 50 % (w/v) aqueous sucrose solution and the solvent control group was fed with 50 % (w/v) aqueous sucrose solution containing 5 % acetone and 0.1 % xanthan. Mortality and behavioural abnormalities were assessed daily during the 10 day exposure period. The chronic effects of XDE 729 Methyl were evaluated by comparing the results of the test item group to those of the solvent control group. Additionally 4 test units without bees but with full diet syringes containing pure 50 % (w/v) aqueous sucrose were placed in the climatic chamber for the evaluation of the evaporation. The syringes of another 4 additional cages without bees but with full diet syringes (50 % (w/v) aqueous sucrose solution containing 5 % acetone and 0.1 % xanthan were placed in the climatic chamber for the evaluation of the evaporation.~~

RESULTS AND DISCUSSION

~~In the control groups (C and C_{sol}), after 10 days of continuous feeding 7.5 and 12.5 % mortality was observed, respectively.~~

~~In the test item treatment groups no statistically significant mortality was observed after 10 days.~~

~~In the controls single affected and apathetic bees were recorded on different assessment dates throughout the test.~~

~~Behavioural abnormalities (affected, apathy, moribund) in the test item treatment groups were observed mainly between assessment one and four. Afterwards only single affected bees were recorded. The overall mean daily consumption of feeding solution over the entire test period of the control groups C and C_{sol} was 33.1 and 24.0 mg/bee/day. At the concentrations of 12.5, 25.0, 50.0, 100 and 200 mg/kg food the overall mean daily consumption of feeding solution was 23.4, 25.1, 21.5, 20.5 and 25.3 mg/bee/day, respectively. In the toxic reference item group, the overall mean daily consumption of feeding solution was 18.8 mg/bee/day.~~

~~At the end of the 10 day test period, the accumulated uptake of the test item at the concentrations of 12.5, 25.0, 50.0, 100 and 200 mg/kg was 2.94, 6.30, 10.81, 20.50 and 50.68 µg/bee, respectively. The corresponding daily mean uptake was therefore 0.29, 0.63, 1.08, 2.05 and 5.07 µg/bee/day, respectively.~~

~~The mean weight of surviving bees was 92.8 mg in the solvent control group and 95.4, 94.1, 95.6, 93.3 and 95.9 mg in the test item groups of 12.5, 25.0, 50.0, 100 and 200 mg/kg food.~~

Table 23: Toxicity of XDE-729 Methyl to honey bees in the chronic oral toxicity test

Treatment Daily		Oral 10-day test									
Nominal mg/kg food	Measured daily-mean µg/bee	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	2.5	2.5	2.5	5.0	5.0	7.5	7.5	7.5
C _{sol}	0	0.0	0.0	0.0	0.0	0.0	5.0	7.5	7.5	12.5	12.5
12.5	0.29	0.0	0.0	0.0	0.0	7.5	7.5	10.0	10.0	10.0	10.0
25.0	0.63	0.0	2.5	5.0	5.0	5.0	7.5	7.5	7.5	7.5	7.5
50.0	1.08	0.0	0.0	0.0	0.0	0.0	2.5	2.5	5.0	5.0	5.0
100	2.05	0.0	0.0	0.0	2.5	2.5	2.5	2.5	2.5	2.5	2.5
200	5.07	0.0	0.0	0.0	0.0	2.5	2.5	2.5	2.5	2.5	2.5
Reference Item		0.0	0.0	0.0	10.0	25.0	60.0	82.5	90.0	100.0	100.0
10-day LD ₅₀		n.d.									
10-day NOEDD		≥ 5.07 µg/bee/day									
10-day LC ₅₀		n.d.									
10-day NOEC		≥ 200 mg/kg food									

n.d. = not determined

Table 24: Effect of XDE-729 Methyl on diet consumption in honey bees in the chronic oral toxicity test

Treatment		Oral 10-day test									
Nominal mg/kg food	Measured daily-mean µg/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	20.4	23.4	27.6	30.1	33.3	34.8	29.9	48.3	40.6	43.0
C _{sol}	0	11.1	21.1	22.4	20.0	29.7	29.4	23.7	28.2	27.9	26.4
12.5	0.29	5.3	13.8	24.8	22.0	32.0	25.8	31.7	23.0	27.8	27.4
25.0	0.63	5.6	21.5	27.1	20.6	29.6	26.5	28.2	26.8	33.7	31.4
50.0	1.08	8.6	17.3	22.6	24.5	28.3	17.4	22.9	22.6	27.3	23.9
100	2.05	6.4	15.7	26.0	18.4	20.7	15.1	23.0	24.7	27.1	27.9
200	5.07	10.2	24.8	25.5	27.3	27.2	21.4	25.7	27.2	37.3	26.8
Reference Item		12.5	34.7	16.0	23.1	21.6	17.2	0.30	31.5	9.50	-
10-day NOEC, food consumption		≥ 200 mg/kg food									

- = all bees were dead

Table 25: Effect of XDE-729 Methyl on weight of surviving bees in honey bees in the chronic oral toxicity test

Treatment		Oral 10-day test			
Nominal mg/kg food	Measured daily-mean µg/bee	Mean weight surviving bees (mg)			
		Replicate 1	Replicate 2	Replicate 3	Replicate 4
C _{sol}	0	87.0	98.8	92.2	93.1
12.5	0.29	92.9	93.1	101.2	93.2
25.0	0.63	93.9	92.7	93.2	96.6
50.0	1.08	96.9	92.7	99.5	92.9
100	2.05	96.5	96.7	89.4	90.8
200	5.07	97.2	88.9	98.9	97.9
10-day NOEC, weight surviving bees		≥ 200 mg/kg food			

Table 26: Sublethal effects of XDE 729 Methyl to honey bees in the chronic oral toxicity test

Treatment		Oral 10-day test									
Nominal mg/kg food	Measured daily-mean µg/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	0	0	0	1a	0	0	0	0
C _{sol}	0	0	0	3ap	0	0	0	0	0	0	0
12.5	0.29	19ap	4ap	1ap	1a	0	0	1a	0	0	0
25.0	0.63	11ap, 1a	5ap	3a	2a	0	0	0	0	0	0
50.0	1.08	4ap	5ap	7ap, 2a	4a	1a	0	0	0	0	0
100	2.05	4ap, 1a	3ap, 1a	12ap, 1a, 1m	7a	1a	0	0	0	0	0
200	5.07	12ap, 1a	9ap	11ap, 1a	3a	0	0	0	0	0	0
Reference Item	0	0	0	5ap, 7a	10a, 1m	9a	16a	7a	4a	-	-

a = affected

ap = apathetic

m = moribund

- = all bees were dead

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 XDE 729 Methyl at the treatment levels of 12.5, 25.0, 50.0, 100 and 200 mg/kg food caused no adverse effects regarding mortality, weight of surviving bees and food consumption.

The LOEC for mortality after 10 days of continuous exposure could not be statistically determined. Therefore, the NOEC was determined to be ≥ 200 mg/kg food. The corresponding NOEDD, based on the actual consumption of the respective feeding solutions, was determined to be ≥ 5.07 µg/bee/day.

The LC₁₀ as well as the LDD₁₀ after 10 days of continuous exposure could not be determined due to the lack of a clear dose response relationship.

The LC₅₀ as well as the LDD₅₀ after 10 days of continuous exposure could not be determined since the observed mortalities were below 50% in all test item groups.

The LOEC based on the overall mean consumption of feeding solution after 10 days of continuous exposure could not be statistically determined. Therefore, the NOEC was determined to be ≥ 200 mg/kg food.

The LOEC based on the weight of surviving bees could not be statistically determined. Therefore, the NOEC was determined to be ≥ 200 mg/kg food.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Honey-bee	<i>Apis mellifera</i>	XDE 729 Methyl	10 days	LC ₅₀	≥ 200	mg/kg food
Honey-bee	<i>Apis mellifera</i>	XDE 729 Methyl	10 days	LDD ₅₀	≥ 5.07	µg/bee/day
Honey-bee	<i>Apis mellifera</i>	XDE 729 Methyl	10 days	NOEC	≥ 200	mg/kg food
Honey-bee	<i>Apis mellifera</i>	XDE 729 Methyl	10 days	NOEDD	≥ 5.07	µg/bee/day

A 2.3.1.2.2 Study 170090: Picloram: A laboratory Study to Determine the Chronic Oral Toxicity to the Adult Worker Honey Bee *Apis mellifera* L. (Hymenoptera: Apidae).

Comments of zRMS:	<p>The study was submitted by the Applicant in order to address the chronic toxicity of picloram to bees. However, the study was not validated by the zRMS since GF-4021 contains more than one active substance and for this reason respective study with the formulated product was provided in order to fulfil the data requirements, while the active substance endpoints should be generated in the course of the EU renewal process.</p> <p>The study summary below has been struck through and shaded as being not necessary to finalise the risk assessment at the zonal level.</p>
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Reference:	KCP 10.3.1.2/03
Report:	Leonard, J., Moore, S.; 2017; Picloram: A laboratory study to determine the chronic oral toxicity to the adult worker honey bee <i>Apis mellifera</i> L. (Hymenoptera: Apidae); SynTech Research, LLC, Stilwell, Kansas, USA; Lab Study No. 014SRUS17C0055; DAS Study No. 170090 ; 28 November 2017; Unpublished
Guideline(s):	OECD Draft Test Guideline (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. February 2016
Deviations:	Not evaluated
GLP:	Yes
Acceptability:	Not evaluated, not required for the zonal assessment of GF-4021
Duplication (if vertebrate study):	-

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	Picloram
Purity:	83.5%
Description (physical state):	Tan Solid
Lot/batch no.:	2H16162952 [TSN306376]

Test System

Organism (Species):	Honey bee (<i>Apis mellifera</i>)
Study type:	10-day chronic oral
Study design:	Dose response test; duration 10 days; minimum 3 replicates, each consisting of 10 bees in one cage per test concentration; assessment of mortality, food consumption and behavioural effects daily.
Test concentrations:	Oral: 0 (control), 0 (vehicle control), 33.85, 57.55, 118.1, 292.4, 496.1 µg/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 in the last 6 weeks. The bees were maintained in a clean holding cage at a temperature of approximately 32 to 33°C and 50 to 70% humidity.
Amount of treated diet consumed:	Mean consumption of the treated diets ranged from 1.50 to 38.73 mg of diet. Mean calculated daily dosages ranged from 0.20 to 81.5 µg/bee.

During holding/acclimation and after administration of the test dosages, bees were provided ad libitum a 500 g/L (w/v) sucrose solution in water. The bees for the definitive test were housed in cages containing pre-weighed feeders (syringes) containing approximately 2000 mg of the appropriate control or treated solutions. All control and treatment feeders were exchanged daily with freshly prepared diet. Consumption of the feeding solutions was monitored by weighing the syringe before and after feeding, correcting for evaporation.

Temperature: 32.19 to 32.74 °C
Relative humidity: 58.05 to 69.04%
Photoperiod: The environmental chamber was kept dark except when room lighting was used during observation periods.

~~Dimethoate: 0.84 mg a.i./kg diet~~

0.03N NaOH

Multiple dose testing (5 doses of the test item, 1 untreated sucrose solution control, 1 vehicle control and 1 toxic reference) was conducted in an effort to generate a dose response relationship and to determine the values for the NOEC, LOEC and, if possible, the LC₅₀ and LC₁₀ (as mg a.i./kg diet) and corresponding values for the NOEL, LOEL and, if possible, the LD₅₀ and LD₁₀ (in µg a.i./bee/day). The nominal test item concentrations tested (determined following a non GLP compliant range finding study) were 131, 263, 526, 1052 and 2104 mg a.i./kg sucrose suspension. Picloram was dispersed in a sucrose solution containing 0.03N NaOH and offered with feeders renewed every day. The individual daily consumption was corrected each day by 1) the number of surviving bees at presentation with feeding solution and 2) average evaporation from syringes within the test unit that did not contain bees.

This study met the performance criteria described in the protocol with regard to 10-day honey bee mortality. Cumulative mortality did not exceed 15% in the untreated sucrose solution control (actual value: 6.7%) or vehicle control (actual value: 10.0%) and mean mortality in the toxic reference item resulted in a 10-day mortality higher than 50% (actual value: 100%). Bees treated with picloram exhibited no effect on diet consumption and resulted in 10-day control corrected mortalities of 5.4, 5.4, 16.3, 1.8, and 0.0%. There were no statistically significant differences between mean mortality or diet consumption in the untreated control and the test item applied at 131, 263, 526, 1052, and 2104 mg/kg.

Treatment-Daily Dose µg a.i./bee		Oral 10-day test									
Nominal	Mean Measured	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	3.3	3.3	0	0	0	0	0
Vehicle Control (0)	0	0	0	0	0	0	0	0	6.7	3.3	3.3
3.385	NA	6.7	0	0	0	3.3	0	0	0	0	3.3
5.755	NA	0	0	0	13.3	0	0	0	0	0	0
11.81	NA	13.3	0	0	0	3.3	3.3	3.3	0	0	0
29.24	NA	0	0	0	0	6.7	3.3	0	0	0	0
49.61	NA	0	0	0	0	0	0	6.7	0	0	0
Dimethoate		0	0	0	0	0	33.3	10.0	43.3	13.3	0
10 day LD ₅₀		>496.1 µg a.i./bee									
10 day NOED		496.1 µg a.i./bee									
10 day LC ₅₀		>2104 mg a.i./kg diet									
10 day NOEC		2104 mg a.i./kg diet									

NA = Not Applicable

Table 28: Effect of picloram on diet consumption in honey bees in the chronic oral toxicity test

Treatment Daily Dose µg a.i./bee		Oral 10 day test									
Nominal	Mean Measured	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	32.39	31.75	36.71	32.78	28.75	30.66	34.11	21.10	29.49	18.36
Vehicle Control (0)	0	28.70	29.86	31.41	25.23	29.89	24.77	35.57	32.42	28.63	1.638
3.385	NA	25.52	28.67	30.26	37.23	32.75	16.79	33.78	20.32	31.54	1.501
5.755	NA	27.15	27.09	20.17	29.62	17.58	15.73	28.63	22.21	28.86	1.786
11.81	NA	28.77	14.05	35.35	26.90	16.47	24.38	27.81	14.01	32.36	4.372
29.24	NA	26.89	25.71	31.06	38.08	23.29	27.19	37.66	25.19	33.91	8.998
49.61	NA	27.29	26.10	25.20	21.84	23.23	17.58	38.73	16.42	24.33	15.06
Reference Item		25.83	26.92	12.25	12.48	9.847	6.307	25.60	8.292	-	-
10 day NOED, diet consumption		496 µg a.i./bee									
10 day NOEC, diet consumption		2104 mg a.i./kg diet									

NA=Not Applicable

Table 29: Sublethal effects of picloram to honey bees in the chronic oral toxicity test

Treatment Daily Dose µg/bee		Oral 10 day test		
Nominal	Mean Measured	Sublethal effects (Number of bees, Day observed)		
		On back	Lethargic	Other
3.385	NA	0	0	1 bee, day 5
3.385	NA	0	0	1 bee, day 6
Dimethoate	NA	0	0	2 bees, day 4
Dimethoate	NA	0	0	2 bees, day 7
Dimethoate	NA	0	0	4 bees, day 8

NA=Not Applicable

CONCLUSION

There were no significant differences between mean mortality or diet consumption in the untreated control and applied test item concentrations of 131, 253, 526, 1052, and 2104 mg picloram/kg sucrose diet. Based on survival data the NOEC was 2104 mg a.i./kg sucrose solution, the LOEC was >2104 mg a.i./kg sucrose solution and the LC₅₀ was >2104 mg a.i./kg sucrose solution. This corresponded to a 10 day cumulative NOED of 496.1 µg a.i./bee, a 10 day cumulative LOED of >496.1 µg a.i./bee and an LD₅₀ of >496.1 µg a.i./bee. The daily NOED was 49.61 µg a.i./bee/day and the daily LOED was >49.61 µg a.i./bee/day. Based on diet consumption, the NOEC was 2104 mg a.i./kg sucrose solution, the LOEC was >2104 mg a.i./kg sucrose solution and the LC₅₀ was >2104 mg a.i./kg sucrose solution.

Common name	Species	Test item	Time scale	Endpoint	Toxicity value	Units of test item
Honey-bee	<i>Apis mellifera</i>	Picloram	10 days	LD ₅₀	>496.1	µg/bee
Honey-Bee	<i>Apis mellifera</i>	Picloram	10 days	NOED	496.1	µg/bee
Honey-Bee	<i>Apis mellifera</i>	Picloram	10 days	LC ₅₀	>2104	mg/kg diet
Honey Bee	<i>Apis mellifera</i>	Picloram	10 days	NOEC	2104	mg/kg diet

A 2.3.1.2.3 Study 170092: Aminopyralid: A laboratory Study to Determine the Chronic Oral Toxicity to the Adult Worker Honey Bee *Apis mellifera* L. (Hymenoptera: Apidae).

Comments of zRMS:	<p>The study was submitted by the Applicant in order to address the chronic toxicity of aminopyralid to bees. However, the study was not validated by the zRMS since GF-4021 contains more than one active substance and for this reason respective study with the formulated product was provided in order to fulfil the data requirements, while the active substance endpoints should be generated in the course of the EU renewal process.</p> <p>The study summary below has been struck through and shaded as being not necessary to finalise the risk assessment at the zonal level.</p>
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Reference:	KCP 10.3.1.2/04
Report:	Leonard, J., Moore, S.; 2017; Aminopyralid: A laboratory study to determine the chronic oral toxicity to the adult worker honey bee <i>Apis mellifera</i> L. (Hymenoptera: Apidae); SynTech Research, LLC, Stilwell, Kansas, USA; Lab Study No. 014SRUS17C0063; DAS Study No. 170092; 16 October 2017; Unpublished
Guideline(s):	OECD Draft Test Guideline (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. February 2016
Deviations:	Not evaluated
GLP:	Yes
Acceptability:	Not evaluated, not required for the zonal assessment of GF-4021
Duplication (if vertebrate study):	-

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	Aminopyralid
Purity:	94.5%
Description (physical state):	White solid
Lot/batch no.:	F0031-143 [TSN102319]

Test System

Organism (Species):	Honey bee (<i>Apis mellifera</i>)
Study type:	Chronic oral
Study design:	Limit test; duration 10 days; minimum 5 replicates, each consisting of 10 bees in one cage per test concentration; assessment of mortality, food consumption and behavioural effects daily.
Test concentrations:	Oral: 0 (control), 742.0 µg /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 in the last four weeks. The bees were maintained in a clean holding cage at a temperature of approximately 32 to 33°C and 50 to 70% humidity.
Amount of treated diet consumed:	Mean consumption of the treated diets ranged from 19.92 to 59.21 mg of diet. Calculated mean daily dosages ranged from 41.84 to 111.9 µg/bee.

During holding/acclimation and after administration of the test dosages, bees were provided ad libitum a 500 g/L (w/v) sucrose solution in water. The bees for the definitive test were housed in cages containing pre-weighed feeders (syringes) containing approximately 2000 mg of the appropriate control or treated solutions. All control and treatment feeders were exchanged daily with freshly prepared diet. Consumption of the feeding solutions was monitored by weighing the syringe before and after feeding, correcting for evaporation.

~~Temperature: 32 to 33°C~~~~Relative humidity: 45 to 80%~~

Photoperiod: The environmental chamber was kept dark except when room lighting was used during observation periods.

~~Dimethoate: 0.84 mg a.i./kg diet~~

NA

A single dose limit test was conducted (1 dose of the test item, 1 untreated sucrose solution control and 1 toxic reference) to determine the values for the NOEC and LOEC (as mg a.i./kg diet) and corresponding values for the NOEL and LOEL (in µg a.i./bee/day). The nominal test item concentration tested (determined following a non GLP compliant range finding study) is 2100 mg a.i./kg sucrose solution. Aminopyralid was dissolved in a 50% sucrose solution and offered with feeders renewed every day. The individual daily consumption was corrected each day by 1) the number of surviving bees at each date of diet presentation and 2) average evaporation from syringes within the test unit that did not contain bees.

This study met the performance criteria described in the protocol with regard to 10-day honey bee mortality. Cumulative mortality did not exceed 15% in the untreated sucrose solution control (actual value: 4.0%) and mean mortality in the toxic reference item resulted in a 10-day mortality higher than 50% (actual value: 90%). Treatment with a single dose limit concentration of aminopyralid did not exhibit an effect on diet consumption and resulted in 10-day mortality of 6.0%. There were no statistically significant differences between mean mortality or diet consumption in the untreated control and the test item applied at 2100 mg aminopyralid/kg sucrose diet.

Treatment-Daily Dose µg a.i./bee		Oral 10-day test										
Nominal	Mean Measured	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	0	0	4.0
742.0	742.0	0	0	0	0	2.0	0	0	0	0	0	4.0
dimethoate		0	0	0	3.3	16.7	16.7	20.0	33.3	0	0	
10 day LD ₅₀		>742.0 µg a.i./bee										
10 day NOED		742.0 µg a.i./bee										
10 day LC ₅₀		>2100 mg a.i./kg diet										
10 day NOEC		2100 mg a.i./kg diet										

Table 31: Effect of aminopyralid on diet consumption in honey bees in the chronic oral toxicity test

Treatment Daily Dose µg a.i./bee		Oral 10-day test									
Nominal	Mean Measured	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	28.90	26.98	35.34	30.17	31.73	54.58	41.13	42.33	44.33	58.91
742.0	742.0	26.20	24.0	24.77	19.92	30.94	49.15	37.03	44.60	37.60	59.21
0.84 mg/kg dimethoate		30.37	27.28	12.55	16.87	17.94	22.38	12.08	3.97	15.24	22.31
10-day NOED, diet consumption		742.0 µg a.i./bee									
10-day NOEC, diet consumption		2100 mg a.i./kg diet									

Table 32: Sublethal effects of aminopyralid to honey bees in the chronic oral toxicity test

Treatment Daily Dose µg/bee		Oral 10-day test		
Nominal	Mean Measured	Sublethal effects (Number of bees, Day observed)		
		On-back	Lethargic	Other
Control (0)	0	0	0	0
742.0	742.0	0	0	1 moribund, day 4

CONCLUSION

There were no significant differences between mean mortality or diet consumption in the untreated control and the test item applied at single limit concentration of 2100 mg aminopyralid/kg sucrose diet. Based on survival data, the NOEC was 2100 mg a.i./kg sucrose solution and the LOEC was >2100 mg a.i./kg sucrose solution. This corresponded to a 10-day cumulative NOED of 742.0 µg a.i./bee and a 10-day cumulative LOED of >742.0 µg a.i./bee. The daily NOED was 74.20 µg a.i./bee/day and the daily LOED was >74.20 µg a.i./bee/day. Based on diet consumption, the NOEC was 2100 mg a.i./kg sucrose solution and the LOEC was >2100 mg a.i./kg sucrose solution.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Honey-bee	<i>Apis mellifera</i>	Aminopyralid	10 days	LD ₅₀	>742.0	µg/bee
Honey-Bee	<i>Apis mellifera</i>	Aminopyralid	10 days	NOED	742.0	µg/bee
Honey Bee	<i>Apis mellifera</i>	Aminopyralid	10 days	LC ₅₀	>2100	mg/kg diet
Honey Bee	<i>Apis mellifera</i>	Aminopyralid	10 days	NOEC	2100	mg/kg diet

A 2.3.1.3 KCP 10.3.1.3 Effects on honey bee development and other honey bee life stages

A 2.3.1.3.1 Study 200623: GF-4021 - Honey Bee (*Apis mellifera* L.) 22 Day Larval Toxicity Test (Repeated Exposure)

Comments of zRMS:	<p>The study was performed in line with OECD 239 with following deviations:</p> <ul style="list-style-type: none"> for the toxic reference item groups mortality but no other parameters were assessed, no emergence boxes were used from day 15 to enable the assignment of each emerged bee to the respective replicate, the temperature was outside the recommended range of 34-35°C and the relative humidity was considerably below or slightly above the recommended ranges. <p>Listed deviations are considered to have no impact on the outcome of the study as no effects occurred in the untreated controls and all validity criteria were met.</p> <p>Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>NOED = 80.1 µg product/larvae</p>
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Reference:	KCP 10.3.1.3/01
Report:	Wendling, K.; 2021b; GF-4021 - Honey Bee (<i>Apis mellifera</i> L.) 22 Day Larval Toxicity Test (Repeated Exposure); Eurofins Agrosience Services Ecotox GmbH, Eutinger Str. 24, D-75223 Niefern-Öschelbronn, Germany; Lab Study No. S20-00660; DAS Study No. 200623 ; 23 February 2021; Unpublished.
Guideline(s):	OECD Guidance Document 239 (2016)
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. Minor short-term deviations from the recommended temperature range of 34-35°C occurred (max: 35.6°C). These deviations are not considered to have had an impact on the study outcome. For zRMS opinion, see commenting box above
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study):	-

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-4021
Purity:	aminopyralid: 31 g/L, 3.3 % w/w; halauxifen-methyl: 10 g/L, 1.08 % w/w; picloram: 48 g/L, 5.1 % w/w
Description (physical state):	liquid / amber; EC
Lot/batch no.:	ENBK-170903-012 [TSN401447]

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; 5 test item groups (T1 to T5); Replicates per treatment group 3 (larvae from 3 different colonies each representing a replicate), Test organisms per replicate: 16; 3 or more replicates, each starting with at least 12 synchronized 1st instar larvae per test concentration ; assessment of mortality and behavioural effects daily after administration of the test item on days 3, 4, 5, and 6 and on days 7, 8, 15 and adult emergence on day 22. Visual assessment of uneaten food on day 8 prior to transfer of the test plates into pupal desiccator. Monitoring of pupal development and adult emergence (eclosion) until day 22. Weighing of emerged bees on day 22.
Test concentrations:	0 (control, solvent control), 33.3, 83.2, 208, 520 and 1300 mg product/kg diet, equivalent to 5.13, 12.8, 32.0, 80.1 and 200 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 <i>Varroa</i> mites or for disease for at least 4 weeks prior to study initiation.

Analytical verification:

Aminopyralid, halauxifen-methyl and picloram were analysed in the stock solution, the test item solutions and control solution as well as in the test item treated larval diet and the diet of the control group by liquid chromatography and mass spectrometric detection (HPLC-MS/MS). Additional verification of the homogeneity (top and bottom sampling of treated diet) and stability (sampling at 24 ± 1 hours after preparation) of the test item in the larval diet.

All mean recoveries of the test item groups were within $\pm 20\%$ of nominal. Therefore, further evaluations were done with nominal concentrations.

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: 33.4^{a} - 35.6^{b} °C (recommended 34 - 35 °C, but not below 23 °C or above 40 °C)

^a deviation < 2 hours

^b deviation ≥ 2 hours on day 10 without impact on study outcome

Relative Humidity:

57.8 - 100% (day 1 to day 8) (target $95 \pm 5\%$),

45.9 – 87.2% (day 8 to day 15) (target: $80 \pm 5\%$),

26.9 – 63.7% (day 15 - day 22) (target: 50 - 80 %)

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-4021 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.

The following methods were used for statistical evaluation:

Dataset	Test on	Test name	Test direction	α	Pre-conditions for hypothesis test
Larval mortality on day 8, mortality on day 15, pupal mortality from day 8 through 22	Two sample comparison between controls	Fisher's Exact Test	two-sided	0.050	confirmed
	monotonicity (linear trend) significance	trend analysis by contrasts		0.01	rejected
		Chi ² test with Bonferroni-Holm adjustment	one-sided greater	0.05	-
Adult emergence on day 22	Two sample comparison between controls	Fisher's Exact Test	two-sided	0.050	confirmed
	monotonicity (linear trend)	trend analysis by contrasts	-	0.01	confirmed
	extrabinomial variance	Tarone test	-	0.01	confirmed
	significance	Cochran-Armitage test	one-sided greater	0.05	-
Bee weight	Two sample comparison between controls	Student's t-test	two-sided	0.05	confirmed
	normal distribution	Shapiro-Wilk's test	-	0.01	confirmed
	variance	Levene's test	-	0.01	confirmed
	homogeneity	trend analysis by contrasts	-	0.01	confirmed
	monotonicity (linear trend) significance	Dunnett's t-test	two-sided	0.05	-
Dataset	Endpoint	Regression analysis	Regression function used		
Adult emergence	EC _{10/20/50}	none	none	EC _{10,20} could not be reliably determined since there was no sufficient dose-response relationship within the tested concentration range and hence are not reported	
				EC ₅₀ could not be determined since the inhibition in emergence was below 50 % at the highest concentration tested compared to the control	

RESULTS AND DISCUSSION

On day 8, larval mortality was 10.4% in the control group and 0.0% in the solvent control group. Larval mortality in the dimethoate reference item group was 100.0%. On day 22, the adult emergence rate in the control and solvent control group was 87.5 and 95.8%, respectively. The adult emergence rate in the fenoxycarb reference item group was 0.0 %.

Compared to the pooled control groups (C and CS), the adult emergence rate on day 22 was statistically significantly different in the highest test item group of 1300 mg product/kg diet (Cochran-Armitage test with Rao-Scott adjustment, one sided greater, $\alpha = 0.05$).

Weight of emerged bees was statistically significantly different compared to the pooled control groups at the highest concentration tested (Dunnett's t-test, two-sided, $\alpha = 0.05$).

During the assessments of mortality and emergence no other test item related observations such as deviating sizes, appearances and malformations of the test organisms were made.

On day 8 uneaten food was observed in the control group and in the test item groups with concentrations of 208, 520 and 1300 mg product/kg diet.

Table 33: Toxicity of GF-4021 to honey bee larvae in a chronic exposure toxicity test

Treatment		Chronic larval exposure toxicity		
Concentration (mg product/kg diet)	Cumulative Dose (μg product/larva per developmental period)	Mortality (%) (Corrected Mortality (%)) ^a		Adult Emergence (%)
		Day 8	Day 15	Day 22
Control (0)		10.4	12.5	87.5
Solvent control (0)		0.0 (n.a.)	2.1 (n.a.)	95.8
33.3	5.13	2.1 (-9.3)	4.2 (-9.5)	93.8
83.2	12.8	8.3 (-2.3)	10.4 (-2.4)	85.4
208	32.0	6.3 (-4.6)	10.4 (-2.4)	87.5
520	80.1	6.3 (-4.6)	12.5 (0.0)	85.4
1300	200	20.8 ^b (11.6)	31.3 ^b (21.5)	62.5 ^c
Reference item (7.39 μg dimethoate/larva per developmental period, nominal)		100.0 (100.0)	---	---
Reference item (0.0493 μg fenoxycarb/larva per developmental period, nominal)		6.3 (6.3)	8.3 (6.3)	0.0
22-day NOED		80.1 μg product/larva per developmental period		
22-day ED ₅₀		> 200 μg product/larva per developmental period		
22-day NOEC		520 mg product/kg diet		
22-day EC ₅₀		> 1300 mg product/kg diet		

n.a.: not applicable

^a mortality corrected for control (C) mortality according to the formula of Abbott (1925) modified by Schneider Orelli (1947)
negative values indicate a lower mortality compared to the control group (C)

^b statistically significantly increased compared to pooled control groups (C and CS) (Chi² test with Bonferroni-Holm adjustment, one sided greater, $\alpha = 0.05$)

^c statistically significantly increased compared to pooled control groups (C and CS) (Cochran-Armitage test, one sided greater, $\alpha = 0.05$)

Table 34: Effect of GF-4021 on weight of emerged bees in the larval chronic toxicity test

Treatment		Chronic larval exposure toxicity			
Concentration (mg product/kg diet)	Cumulative Dose (µg product/larva per developmental period)	Mean weight emerged bees (mg)			
		Replicate 1	Replicate 2	Replicate 3	Mean per Treatment Group ^a
Control (0)		0.0921	0.0863	0.0896	0.0891
Solvent Control (0)		0.0883	0.0899	0.0898	0.0893
33.3	5.13	0.0882	0.0829	0.0914	0.0875
83.2	12.8	0.0889	0.0804	0.0890	0.0856
208	32.0	0.0890	0.0848	0.0812	0.0849
520	80.1	0.0849	0.0820	0.0843	0.0838
1300	200	0.0790	0.0706	0.0842	0.0777 ^b
10 day NOED _{bee weight}		80.1 µg product/larva per developmental period			
10 day NOEC _{bee weight}		520 mg product/kg diet			

^a calculation based on the individual weight

^b statistically significantly increased compared to pooled control groups (C and CS) (Dunnett's t-test, two-sided, $\alpha = 0.05$)

Table 35: Uneaten food, developmental and behavioural effects in the chronic exposure larval toxicity test for GF-4021

Treatment		Chronic larval exposure toxicity		
Concentration (mg product/kg diet)	Cumulative Dose (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)		no	none	none
33.3	5.13	no	none	none
83.2	12.8	no	none	none
208	32.0	yes	none	none
520	80.1	yes	none	none
1300	200	yes	none	none
Reference item (7.39 µg dimethoate/larva per developmental period)		no	none	none
Reference item (0.0493 µg fenoxycarb/larva per developmental period)		no	none	none

CONCLUSION

All validity criteria were met, therefore, the study was valid.

The mean measured concentrations of the test item in the larval diet were within $\pm 20\%$ of nominal. Therefore, the concentrations of the test item solutions and the concentration of the test item in the larval diet were confirmed and the endpoints are based on nominal concentrations.

In a repeated exposure larval toxicity test with GF-4021 and a duration of 22 days the NOEC for adult emergence on day 22 was determined to be 520 mg product/kg diet, equivalent to a NOED of 80.1 µg product/larva per developmental period.

The EC_{10,20} and the corresponding ED_{10,20} for adult emergence on day 22 could not be determined due to the lack of a clear concentration/dose-response relationship. The EC₅₀ was considered >1300 mg product/kg diet, equivalent to an ED₅₀ of >200 µg product/larva per developmental period.

The NOEC_{bee weight} was determined to be 520 mg product/kg diet, equivalent to a NOED_{bee weight} of 80.1 µg product/larva per developmental period. The LOEC_{bee weight} and LOED_{bee weight} on day 22 were

determined to be 1300 mg product/kg diet and 200 µg product/larva per developmental period, respectively.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Honey bee	<i>Apis mellifera</i>	GF-4021	22 day	NOED	80.1	µg/larva/developmental period
Honey bee	<i>Apis mellifera</i>	GF-4021	22 day	NOEC	520	mg/kg diet

A 2.3.1.3.1 Study 170073: XDE-729 Methyl – Honey Bee (*Apis mellifera* L.) 22 Day Larval Toxicity Test (Repeated Exposure).

Comments of zRMS:	<p>The study was submitted by the Applicant in order to address the toxicity of halauxifen-methyl to bee larvae. However, the study was not validated by the zRMS since GF-4021 contains more than one active substance and for this reason respective study with the formulated product was provided in order to fulfil the data requirements, while the active substance endpoints should be generated in the course of the EU renewal process.</p> <p>The study summary below has been struck through and shaded as being not necessary to finalise the risk assessment at the zonal level.</p>
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Reference:	KCP 10.3.1.3/02
Report:	Oberrauch, S.; 2018; XDE-729 methyl - Honey Bee (<i>Apis mellifera</i> L.) 22 Day Larval Toxicity Test (Repeated Exposure); Eurofins Agrosience Services EcoChem GmbH / Eurofins Agrosience Services Ecotox GmbH, Eutinger Str. 24, D-75223 Niefern-Öschelbronn, Germany; Lab Study No. S17-00206; DAS Study No. 170073 ; 01 February 2018; Unpublished.
Guideline(s):	OECD Guidance Document 239 on Honey bee (<i>Apis mellifera</i>) Larval Toxicity Test, Repeated Exposure (2016)
Deviations:	Not evaluated
GLP:	Yes
Acceptability:	Not evaluated, not required for the zonal assessment of GF-4021
Duplication (if vertebrate study):	-

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	XDE-729-methyl
Purity:	99.7 % w/w
Description (physical state):	solid / white
Lot/batch no.:	YC2-106153-95 (TSN301574)

Test System

Organism (Species):	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 1st instar larvae per test concentration; assessment of mortality and behavioural effects daily after administration of the test item on days 3, 4, 5, and 6 and on days 7, 8, 15 and 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), 3.84, 9.60, 24.0, 60.0 and 150 mg XDE 729 methyl/kg diet equivalent to 0.591, 1.48, 3.70, 9.24 and 23.1 µg XDE 729 methyl/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:	XDE 729 methyl was analysed in the stock solution, 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 and solvent 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. <p>The analytical verification of XDE 729 methyl resulted in recoveries of 99 to 119 % (solutions) and 91 to 106 % (diet) of the nominal value. The measured concentrations 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 81 to 107 %. The analytical dose verification of the aged larval diet (24 h) resulted in a recovery rate of 91 % of the nominal concentration at day 4. Therefore the stability of the test item in the larval diet was proven for this period.</p>
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. <p>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.</p>
Environmental conditions:	Temperature: 32.8–35.1 °C

Reference substance:

Relative Humidity: 49.9 – 100.0 % (day 1 to day 8), 56.1 – 88.8 % (day 8 to day 15), 35.7 – 70.5 % (day 15 – day 22)
Photoperiod: constant darkness except during grafting, feeding and assessments.
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 XDE 729 methyl 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 solvent control group.

RESULTS AND DISCUSSION

On day 8 the cumulative mortality was 4.2 % in the control, 6.3 % in the solvent control, 97.9 % in the dimethoate reference item group 0.0 % in the fenoxycarb reference item group. On day 22, the adult emergence rate in the control and solvent control group was 91.7 and 77.1 %, respectively. The adult emergence rate in the fenoxycarb reference item group was 2.1%.

Compared to the solvent control group, the adult emergence rate on day 22 was not statistically significantly different in the highest test item group of 150 mg XDE 729 methyl/kg diet (multiple Chi² test with Bonferroni Holm adjustment, one sided greater, $\alpha = 0.05$). Therefore, the NOEC for adult emergence on day 22 was determined as ≥ 150 mg XDE 729 methyl/kg diet, equivalent to a NOED of ≥ 23.1 µg XDE 729 methyl/larva per developmental period.

Table 36: Toxicity of XDE 729 methyl to honey bee larvae in a chronic exposure toxicity test

Nominal Treatment		Chronic larval exposure toxicity		
mg XDE 729 methyl/kg diet	µg XDE 729 methyl/larva per developmental period	Mortality (%) (Corrected Mortality (%))		Emergence (%)
		Day 8	Day 15	Day 22
Control (0)		4.2 (n.a.)	8.3 (n.a.)	91.7
Solvent Control (0)		6.3 (n.a.)	22.9 (n.a.)	77.1
3.84	0.591	8.3 (2.1)	22.9 (0.0)	75.0
9.60	1.48	4.2 (-2.2)	37.5 (18.9)	62.5
24.0	3.70	4.2 (-2.2)	27.1 (5.4)	70.8
60.0	9.24	6.3 (0.0)	31.3 (10.9)	64.6
150	23.1	2.1 (-4.5)	14.6 (-10.8)	83.3
Reference item (7.39 µg dimethoate/larva per developmental period, nominal)		97.9 (97.8)	—	—
Reference item (0.0493 µg fenoxycarb/larva per developmental period, nominal)		0.0 (-6.7)	6.3 (-21.5)	2.1
22-day NOED, nominal treatment		≥ 23.1 µg XDE 729 methyl/larva per developmental period, equivalent to ≥ 150 mg XDE 729 methyl/kg diet		

* Significantly different compared to solvent control (multiple Chi² test with Bonferroni Holm adjustment, one sided greater, $\alpha = 0.05$)

n.a. — not applicable

Table 37: — Uncaten food, developmental and behavioural effects in the chronic exposure larval toxicity test for XDE 729 methyl

Nominal Treatment		Chronic larval exposure toxicity		
mg XDE 729 methyl/kg diet	µg XDE 729 methyl/larva per developmental period	Uncaten food observed on day 8	Behavioural effects (day)	Developmental effects (day)
Control (0)		yes	none	none
Solvent Control (0)		yes	none	none
3.84	0.591	yes	none	none
9.60	1.48	yes	none	none
24.0	3.70	yes	none	none
60.0	9.24	yes	none	none
150	23.1	yes	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

CONCLUSION

In a repeated exposure larval toxicity test with XDE 729 methyl and a duration of 22 days, the NOEC for adult emergence was determined as ≥ 150 mg XDE 729 methyl/kg diet, equivalent to a NOED of ≥ 23.1 µg XDE 729 methyl/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>	XDE 729 methyl	22 day	NOED	≥ 23.1	µg/larva/developmental period
Honey bee	<i>Apis mellifera</i>	XDE 729 methyl	22 day	NOEC	≥ 150	mg/kg diet

A 2.3.1.3.2 Study 170091: Picloram: A Repeated-Exposure Laboratory Toxicity Study in Larvae, Pupae and Emergent Adults of the Honey Bee *Apis mellifera* Linnaeus (Hymenoptera: Apidae).

Comments of zRMS:	<p>The study was submitted by the Applicant in order to address the toxicity of picloram to bee larvae. However, the study was not validated by the zRMS since GF-4021 contains more than one active substance and for this reason respective study with the formulated product was provided in order to fulfil the data requirements, while the active substance endpoints should be generated in the course of the EU renewal process.</p> <p>The study summary below has been struck through and shaded as being not necessary to finalise the risk assessment at the zonal level.</p>
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Reference:	KCP 10.3.1.3/03
Report:	Leonard, J., Moore, S.; 2017; Picloram: A repeated-exposure laboratory toxicity study in larvae, pupae and emergent adults of the honey bee <i>Apis mellifera</i> Linnaeus. (Hymenoptera: Apidae); SynTech research Laboratory Services LLC, Ecotoxicology, 17745 South Metcalf Avenue, Stilwell, Kansas, 66085-9104, USA; Lab Study No. 014SRUS17C0056; DAS Study No. 170091; 28 November 2017; Unpublished.
Guideline(s):	OECD Guidance No. 239 ENV/JM/MONO(2016)34
Deviations:	Not evaluated
GLP:	Yes
Acceptability:	Not evaluated, not required for the zonal assessment of GF-4021
Duplication (if vertebrate study):	-

MATERIALS AND METHODS

Test Item(s)

Test item (Common name): Picloram
Purity: 83.5%
Description (physical state): White powder
Lot/batch no.: Lot No. 2H16162952 [TSN306376]

Test System

Organism (*Species*): Honey bee (*Apis mellifera*)
Study type: Chronic Larval – repeated exposure
Study design: Dose response test; duration 22 days; 3 or more replicates, each starting with at least 12 synchronized 1st instar larvae per test concentration; assessment of mortality and behavioural effects daily after administration of the test item on Days 3, 4, 5, and 6. Visual assessment of uneaten food at the end of Day 8 prior to transfer to pupal incubation plate. Monitoring of pupal development and adult emergence (eclosion) until Day 22.
Test concentrations: 0 (control), 0 (vehicle control), 6.25, 12.5, 25.0, 50.0, 100 µg /larva equivalent to 0, 0, 40.6, 81.1, 162, 325, 649 mg/kg diet
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 four weeks prior to study initiation.
Analytical verification: LC MS/MS
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 dissolved or homogeneously dispersed in 20 to 50 µL/larvae of diet B or C depending upon the day of incubation.
Environmental conditions: Temperature: 34 to 35°C
Relative Humidity: 90 to 100% for larval phase
75 to 85% for pupal phase
50 to 55% for adult emergence
Photoperiod: The climate cabinet was kept dark.
Reference substance: Dimethoate: 7.6 µg /larva, 48 mg/kg diet
Fenoxycarb: 0.039 µg /larva, 0.25 mg/kg diet
Vehicle: 0.03N NaOH

Methodology

This study assessed the lethal and sublethal effects of picloram on the brood of the honey bee *Apis mellifera* (Hymenoptera: Apidae), when mixed with artificial diet and fed to the larvae. On days 3, 4, 5 and 6 of the larval rearing period, larvae were fed diet supplemented with picloram at the required doses in addition to an untreated control, vehicle control and toxic reference compounds. Multiple

dose testing was conducted in an effort to generate a dose response relationship and to determine the No-Observed Effect Concentration (NOEC). The nominal test item concentrations tested were 40.6, 81.1, 162, 325, 649 mg a.i./kg diet equivalent to 6.25, 12.5, 25.0, 50.0, 100 ug a.i./larva on the entire larval rearing period. Two toxic reference items, dimethoate and fenoxycarb, were included to indicate relative susceptibility of the test organisms and the test system, and to ensure that the study has the ability to detect effects of the test item if they occur. The nominal dose of dimethoate tested was 7.6 ug dimethoate/larva, corresponding to a concentration of 48.0 mg a.i./kg. The nominal dose of fenoxycarb tested was 0.039 ug fenoxycarb/larva, corresponding to a concentration of 0.25 mg a.i./kg. Picloram was dispersed in water and a vehicle (0.03 N sodium hydroxide), and then further dispersed in larval diet on days 3, 4, 5 and 6 of the study.

RESULTS AND DISCUSSION

This study met the performance criteria described in the protocol with regard to control day 8 larval mortality <15% averaged across replicates, control adult bee day 22 emergences >70% averaged across replicates, day 8 mortality for dimethoate ≥50% and for fenoxycarb day 22 emergence ≤20% averaged across replicates. The study also met the OECD draft guidance document performance criteria for controls of “cumulative larval mortality from D3 to D8 should be ≤15% across replicates” and “the adult emergence rate on D22 should be ≥70% on D22 across replicates”. Untreated control mortality on day 8 was 0.0%, within the acceptable validity criteria described in the 2016 guidance and protocol. Adult bee emergence at day 22 was 79.2% for the untreated control, which was within the acceptable range described in the 2016 guidance and protocol. There was no statistically significant difference between mean mortality in the negative control and any of the applied concentrations of test item. Control corrected 22-day mortality in the fenoxycarb treated group was 100%, which is within the acceptable validity criteria described in the 2016 guidance and protocol. Control corrected 8-day mortality in the dimethoate treated group was 72.9%, which was within the acceptable validity criteria described in the 2016 guidance and protocol.

Table 38: Toxicity of Picloram to honey bee larvae in a chronic exposure toxicity test

Nominal treatment (Mean-Measured Treatment)		Mortality (%) (Corrected Mortality (%))		
µg/larva	mg/kg	Day 8	Day 15	Day 22
Control (0)		0.0 (0.0)	12.5 (0.0)	20.8 (0.0)
Vehicle (NaOH) Control (0)		4.2 (2.1)	18.8 (3.7)	20.8 (0.0)
2.984	21.29	4.2 (2.1)	14.6 (0.0)	18.8 (0.0)
6.211	43.91	2.1 (0.0)	10.4 (0.0)	16.7 (0.0)
12.25	86.41	4.2 (2.1)	8.3 (0.0)	14.6 (0.0)
25.88	181.2	2.1 (0.0)	12.5 (0.0)	20.8 (0.0)
62.70	407.3	0.0 (0.0)	16.7 (1.2)	18.8 (0.0)
7.6 µg dimethoate	48	72.9 (72.3)	81.3 (77.8)	89.6 (86.8)
0.039 µg fenoxycarb	0.25	0.0 (0.0)	6.3 (0.0)	100 (100)
8-day LD ₅₀ , mean measured treatment		>62.70 µg/larva equivalent to >407.3 mg/kg diet		
22-day NOED, mean measured treatment		62.70 µg/larva equivalent to 407.3 mg/kg diet		

Table 39: Uneaten food, developmental and behavioural effects in the chronic exposure larval toxicity test for Picloram

Mean measured treatment		Uneaten food observed on Day 8/9 (%)	Behavioural effects (day)	Developmental effects (day)
µg/larva	mg/kg			
Control (0)		0.0	None	None
Acetone Control (0)		2.1	None	None
Vehicle Control (0)		2.1	None	None
2.984	21.29	4.2	None	None
6.211	43.91	0.0	None	None
12.25	86.41	2.1	None	None
25.88	181.2	8.3	None	None
62.70	407.3	0.0	None	None
7.6 µg dimethoate	48	2.1	None	None
0.039 µg fenoxycarb	0.25	2.1	None	None

CONCLUSION

There was no statistically significant difference between mean mortality in the negative control and any of the applied concentrations picloram. Thus the NOEC based on mean measured concentrations was 407.3 mg a.i./kg and the LOEC was >407.3 mg a.i./kg for 22 day survival. For 8 day survival, the NOEC based on mean measured concentrations was 407.3 mg a.i./kg and the LOEC was >407.3 mg a.i./kg. The mean measured LC₁₀ was calculated to be >407.3 mg a.i./kg larval diet for 8 day survival and >407.3 mg a.i./kg larval diet for 22 day survival.

This corresponds to a cumulative NOED based on measured concentrations of 62.70 µg a.i./larva, a LOED of >62.70 µg a.i./larva for 22 day survival. For 8 day larval survival, the cumulative NOED based on measured concentrations was 62.70 µg a.i./larva, the LOED was >62.70 a.i./larva and the LD₁₀ was >62.70 µg a.i./larva.

Common name	Species	Test item	Time scale	Endpoint	Toxicity value	Units of test item
Honey bee	<i>Apis mellifera</i>	Picloram	22 day	NOED	62.70	µg/larva
Honey bee	<i>Apis mellifera</i>	Picloram	22 day	NOEC	407.3	mg/kg

A 2.3.1.3.3 Study 170413: Aminopyralid: A Repeated-Exposure Laboratory Toxicity Study in Larvae, Pupae and Emergent Adults of the Honey Bee *Apis mellifera* Linnaeus (Hymenoptera: Apidae).

Comments of zRMS:	<p>The study was submitted by the Applicant in order to address the toxicity of aminopyralid to bee larvae. However, the study was not validated by the zRMS since GF-4021 contains more than one active substance and for this reason respective study with the formulated product was provided in order to fulfil the data requirements, while the active substance endpoints should be generated in the course of the EU renewal process.</p> <p>The study summary below has been struck through and shaded as being not necessary to finalise the risk assessment at the zonal level.</p>
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Reference:	KCP 10.3.1.3/04
Report:	Leonard, J., Moore, S.; 2017; Aminopyralid: A repeated-exposure laboratory toxicity study in larvae, pupae and emergent adults of the honey bee <i>Apis mellifera</i> Linnaeus. (Hymenoptera: Apidae); SynTech Research, LLC, Stilwell, Kansas, USA; Lab Study No. 014SRUS17C0034; DAS Study No. 170413 ; 16 October 2017; Unpublished.
Guideline(s):	OECD Guidance No. 239 ENV/JM/MONO(2016)34
Deviations:	Not evaluated
GLP:	Yes
Acceptability:	Not evaluated, not required for the zonal assessment of GF-4021
Duplication (if vertebrate study):	-

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	Aminopyralid
Purity:	94.5%
Description (physical state):	White solid
Lot/batch no.:	F0031-143 [TSN102319]

Test System

Organism (Species):	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 synchronized 1st instar larvae per test concentration; assessment of mortality and behavioural effects daily after administration of the test item on Days 3, 4, 5, and 6. Visual assessment of uneaten food at the end of Day 8 prior to transfer to pupal incubation plate. Monitoring of pupal development and adult emergence (eclosion) until Day 22.
Test concentrations:	0 (control), 0 (vehicle), 6.25, 12.5, 25.0, 50.0, 100 µg /larva equivalent to 0, 0, 40.6, 81.1, 162, 325, 649 aminopyralid mg/kg diet
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 four weeks prior to study initiation.
Analytical verification:	LC MS/MS
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 dissolved or homogeneously dispersed in 20 to 50 µL/larvae of diet B or C depending upon the day of incubation.
Environmental conditions:	Temperature: 34 to 35°C Relative Humidity: 90 to 100% for larval phase 75 to 85% for pupal phase 50 to 55% for adult emergence Photoperiod: The climate cabinet was kept dark.
Reference substance:	Dimethoate: 7.6 µg /larva, 48 mg/kg diet Fenoxycarb: 0.039 µg /larva, 0.25 mg/kg diet

Methodology

This study assessed the lethal and sublethal effects of aminopyralid on the brood of the honey bee *Apis mellifera* (Hymenoptera: Apidae), when mixed with artificial diet and fed to the larvae. On days 3, 4, 5 and 6 of the larval rearing period, larvae were fed diet supplemented with aminopyralid at the required doses in addition to an untreated control, a vehicle (0.027N NaOH) control and toxic reference compounds. Multiple dose testing was conducted in an effort to generate a dose response relationship and to determine the No Observed Effect Concentration (NOEC). The nominal test item concentrations tested were 40.6, 81.1, 162, 325 and 649 mg a.i./kg diet, equivalent to 6.25, 12.5, 25.0, 50.0 and 100 µg a.i./larva on the entire larval rearing period. Two toxic reference items, dimethoate and fenoxycarb, were included to indicate relative susceptibility of the test organisms and the test system, and to ensure that the study has the ability to detect effects of the test item if they occur. The nominal dose of dimethoate tested was 7.6 µg dimethoate/larva, corresponding to a concentration of 48.0 mg a.i./kg. The nominal dose of fenoxycarb tested was 0.039 µg fenoxycarb/larva, corresponding to a concentration of 0.25 mg a.i./kg. Aminopyralid was dissolved in 0.027N NaOH, and then dispersed in larval diet on days 3, 4, 5, and 6 of the study.

RESULTS AND DISCUSSION

This study met the performance criteria described in the protocol with regard to control day 8 larval mortality <15% averaged across replicates, control adult bee day 22 emergence >70% averaged across replicates, day 8 mortality for dimethoate ≥50% and for fenoxycarb day 22 emergence ≤20% averaged across replicates. The study also met the OECD draft guidance document performance criteria for controls of “cumulative larval mortality from D3 to D8 should be ≤15% across replicates” and “the adult emergence rate on D22 should be ≥70% on D22 across replicates”. Untreated control mortality on day 8 was 8.3% and 2.1% in the vehicle control, both within the acceptable validity criteria described in the 2016 guidance and protocol. Adult bee emergence at day 22 was 85.4% for the untreated control and 81.2% for the vehicle control, which were within the acceptable range described in the 2016 guidance and protocol. There was no statistically significant difference between mean mortality in the pooled negative controls and any of the applied concentrations of test item. Control corrected 22-day mortality in the fenoxycarb treated group was 100%, which is within the acceptable validity criteria described in the 2016 guidance and protocol. Control corrected 8-day mortality in the dimethoate treated group was 100%, which was within the acceptable validity criteria described in the 2016 guidance and protocol.

Table 40: Toxicity of aminopyralid to honey bee larvae in a chronic exposure toxicity test

Nominal treatment (Mean Measured Treatment)		Mortality (%) (Corrected Mortality (%))		
µg/larva	mg/kg	Day 8	Day 15	Day 22
Control (0)		8.3 (3.3)	10.4 (1.1)	14.6 (0.0)
Vehicle control (0)		2.1 (0.0)	8.3 (0.0)	18.8 (2.5)
Aminopyralid 6.25 (5.85)	40.6 (38.09)	2.1 (0.0)	14.6 (5.7)	20.8 (4.9)
Aminopyralid 12.5 (11.8)	81.1 (75.87)	2.1 (0.0)	12.5 (3.4)	18.8 (2.5)
Aminopyralid 25.0 (23.3)	162 (151.9)	8.3 (3.3)	10.4 (1.1)	20.8 (4.9)
Aminopyralid 50.0 (45.5)	325 (299.3)	2.1 (0.0)	6.3 (0.0)	16.7 (0.0)
Aminopyralid 100 (92.8)	649 (610.0)	10.4 (5.5)	18.8 (10.3)	25.0 (10.0)
7.6 µg dimethoate	48 mg/kg dimethoate	100 (100)	100 (100)	100 (100)
0.039 µg fenoxycarb	0.25 mg/kg fenoxycarb	6.3 (1.1)	64.6 (60.9)	100 (100)
8-day LD ₁₀ , nominal treatment		>100 µg/larva equivalent to 649 mg/kg diet		
8-day LD ₁₀ , mean measured treatment		>92.8 µg/larva equivalent to 610.0 mg/kg diet		
22-day NOED, nominal treatment		100 µg/larva equivalent to 649 mg/kg diet		
22-day NOED, mean measured treatment		92.8 µg/larva equivalent to 610.0 mg/kg diet		

Table 41: Unaten food, developmental and behavioural effects in the chronic exposure larval toxicity test for aminopyralid

Nominal treatment		Unaten food observed on Day 8? (%)	Behavioral effects (day)	Developmental effects (day)
µg/larva	mg/kg			
Control (0)		0	None	None
Vehicle control (0)		4.2	None	None
Aminopyralid 6.25 (5.85)	40.6 (38.09)	0	None	None
Aminopyralid 12.5 (11.8)	81.1 (75.87)	4.2	None	None
Aminopyralid 25.0 (23.3)	162 (151.9)	0	None	None
Aminopyralid 50.0 (45.5)	325 (299.3)	2.1	None	None
Aminopyralid 100 (92.8)	649 (610.0)	4.2	None	None
7.6 µg dimethoate	48 mg/kg dimethoate	0	None	None
0.039 µg fenoxycarb	0.25 mg/kg fenoxycarb	2.1	None	None

CONCLUSION

There was no statistically significant difference between mean mortality in the pooled negative controls and any of the applied concentrations aminopyralid. Thus the NOEC based on nominal concentrations was 649.0 mg a.i./kg and the LOEC was >649.0 mg a.i./kg for 22-day survival. For 8-day larval survival, the NOEC based on nominal concentrations was 649.0 mg a.i./kg and the LOEC

was >649.0 mg a.i./kg. The nominal LC10 was calculated to be >649.0 mg a.i./kg larval diet for 8-day survival and 649.0 mg a.i./kg larval diet for 22-day survival. This corresponds to a cumulative NOED based on measured concentrations of 100 µg a.i./larva, a LOED of >100 µg a.i./larva and the LD10 based on measured concentrations was calculated to be 100 µg a.i./larva for 22-day survival. For 8-day larval survival, the cumulative NOED based on measured concentrations was 100 µg a.i./larva, the LOED was >100 µg a.i./larva and the LD10 was >100 µg a.i./larva.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Honey-bee	<i>Apis mellifera</i>	Aminopyralid	22-day	NOED	100	µg/larva
Honey-bee	<i>Apis mellifera</i>	Aminopyralid	22-day	NOEC	649.0	mg/kg

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

Not required to characterise the product in the current submission.

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

Not required to characterise the product in the current submission.

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

Not required to characterise the product in the current submission.

A 2.3.2 KCP 10.3.2 Effects on non-target arthropods other than bees

A 2.3.2.1 KCP 10.3.2.1 Standard laboratory testing for non-target arthropods

A 2.3.2.1.1 Study 190467: GF-4021: A Rate-Response Laboratory Study of the Effects of Fresh Residues on the Predatory Mite *Typhlodromus pyri* (Acari: Phytoseiidae).

Comments of zRMS:	<p>The study was performed in line with the respective guideline with no major deviations.</p> <p>Minor fluctuations in relative humidity below the threshold range of 60-90% were observed (exact values not given in the test report), but their duration was short (<2 hours) and is considered to have no impact on the results of the study, especially all validity criteria were met.</p> <p>The study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>LR₅₀ > 250 mL product/ha ER₅₀ > 250 mL product/ha</p>
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Reference:	KCP 10.3.2/01
Report:	Fallowfield L.; 2020; GF-4021: A Rate-Response Laboratory Study of the Effects of Fresh Residues on the Predatory Mite <i>Typhlodromus pyri</i> (Acari: Phytoseiidae); Mambo-Tox, A Division of Cawood Scientific Ltd, ; Lab Study No. DOW-19-22; DAS Study No. 190467 ; 16 March 2020; Unpublished.
Guideline(s):	Blümel, S. <i>et al.</i> (2000)
Deviations:	For short periods of time (<2 hours) the relative humidity was outside the target range

	of 60-90%. This is considered to have no impact on the study results since all validity criteria were met.
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study):	-

MATERIALS AND METHODS

Test Item(s)

Test item (Common name): GF-4021
Purity: 3.3% aminopyralid, 1.08% halauxifen-methyl, 5.1% picloram
Description (physical state): Clear amber fluid (EC)
Lot/batch no.: ENBK-170903-012 (TSN401447)

Test System

Organism (*Species*): Predatory mite (*Typhlodromus pyri*)
Study type: Tier 1 laboratory study, glass plates for mortality and fecundity
Study design: Assessments of mortality measured 7 days after treatment and egg production 14 days after treatment.
3 replicates, each consisting of 20 mites in one arena, per test concentration.
Test concentrations: 0 (control), 87.5, 114, 148, 192 and 250 mL test item/ha
Environmental conditions: Temperature: 23.6-25.2°C
Relative humidity: 60-78%
Photoperiod: 16 h (700-1200 lux)
Feeding: fruit-tree pollen
Reference substance: Dimethoate

Methodology

GF-4021 was evaluated at five rates, equivalent to 87.5, 114, 148, 192 and 250 mL test item/ha. Also included in the test were a water-treated control and a toxic reference treatment of dimethoate (nominally 400 g/L content of a.s.), applied at a rate of 15 mL formulation/ha. All treatments were applied to glass plates, at a volume rate equivalent to 200 L spray solution/ha. Once the target plates had been given time to dry, they were used to make test arenas, with their treated surface facing upwards.

Twenty protonymphal *T. pyri* were placed on each replicate arena, with three replicates (60 mites in total) prepared per treatment. The mites were fed regularly with untreated pollen. Their survival was assessed over a 7-day period, by which time the mites in the control were adult. The sex of the adult mites was then determined, and they were left *in situ* so that their reproduction could be assessed over a further 7 days. Assessments of oviposition activities were carried out at 10, 13 and 14 DAT. The mean number of eggs produced per female between 7 and 14 days after treatment (DAT) was calculated. These reproduction assessments were made for mites from the control and all test-item rates that had resulted in $\leq 50\%$ corrected mortality.

In order to determine the no-observed-effect rate (NOER) for mortality, the percentage mortality in each test item treatment was compared to the control using Step-down Cochran-Armitage Test Procedure ($\alpha = 0.05$, one sided, $>$ control). Fisher's Exact Binomial Test was used for the toxic reference ($\alpha = 0.05$, one-sided, $>$ control). Due to the outcome of the bioassay, a regression analysis to calculate the median lethal rate (LR₅₀) was not considered appropriate. The LR₅₀ value was therefore

extrapolated from the data. In order to determine the NOER for reproduction, the data were first checked for normality (Shapiro-Wilk test) and for homogeneity of variance (Levene's test), before being compared by Multiple Sequentially-rejective t-test After Bonferroni-Holm ($\alpha = 0.05$, one-sided, $< \text{control}$). The median effect rate (ER_{50}) with respect to reproduction was extrapolated from the data. Analyses were performed using validated computer software ToxRatPro.

RESULTS AND DISCUSSION

At 7 days there was 1.7% mortality in the control treatment, compared with 13.3%, 5.0%, 28.3%, 18.3% and 43.3% mortality in the 87.5, 114, 148, 192 and 250 mL test item/ha treatment rates of GF-4021, respectively. Corrected mortality in the test-item treatments was 11.9%, 3.4%, 27.1%, 16.9% and 42.4%, respectively. Therefore, the 7-day LR_{50} value was > 250 mL test item/ha, the highest rate tested. Statistically, the 250, 192 and 148 mL test item/ha treatment rates differed significantly from the control. The NOER with respect to mite survival was therefore 114 mL test item/ha.

The mean number of eggs produced per female was 5.3 in the control treatment, compared with 4.2, 4.0, 3.7, 2.8 and 3.8 eggs per female in the 87.5, 114, 148, 192 and 250 mL test item/ha rates of GF-4021, respectively. Relative to the control, the respective decrease in reproduction was equivalent to 20.5%, 24.6%, 30.1%, 47.7% and 28.3%. Therefore, the ER_{50} value was > 250 mL test item/ha, the highest rate tested. Statistically, none of the results differed significantly from the control. The NOER with respect to reproduction was therefore 250 mL test item/ha.

All the study validity criteria were met: a) Mortality in the control treatment over the initial 7 days should not exceed 20% (actual value was 1.7% mortality at 7 DAT); b) Corrected mortality in the toxic reference treatment over the initial 7 days should be 50-100% (actual value was 89.8% at 7 DAT); and c) The mean cumulative number of eggs produced between 7 and 14 days should be equal to or greater than 4.0 per female in the control treatment (actual number of eggs per female was 5.3).

Table 42: Effects of GF-4021 on the survival of *Typhlodromus pyri*

Test concentrations (mL test item/ha)	Mean % Mortality	Abbott corrected % mortality
Control	1.7	-
87.5	13.3	11.9
114	5.0	3.4
148	28.3 *	27.1
192	18.3 *	16.9
250	43.3 *	42.4
Toxic Reference	90.0 *	89.8

* Statistically different from the control ($\alpha = 0.05$).

Table 43: Effects of GF-4021 on the fecundity of *Typhlodromus pyri*

Test concentrations (mL test item/ha)	Mean no. of eggs per female	% Difference compared to control ¹
Control	5.3	-
87.5	4.2	20.5
114	4.0	24.6
148	3.7	30.1
192	2.8	47.7
250	3.8	28.3

¹ Positive values indicate a worse performance compared to the control.

CONCLUSION

In a laboratory test to determine the effects of freshly-dried residues of GF-4021 on the predatory mite *Typhlodromus pyri*, the 7-day LR_{50} value was > 250 mL test item/ha, the highest rate tested. The ER_{50} value was also > 250 mL test item/ha. Based on statistical comparison with the control, the NOER with respect to mite survival was 114 mL test item/ha and the NOER for reproduction was 250 mL test item/ha, the highest rate tested.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Predatory mite	<i>Typhlodromus pyri</i>	GF-4021	7 days	LR ₅₀	> 250	mL/ha
Predatory mite	<i>Typhlodromus pyri</i>	GF-4021	7-14 days	ER ₅₀	> 250	mL/ha

A 2.3.2.1.2 Study 190464: GF-4021: A Rate-Response Laboratory Study of the Effects of Fresh Residues on the Parasitic Wasp *Aphidius rhopalosiphi* (Hymenoptera, Braconidae).

Comments of zRMS:	<p>The study was performed in line with the respective guideline with no deviations.</p> <p>All validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>LR₅₀ = 192.2 mL product/ha ER₅₀ > 192 mL product/ha</p>
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Reference:	KCP 10.3.2/02
Report:	Stevens, J.; 2020; GF-4021: A Rate-Response Laboratory Study of the Effects of Fresh Residues on the Parasitic Wasp <i>Aphidius rhopalosiphi</i> (Hymenoptera, Braconidae); Mambo-Tox, A Division of Cawood Scientific Ltd, Southampton, UK; Lab Study No. DOW-19-21; DAS Study No. 190464 ; 02 April 2020; Unpublished.
Guideline(s):	Mead-Briggs <i>et al.</i> (2000)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study):	-

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-4021
Purity:	3.3% aminopyralid, 1.08% halauxifen-methyl, 5.1% picloram
Description (physical state):	Clear yellow fluid (EC)
Lot/batch no.:	ENBK-170903-012 (TSN401447)

Test System

Organism (<i>Species</i>):	Parasitic wasp (<i>Aphidius rhopalosiphi</i>)
Study type:	Tier 1 – glass plate
Study design:	<p>Assessments of mortality measured 48 hrs after treatment and parasitisation 13 days after treatment.</p> <p>4 replicates, each consisting of 10 wasps in one arena per test concentration.</p>
Test concentrations:	0 (control), 87.5, 114, 148, 192 and 250 mL GF-4021/ha
Environmental conditions:	<p>Temperature: 20.4-21.7°C</p> <p>Relative humidity: 70-75% for mortality-assessment phase</p> <p>Photoperiod: 16 h (Exposure: 942-1016 lux; Oviposition: 2010-2112 lux; Fecundity: 4772-4846 lux)</p> <p>Feeding: 1:3 v/v solution of honey in water on cotton wool</p>

Reference substance: BAS 152 65 I (400 g/L dimethoate), applied at 0.1 mL product/ha

Methodology

GF-4021 was evaluated at five application rates, equivalent to 87.5, 114, 148, 192 and 250 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 0.10 mL product/ha. Treatments were applied using a laboratory track-sprayer to glass plates which were left to dry and then used to construct the test arenas.

Ten wasps (including a minimum of five females) were confined in each arena, with four replicates (i.e. a total of 40 wasps) prepared for each treatment. Wasp mortality was assessed after 2, 24 and 48 h. To assess sub-lethal effects on reproduction, assessments were then carried out for the control and for the test item treatment rates of 87.5, 114, 148 and 192 mL/ha. Fifteen female wasps were confined individually over untreated aphid-infested barley plants for 24 h, before being removed. The plants were left for a further 10 days before the number of aphid mummies that had developed on plants where wasps had been found alive after the 24 h oviposition period was recorded.

In order to determine the no-observed-effect rate (NOER) for mortality, the percentage mortality in each test item treatment was compared to the control using multiple sequentially-rejective Fisher test after Bonferroni-Holm (one-sided, $>$ control, $\alpha = 0.05$). Fisher's exact binomial test was used for the toxic reference ($\alpha = 0.05$, one-sided, $>$ control). The 48-h median lethal rate (LR₅₀) for the test item was derived by linear regression analysis according to the Weibull model. In order to determine the NOER for reproduction, the data were first checked for normality (Shapiro-Wilk test, $\alpha = 0.05$) and for homogeneity of variance (Levene's test, $\alpha = 0.05$). As normality could not be assumed, the data were compared by multiple sequentially-rejective U-test after Bonferroni-Holm ($\alpha = 0.05$, one-sided, $<$ control). The median effect rate (ER₅₀) with respect to reproduction was visually extrapolated from the data. Analyses were performed using validated computer software ToxRatPro.

RESULTS AND DISCUSSION

At 48 h, there was 0.0% mortality in the control treatment, compared with 5.0%, 0.0%, 32.5%, 40.0% and 90.0% mortality in the 87.5, 114, 148, 192 and 250 mL GF-4021/ha treatment rates, respectively. In the toxic reference treatment, 95.0% mortality was observed at 48 h. The 48-h median lethal rate (LR₅₀) for GF-4021 was 192.2 mL test item/ha (with 95% confidence limits of 143.4 and 240.1 mL test item/ha). The result for the test-item treatment rate of 250, 192 and 148 mL/ha differed significantly from the control. Therefore, the *no-observed-effect rate* (NOER) with respect to wasp survival was 114 mL product/ha.

In the reproduction assessments, the mean number of mummies produced per surviving female was 43.3 in the control, compared with 38.1, 40.5, 43.4 and 46.5 in the 87.5, 114, 148, and 192 mL GF-4021/ha treatment rates, respectively. Relative to the control, there was a decrease in reproduction of 11.9% and 6.3% in the 87.5 and 114 mL test item/ha, respectively; and an increase in reproduction of 0.3% and 7.5% in the 148 and 192 mL test item/ha treatments respectively. Therefore, the ER₅₀ value based on reproductive performance was >192 mL test item/ha. When compared statistically, none of the results for the test-item treatments differed significantly from the control. Therefore, the NOER value with respect to reproduction was 192 mL test item/ha, the highest rate tested for reproduction.

All the study validity criteria were met: a) Mortality within the control treatment should not exceed 13% (i.e. 5 wasps from 40) at 48 h (actual value was 0.0% mortality at 48 h); b) Corrected mortality within the toxic reference treatment should exceed 50% at 48 h (actual value was 95.0% at 48 h); and c) For the reproduction assessments, the mean number of mummies in the control treatment should be a minimum of 5.0 per surviving female and there should not be more than two zero values in the control treatment (actual mean value was 43.3 mummies per surviving female in the control and there were no zero values in the control).

Table 44: Effects of GF-4021 on the survival of *Aphidius rhopalosiphi*

Test concentrations (mL/ha)	% Mortality at 48 h	Abbott corrected % mortality +
Control	0.0	-
87.5	5.0	5.0
114	0.0	0.0
148	32.5 *	32.5
192	40.0 *	40.0
250	90.0 *	90.0
Toxic Reference	95.0 *	95.0

* Statistically different from the control

Table 45: Effects of GF-4021 on the parasitism rate of *Aphidius rhopalosiphi*

Test concentrations (mL/ha)	Mean no. of mummies per female	% Difference compared to control +
Control	43.3	-
87.5	38.1	11.9
114	40.5	6.3
148	43.4	-0.3
192	46.5	-7.5

+ (Positive values indicate worse performance compared to control)

CONCLUSION

In a laboratory test to determine the effects of fresh residues of GF-4021 on the parasitoid wasp *Aphidius rhopalosiphi*, the 48-h LR₅₀ value was 192.2 mL test item/ha, with 95% confidence limits of 143.4 and 240.1 mL test item/ha. Based on statistical comparisons with the control, the NOER value with respect to wasp survival was 114 mL test item/ha.

In assessments of the reproductive performance of surviving wasps, the ER₅₀ value for GF-4021 was > 192 mL test item/ha. Based on statistical comparison with the control, the NOER value for reproduction was 192 mL test item/ha, the highest rate tested for reproduction.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Parasitic wasp	<i>Aphidius rhopalosiphi</i>	GF-4021	48 hr	LR ₅₀	192.2	mL/ha
Parasitic wasp	<i>Aphidius rhopalosiphi</i>	GF-4021	13 days	ER ₅₀	>192	mL/ha

A 2.3.2.2 KCP 10.3.2.2 Extended laboratory testing, aged residues studies with non-target arthropods

Not required to characterise the product in the current submission.

A 2.3.2.3 KCP 10.3.2.3 Semi-field studies with non-target arthropods

Not required to characterise the product in the current submission.

A 2.3.2.4 KCP 10.3.2.4 Field studies with non-target arthropods

Not required to characterise the product in the current submission.

A 2.3.2.5 KCP 10.3.2.5 Other routes of exposure for non-target arthropods

Not required to characterise the product in the current submission.

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 190475: GF-4021: Determination of Chronic Toxicity to the Earthworm *Eisenia andrei* (Oligochaeta: Lumbricidae) in an artificial soil substrate.

Comments of zRMS:	<p>The study was performed in line with OECD 222 with no deviations.</p> <p>The test design was relevant to derive both NOEC and ECx values (8 concentrations, 8 replicates for control, 4 replicates per treatment group). However, the ECx values could not be calculated due to effects <10% in majority of test groups and lack of the dose-response.</p> <p>All validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>NOEC = 40 mg product/kg soil d.w.</p>
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Reference:	KCP 10.4.1.1/01
Report:	McCormac, A.; 2020; GF-4021: Determination of Chronic Toxicity to the Earthworm <i>Eisenia andrei</i> (Oligochaeta: Lumbricidae) in an Artificial Soil Substrate; Mambo-Tox, A Division of Cawood Scientific Ltd., Southampton, UK; Lab Study No. DOW-19-24; DAS Study No. 190475 ; 25 March 2020; Unpublished.
Guideline(s):	OECD 222 (2016)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study):	

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-4021
Purity:	3.3% aminopyralid, 1.08% halauxifen-methyl, 5.1% picloram.
Description (physical state):	Clear yellow fluid
Lot/batch no.:	ENBK-170903-012 (TSN401447)

Test System

Organism (<i>Species</i>):	Earthworm (<i>Eisenia andrei</i>)
Study type:	56-day earthworm chronic 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 control.
Test concentrations:	0 (control), 0.7, 1.2, 2.1, 3.8, 7.0, 12.0, 22.0 and 40.0 mg GF-4021/kg soil dry weight.

Soil parameters:	Artificial soil according to OECD 222 (10% peat) pH at initiation: 6.0 – 6.2 pH at termination: 5.4 – 5.5 Water content at initiation: 50% WHC _{max} Water content at termination: 46-56% WHC _{max}
Environmental conditions:	Temperature: 18.9-20.3°C Relative humidity: not applicable Light intensity: 550-720 lux Photoperiod: 16 h Feeding: oat flakes 3 g + horse manure 2 g at 1 DAT; oat flakes 5 g at 1, 2 and 3 weeks, 10 g at 28 DAT.
Reference substance:	carbendazim (evaluated in separate GLP-compliant study)

Methodology

The test item was evaluated at eight concentrations. These variants were compared to a water-treated control. Treatments were incorporated into an artificial soil substrate containing 10% w/w peat held within clear polystyrene plastic boxes (17.1 cm × 11.3 cm in area, by 6 cm deep) with ventilated lids (n = 8 for control; n = 4 per test item treatment). Soil moisture content was maintained at 50% (± 10%) of the maximum water-holding capacity throughout the bioassay.

Ten adult *E. andrei* (approximately 6.5 months old, with a fresh weight in the range of 250-600 mg and with a visible clitellum) were introduced to each arena. Finely ground oat flakes plus dried horse manure was provided as food at 1 DAT, and oat flakes alone were provided after 1, 2 and 3 weeks of the bioassay. At 28 DAT, mortality, behaviour, condition and biomass change of the original adult worms were assessed. The test soil, with any cocoons or juvenile worms, was returned to the test chambers and a final supply of oat flakes provided. After a further 28 days (i.e. 56 DAT), the number of juvenile worms that had developed in each replicate arena was assessed.

Statistical analyses were performed using the validated computer software ToxRatPro (version 3.3.0). Mortality for each test item treatment concentration was compared to that in the control treatment using Multiple sequentially-rejective Fisher test after Bonferroni-Holm (one-sided, > control, $\alpha = 0.05$). The LC₅₀ value was derived by extrapolation from the data. Body weight and reproduction data were checked for normality (Shapiro Wilk test, $\alpha = 0.01$) and for equality of variance (Levene's test, $\alpha = 0.01$). Trend analysis by contrasts (monotonicity of concentration/response) revealed a significant linear trend ($\alpha = 0.05$) for body weight but not reproduction. Comparison of the individual test item treatment groups to the control was made using Williams multiple sequential t-test (one-sided, < control, $\alpha = 0.05$) for body weight, and Dunnett's multiple t-test (one-sided, < control, $\alpha = 0.05$) for reproduction. The EC₅₀ value was derived by extrapolation from the data, and the EC₂₀ or EC₁₀ values could not be determined.

RESULTS AND DISCUSSION

At 28 DAT, the percentage mortality in the control was 0%, compared to a range of 0-3% mortality in the 0.7-40.0 mg test item/kg soil dry weight treatment concentrations of GF-4021. Therefore, the LC₅₀ value was > 40.0 mg test item/kg soil dry weight. There was no significant mortality or biomass change, compared to the control, in any of the test-item treatment concentrations up to and including the highest tested, i.e. 40.0 mg test item/kg soil dry weight. There was also no observed loss in condition or change in behaviour amongst the earthworms in any of the test-item treatments. Therefore, the NOEC value for adult mortality and the NOEC value for worm growth and condition were both 40.0 mg test item/kg soil dry weight, the highest concentration tested.

At 56 DAT, the percentage reduction in the numbers of juveniles relative to the control was < 50% for all test item treatment concentrations, up to and including 40.0 mg test item/kg soil dry weight, the

highest concentration tested. Therefore, it was not possible to calculate the median effect concentration value (EC₅₀) by regression analysis and it was considered, by extrapolation of the data, to be > 40.0 mg test item/kg soil dry weight. The EC₂₀ and EC₁₀ values could not be determined due to a lack of a clear dose-response with effects > 10% or > 20% on reproduction. At 56 DAT, the numbers of juveniles were not significantly reduced relative to the control at all treatment concentrations up to and including 40.0 mg test item/kg soil dry weight. Thus, the NOEC value for effects on reproduction was 40.0 mg test item/kg soil dry weight, the highest concentration tested. The LOEC value was not determined.

All the validity criteria for the study were met: a) control treatment mortality should not exceed 10% at 28 days (the actual level of the control data was 0%); b) the number of juveniles recorded in the control treatment should be at least 30 per replicate (the actual minimum number of juveniles recorded in an individual control arena was 162); and c) the coefficient of variation for the results of reproduction in the control treatment replicates should not exceed 30% (the actual CV for the control data was 13.3%).

Table 46: Effects of GF-4021 on earthworm survival, biomass and reproduction

Test concentrations (mg GF-4021/kg soil dry weight)	% Mortality after 28 days ¹	Mean % Bodyweight change after 28 days ²	Mean no. of juveniles at day 56	% Change in number of juveniles compared to control ³
Untreated control	0	21	202	-
0.7	0	25	205	1.4
1.2	0	24	215	6.1
2.1	0	17	185	-8.4
3.8	0	23	200	-1.2
7.0	0	16	189	-6.4
12.0	3	19	168	-17.0
22.0	0	15	195	-3.7
40.0	3	23	192	-4.9

¹ Mortality: Multiple sequentially-rejective Fisher test after Bonferroni-Holm, one-sided, > control, $\alpha = 0.05$

² Bodyweight: A positive value indicates an increase in adult bodyweight relative to 0 DAT; Williams multiple sequential t-test, one-sided, < control, $\alpha = 0.05$.

³ % Change in juvenile numbers: A negative value indicates a decrease, and a positive value an increase in reproduction relative to the control mean; Dunnett's multiple t-test, one-sided < control, $\alpha = 0.05$.

There were no statistically significant differences from the control for any of the parameters.

CONCLUSION

The chronic effects of GF-4021 on the earthworm *Eisenia andrei* were evaluated under laboratory test conditions using an artificial soil substrate containing 10% w/w organic matter. The LC₅₀ value for GF-4021 was > 40.0 mg test item/kg soil dry weight. In terms of effects on earthworm survival, behaviour and adult biomass, the NOEC value was 40.0 mg test item/kg soil dry weight, the highest concentration tested. In terms of effects on earthworm reproduction, the EC₅₀ value for GF-4021 was > 40.0 mg test item/kg soil dry weight, the NOEC value was 40.0 mg test item/kg soil dry weight, the highest concentration tested. Taking into account all of these assessment criteria, it was concluded that the overall NOEC value for GF-4021 was 40.0 mg test item/kg soil dry weight.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Earthworm	<i>Eisenia andrei</i>	GF-4021	56-day	NOEC	40.0	mg/kg soil dw

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

Not required to characterise the product in the current submission.

**A 2.4.2 KCP 10.4.2 Effects on non-target soil meso- and macrofauna
(other than earthworms)**

A 2.4.2.1 KCP 10.4.2.1 Species level testing

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

Not required to characterise the product in the current submission.

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

A 2.5.1 Study 190194: GF-4021: Effects on the Activity of the Soil Microflora in the Laboratory.

Comments of zRMS:	<p>As studies on effects on soil carbon transformation are no longer a data requirement, the part of the study referring to carbon transformation was not evaluated and was removed from the summary below.</p> <p>The part of the study referring to nitrogen transformation was performed fully in line with OECD 216 with no deviations.</p> <p>All validity criteria were met and 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 1.58 mg product/kg soil d.w.</p>
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Reference:	KCP 10.5
Report:	Hammesfahr, U.; 2020; GF-4021: Effects on the Activity of the Soil Microflora in the Laboratory; ibacon GmbH, Rossdorf, Germany; Lab Study No. 141841080; DAS Study No. 190194 ; 06 April 2020; Unpublished.
Guideline(s):	OECD 216 (2000)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable (evaluated only the part of the study investigating effects on nitrogen transformation)
Duplication (if vertebrate study):	

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-4021
Purity:	3.3% aminopyralid; 1.08% halauxifen-methyl; 5.1% picloram
Description (physical state):	Amber liquid
Lot/batch no.:	ENBK-170903-012 (TSN401447)

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
Study duration:	28 days
Parameters measured:	<p>Nitrogen transformation:</p> <p>analysis of nitrate, nitrite and ammonium in extracted soil samples, via Continuous Flow Analyser (AA3, XY-2 / XY-3Sampler); limits of quantification:</p> <p>NO₃-N: 0.134 mg/kg soil dry weight</p> <p>NO₂-N: 0.425 mg/kg soil dry weight</p> <p>NH₄-N: 0.081 mg/kg soil dry weight</p> <p>soil water content</p> <p>pH</p>

Observation intervals:	0, 7, 14 and 28 days
Test concentrations:	0.32 and 1.58 mg GF-4021/kg soil dry weight
Toxic reference:	Sodium chloride at a concentration of 16 g/kg soil dry weight (conducted as a separate quality control study within a year from the present study)
Method of test item application:	Incorporation into the soil
Environmental conditions:	Conducted in the dark. Temperature: $20 \pm 2^{\circ}\text{C}$ pH: 7.3 to 7.4 Soil source: The soil batch used in this study was according to the guidelines and was taken from fallow grassland: District authority: Rhineland Palatinate Municipality: Mechtersheim, Germany Location: "In der Speyerer Hohl", No. 977
Soil properties	Water content of soil at start: 46% - 47% of MWHC Water content of soil at end: 46% - 47% of MWHC Clay (%): 11.4 Silt (%): 36.0 Sand (%): 52.6 Organic Carbon (%): 0.89 Microbial biomass (% of total organic carbon): 3.35 Textural classification: Loamy Sand

Methodology

Determination of nitrogen-transformation (ammonium-, nitrite- and nitrate-nitrogen levels) in soil enriched with lucerne meal (concentration in soil 0.5%). Comparison of test item treated soil with a non-treated soil. Three replicates per treatment and concentration. NH_4^{+} -, NO_2^{-} - and NO_3^{-} -nitrogen formed from the nitrification process were determined by means of a Continuous Flow Analyser (AA3, XY-2 / XY-3 Sampler).

Data for the soil nitrification (nitrite, ammonium, nitrate content and nitrate formation rates) were tested for normality and homogeneity of variance using the R/S-Test ($\alpha = 0.01$) and Levene's test ($\alpha = 0.01$), respectively. The Student t-test (two-sample comparison, two sided, $\alpha = 0.05$) was used for comparison of treated and control values for nitrate-N contents, nitrate formation rates, respectively. The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0 ®ToxRat Solutions GmbH.

RESULTS AND DISCUSSION

The cumulative soil nitrate formation rates were below the 25% trigger value given by the OECD 216 guideline by the end of the study. In the last interval between days 0 and 28, the deviations from control were 9.96% and -3.99% for the 0.32 and 1.58 mg/kg soil dry weight test rates of GF-4021, respectively. The deviation was statistically significant different from the control for the low test item rate (Student t-test, $\alpha = 0.05$).

The incremental soil nitrate formation rates were below the 25% trigger value given by the OECD 216 guideline by the end of the study. In the last interval between days 14 and 28, the deviations from control were 0.45% and 2.23% for the 0.32 and 1.58 mg/kg soil dry weight test rates of GF-4021, respectively. There were no statistically significant differences on day 28 between control and both test item rates (Student ttest, $\alpha = 0.05$).

The variation between the replicate control samples was within the validity criterion of 15% for both the nitrogen transformation test (OECD test guidelines 216) throughout the test. The validity of the test system was further confirmed by the sensitivity established in separate positive control experiment using sodium chloride at a concentration of 16 g/kg soil dry weight.

Table 47: Effects of GF-4021 on the nitrate formation rate

Interval sampling days	Control	0.32 mg GF-4021 /kg soil dry weight			1.58 mg GF-4021 /kg soil dry weight		
	[mg NO ₃ -N /kg/day ¹]	[mg NO ₃ -N /kg/day ¹]	[% ²]	[sig ³]	[mg NO ₃ -N /kg/day ¹]	[% ²]	[sig ³]
0-7	0.086	0.407	373.26	*	0.061	-29.07	n.s.
0-14	0.710	0.867	22.11	*	0.627	-11.69	n.s.
0-28	0.803	0.883	9.96	*	0.771	-3.99	n.s.
Interval sampling days	[mg NO ₃ -N /kg/day ¹]	[mg NO ₃ -N /kg/day ¹]	[% ²]	[sig ³]	[mg NO ₃ -N /kg/day ¹]	[% ²]	[sig ³]
0-7	0.086	0.407	373.26	*	0.061	-29.07	n.s.
7-14	1.335	1.328	-0.52	n.s.	1.193	-10.64	n.s.
14-28	0.895	0.899	0.45	n.s.	0.915	2.23	n.s.

¹ mean mg NO₃-N/[kg soil dry weight and day]

² deviation from control (negative value =% inhibition, positive value =% stimulation)

³ statistical significance (Student t-test, two sided, $\alpha = 0.05$): * significant differences from the control; n.s. = no significant differences from the control

CONCLUSION

Based on the results of this study, it is concluded that GF-4021 had no significant impact on soil microorganisms (nitrogen transformation) when applied at test item concentrations up to 1.58 mg/kg soil dry weight. It can be concluded that GF-4021 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-4021	28 day – N transformation	<25% deviation from the control	1.58	mg/kg soil dw

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

Not required to characterise the product in the current submission.

A 2.6.2 KCP 10.6.2 Testing on non-target plants

A 2.6.2.1 Study 190546: GF-4021 Seedling Emergence and Seedling Growth Terrestrial Non Target Plants

Comments of zRMS:	<p>The study was performed in line with OECD 208 with deviations discussed below.</p> <p>It was noted that the temperature exceeded the recommended range of 12-32°C on some occasions. The high temperature occurred during temporary heatwaves with strong winds. Safety measures prevent opening of the vents during strong winds, which meant the temperature in the glasshouse rose above the required level. The control plants were healthy and growing well and for this reason it is not considered that this deviation had impact on the integrity of the study.</p> <p>Further, it was noted that the relative humidity fell below the recommended range of 45-95% on some occasions. The apparent low relative humidity readings were caused by the temperature and air pressure combination, which moved away from the dewpoint temperature, thus reducing the humidity. The relative humidity is dependent on the number of plants in the glasshouse and the amount of watering they receive, which can vary over a period of time. As the plants are watered via saucers, the relative humidity can be affected by the time of day they are watered and can potentially decrease before being re-watered. Consequently, on some occasions, the relative humidity can be below 70% ±25%. The control plants were healthy and grew well and for this study the low relative humidity has not affected the plants. It is thus not considered that this deviation had impact on the integrity of the study.</p> <p>As all validity criteria were met and the control plants for which the above values were outside of the recommended range have not exhibited any adverse effects related to too low relative humidity and too high temperature and all control plants for which the above values were outside of the recommended ranges survived, it is not expected that these deviations had a significant impact on the test results.</p> <p>Recovery rates for aminopyralid, halauxifen-methyl and picloram in the spray solution samples were within the range of 98-103%, 92-100%, 99-102%, respectively; therefore, the endpoints can be expressed as nominal concentrations.</p> <p>The following validity criteria for the test were met:</p> <ul style="list-style-type: none"> • the emergence in the untreated control pots must be at least 70% (observed 85-100%), • the control seedlings must not exhibit any phytotoxic effects (no effects observed in the study), • the mean control plant survival must be at least 90% (observed 94-100%), • the environmental conditions must be identical for each of the species tested (yes). <p>Taking all of the above into account, the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>Lowest shoot fresh weight ER₅₀ = 14.1 mL product/ha (tomato) Lowest phytotoxicity ER₅₀ = 17.2 mL product/ha (tomato) Lowest emergence ER₅₀ = 150 mL product/ha (soybean) Lowest survival ER₅₀ = 76.1 mL product/ha (onion)</p>
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Reference:	KCP 10.6.2/01
Report:	Bramby-Gunary, J; 2020a; GF-4021 Seedling Emergence and Seedling Growth Terrestrial Non Target Plants ; AgroChemex Ltd., Essex, CO11 2NF, United Kingdom.; Lab Study No. ACE-19-079; DAS Study No. 190546 ; 29 October 2020; Unpublished.
Guideline(s):	OECD Guideline 208 (2006)
Deviations:	See zRMS comments above
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study):	-

MATERIALS AND METHODS

Test Item(s)

Test item (Common name): GF-4021
Purity: 3.3 wt% aminopyralid (31.0 g/L), 1.08 wt% halauxifen-methyl (10 g/L) and 5.1 wt% picloram (48 g/L); nominally 32 g/L aminopyralid, 10 g/L halauxifen-methyl and 48 g/L picloram
Description (physical state): Amber liquid. Emulsifiable concentrate (EC)
Lot/batch no.: ENBK-170903-012 (TSN401447)

Test System

Monocotyledonous species: onion, oat, and ryegrass
Dicotyledonous species: sugar beet, oilseed rape, cucumber, carrot, soybean, sunflower, tomato and field bean
Study type: Greenhouse study assessing Seedling Emergence and Seedling Growth
Non-porous plastic pots were used (15 cm ± 5% diameter)
Parameters measured: Emergence counts
Number of dead plants
Shoot fresh weight
Phytotoxicity rating system, if used:
0 % No phytotoxicity
1 - 39 % Slight phytotoxicity
40 - 69 % Moderate phytotoxicity
70 - 99 % Severe phytotoxicity
100 % All plants dead
Growth conditions: Temperature (range): 15.5 – 36.3 °C
Photoperiod: ≥16 hours
Light intensity (range): 0.4 – 87.9 Klux
Relative humidity: 25.1 – 77.4%
Water regime and schedules: daily as required
Water source/type: mains water
Pest control method /fertilisation, if used: none / slow release fertiliser
Growth medium: Soil type: sandy clay loam
Details of nutrient medium, if used: 125 g slow release fertiliser (Osmocote® Pro) was incorporated into 30 litres of soil mix.
pH: 8.0

Test concentrations:	Nominal: 31.25, 62.5, 125, 250 and 500 mL GF-4021/ha (oat, ryegrass, oilseed rape and cucumber), 7.81, 15.63, 31.25, 62.5, 125, 250 and 500 mL GF-4021/ha (onion, sugar beet, carrot, soybean, sunflower and field bean) and 0.49, 0.98, 1.95, 3.91, 7.81, 15.63, 31.25, 62.5, 125 and 250 mL GF-4021/ha (tomato)
Analytical verification:	The spray solutions were analysed to determine the concentration of aminopyralid, halauxifen-methyl and picloram to verify the highest application rate using HPLC with ultraviolet (UV).
Test material application:	Method: Mardrive cabinet track sprayer with 8004E TeeJet even flat fan nozzle. Application interval: N/A Reference chemical (if used): N/A
Seeds:	Source: 1) Moles Seeds (UK) Ltd, Turkey Cock Lane, Stanway, Colchester, Essex, CO3 8PD, United Kingdom. (onion, cucumber, sunflower and tomato) 2) Senova Ltd, 49 North Road, Great Abington, Cambridge CB21 6AS, United Kingdom. (oat) 3) Walnes Seeds Ltd., Moat Farm, Moat Park, Earl Soham, Woodbridge, Suffolk IP13 7SR, United Kingdom. (ryegrass) 4) Lion Seeds, Maldon Road, Maldon, Essex, CM9 6SN, United Kingdom. (sugar beet) 5) Limagrain UK Ltd, Rothwell, Market Rasen Lincolnshire LN7 6DT, United Kingdom. (oilseed rape and field bean) 6) E. W. King & CO. Ltd., Kelvedon, CO5 9PG, United Kingdom. (carrot) 7) Soya UK, Longways House, Burnetts Lane, West End, Southampton, Hampshire, SO30 2HH, United Kingdom. (soybean) Method of seeding: manual Prior seed treatment/sterilisation: none Number of seeds per replicate pot: 5 (onion, oat and ryegrass), 3 (oilseed rape and carrot) and 2 (sugar beet, cucumber, soybean, sunflower, tomato and field bean)
Number of control replicates:	4 (onion, oat and ryegrass), 7 (oilseed rape and carrot) and 10 (sugar beet, cucumber, soybean, sunflower, tomato and field bean)
Number of test concentration replicates:	4 (onion, oat and ryegrass), 7 (oilseed rape and carrot) and 10 (sugar beet, cucumber, soybean, sunflower, tomato and field bean)

Methodology

The methodology was based on OECD Guidelines for the Testing of Chemicals, Test No. 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test, July 2006. The study was conducted to GLP standards.

Four species (oat, ryegrass, oilseed rape and cucumber) were exposed to a deionised water control and five test item concentrations, tomato was exposed to a deionised water control and ten test item concentrations and six species (onion, sugar beet, carrot, soybean, sunflower and field bean) were exposed to a deionised water control and seven test item concentrations. The test duration was 21 days after 50% emergence in the controls.

Emergence, mortality and phytotoxicity were assessed weekly; biomass (fresh weight) were assessed at test termination.

The 50% effect rates (ER₅₀) values were calculated from the data using final emergence, final survival, final visual phytotoxicity (injury) and mean foliar fresh weight per surviving plant per replicate for each species. The values are expressed in millilitre GF-4021 per hectare (mL GF-4021/ha) for each species. The applications were made using a Mardrive cabinet track sprayer with 8004E TeeJet even flat fan nozzle.

RESULTS AND DISCUSSION

The ER₅₀ values determined for oat, ryegrass, sugar beet, oilseed rape, cucumber, sunflower, tomato, and field bean were greater than the highest concentration of GF-4021 tested. As there was not sufficient reduction in the emergence for these species it was not possible to calculate the ER₅₀. Therefore, in these cases, the ER₅₀ value are reported as > 500 mL GF-4021/ha (>250 mL GF-4021/ha for tomato).

The ER₅₀ values based on survival for oat, ryegrass, oilseed rape and cucumber were greater than the highest concentration of GF-4021 tested. As there was no reduction in the survival for these species it was not possible to calculate the ER₅₀. Therefore, in these cases, the ER₅₀ value are reported as > 500 mL GF-4021/ha.

The concentration response of tomato to GF-4021 lead to a decreased in survival of 63% at 250 mL GF-4021/ha. The ER₅₀ value calculated are very slightly greater than the highest concentration tested for this species (250 mL GF-4021/ha). In this case the ER₅₀ value are reported as > 250 mL GF-4021/ha.

The ER₅₀ values based on phytotoxicity and fresh weight for oat and ryegrass were greater than the highest concentration of GF-4021 tested. As there was no phytotoxicity (oat) or not sufficient reduction (ryegrass) it was not possible to calculate the ER₅₀. Therefore, in these cases, the ER₅₀ value are reported as > 500 mL GF-4021/ha.

The concentration response of oilseed rape to GF-4021 lead to 61% visual phytotoxicity at 500 mL GF-4021/ha.

The ER₅₀ value calculated for fresh weight are slightly greater than the highest concentration tested for this species (500 mL GF-4021/ha). In this case the ER₅₀ value are reported as >500 mL GF-4021/ha.

Table 48: Observations Day 21 of Plant: % Emergence, % survival, % visual injury, shoot fresh weight (g): Monocotyledonous species

Treatment	Oat				Ryegrass			
	Emergence	Survival	Visual injury	Shoot fresh weight	Emergence	Survival	Visual injury	Shoot fresh weight
Control	95	100	0	6.06	90	100	0	1.26
31.25	95	100	0	5.80	85	100	0	1.27
62.5	100	100	0	6.27	90	100	3	1.04
125	90	100	0	6.15	100	100	0	1.58
250	100	100	0	5.15	85	100	6	1.32
500	95	100	0	6.37	80	100	15	1.17

	Onion			
Treatment	Emergence	Survival	Visual injury	Shoot fresh weight
Control	85	100	0	0.950
7.81	85	100	0	0.800
15.63	100	100	0	0.877
31.25	100	85	20	0.919
62.5	95	74	43	0.335
125	80	19	85	0.0609
250	70	0	100	-
500	20	0	100	-

– No result, all plants dead.

Table 49: Observations Day 21 of plant: % Emergence, % survival, % visual injury, shoot fresh weight (g): Dicotyledonous species

	Oilseed rape				Cucumber			
Treatment	Emergence	Survival	Visual injury	Shoot fresh weight	Emergence	Survival	Visual injury	Shoot fresh weight
Control	90	100	0	23.0	100	100	0	24.6
31.25	95	100	0	20.9	95	100	2	27.9
62.5	86	100	1	23.1	100	100	14	26.5
125	100	95	17	21.9	100	100	31	25.0
250	90	100	31	18.2	95	100	53	21.5
500	62	100	61	12.5	100	100	79	9.0

	Tomato			
Treatment	Emergence	Survival	Visual injury	Shoot fresh weight
Control	100	100	0	23.7
0.49	100	100	0	24.7
0.98	100	100	0	23.8
1.95	100	100	0	22.7
3.91	100	100	6	22.8
7.81	95	100	20	23.4
15.63	100	100	52	9.46
31.25	95	100	67	5.35
62.5	90	100	79	1.79
125	100	60	90	0.713
250	95	37	97	0.163

	Sugar beet				Carrot				Soybean			
Treatment	Emergence	Survival	Visual injury	Shoot fresh weight	Emergence	Survival	Visual injury	Shoot fresh weight	Emergence	Survival	Visual injury	Shoot fresh weight
Control	95	100	0	16.4	86	94	0	1.45	100	100	0	4.63
7.81	90	100	0	13.8	81	94	0	1.84	100	90	25	3.24
15.63	95	100	13	10.5	81	100	0	2.35	85	100	50	3.05
31.25	95	100	34	9.33	100	95	14	1.21	90	94	57	2.32
62.5	90	100	64	4.12	62	85	21	1.49	80	44	85	1.78
125	95	95	78	2.13	67	57	55	0.60	70	57	86	1.19
250	95	74	87	0.39	33	29	73	0.91	25	0	100	-
500	80	19	97	0.23	19	0	100	-	5	0	100	-

– No result, all plants dead.

Treatment	Sunflower				Field bean			
	Emergence	Survival	Visual injury	Shoot fresh weight	Emergence	Survival	Visual injury	Shoot fresh weight
Control	100	100	0	20.5	95	100	0	11.8
7.81	100	100	0	19.6	100	100	2	12.0
15.63	100	100	0	20.4	100	100	14	12.1
31.25	95	100	6	23.0	95	100	23	10.3
62.5	100	95	33	18.9	85	100	54	7.08
125	100	100	46	13.7	90	83	64	7.23
250	90	72	71	6.88	85	82	69	5.36
500	80	31	91	4.38	90	22	92	3.16

Table 50: Reported ER₅₀ values based on emergence, survival, phytotoxicity and shoot fresh weight (mL GF-4021/ha)

Species	Emergence	Survival	Phytotoxicity	Shoot fresh weight
	ER ₅₀ (95% CL)	ER ₅₀ (95% CL)	ER ₅₀ (95% CL)	ER ₅₀ (95% CL)
Onion	338 (212 – 464)	76.1 (58.8 – 98.5)	68.8 (59.4 – 78.6)	61.3 (59.8 – 62.8)
Oat	>500 (N/A)	>500 (N/A)	>500 (N/A)	>500 (N/A)
Ryegrass	>500 (N/A)	>500 (N/A)	>500 (N/A)	>500 (N/A)
Sugar beet	>500 (N/A)	327 (300 – 356)	44.9 (39.6 – 51.2)	32.4 (21.8 – 48.1)
Oilseed rape	>500 (N/A)	>500 (N/A)	381 (315 – 461)	>500 (N/A)
Cucumber	>500 (N/A)	>500 (N/A)	217 (174 – 272)	416 (341 – 496)
Carrot	222 (120 – 370)	155 (90.2 – 240)	126 (101 – 154)	211 (26.7 – N/A) *
Soybean	150 (101 – 213)	103 (45.3 – 199)	19.1 (14.4 – 25.3)	33.7 (27.4 – 41.1)
Sunflower	>500 (N/A)	375 (257 – 548)	126 (104 – 152)	186 (146 – 247)
Tomato	>250 (N/A)	>250 (N/A)	17.2 (14.9 – 19.9)	14.1 (11.8 – 16.8)
Field bean	>500 (N/A)	336 (234 – 483)	73.0 (51.7 – 103)	161 (104 – 250)

N/A = Not applicable

Where 95% CL is reported, R² was >0.7 , * R² was 0.4139

Regression models used:

Species	Emergence	Survival	Phytotoxicity	Shoot fresh weight
Onion	2P Cumulative Normal	2P Log-Logistic	4P Logistic	3P Cum Log-Normal (Probit)
Oat	N/A	N/A	N/A	N/A
Ryegrass	N/A	N/A	N/A	N/A
Sugar beet	N/A	2P Cum Log-Normal (Probit)	4P Log-Logistic+Hormesis	4P Morgan-Mercer-Flodin
Oilseed rape	N/A	N/A	3P Cum Log-Normal (Probit)	3P Log-Logistic
Cucumber	N/A	N/A	4P Log-Logistic+Threshold	3P OECD Exponential #3
Carrot	2P OECD Exponential #2	2P Log-Gompertz	3P Weibull	4P Log-Logistic+Hormesis
Soybean	2P OECD Exponential #2	2P OECD Exponential #2	3P Cum Log-Normal (Probit)	3P Log-Gompertz
Sunflower	N/A	2P Log-Logistic	3P Cum Log-Normal (Probit)	4P Log-Logistic+Hormesis
Tomato	N/A	2P OECD Exponential #2	4P Log-Logistic+Threshold	4P Log-Logistic
Field bean	N/A	2P Cum Log-Normal (Probit)	3P Cum Log-Normal (Probit)	3P Cum Log-Normal (Probit)

N/A = Not applicable

CONCLUSION

Based on phytotoxicity and fresh weight the most sensitive species tested was tomato with an ER₅₀ value of 17.2 and 14.1 mL GF-4021/ha respectively. Based on emergence the most sensitive species tested was soybean with an ER₅₀ values of 150 mL GF-4021/ha and based on and survival the most sensitive species tested was onion with an ER₅₀ values of 76.1 mL GF-4021/ha respectively.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Tomato	<i>Lycopersicon esculentum</i>	GF-4021	N/A	Shoot fresh weight ER ₅₀	14.1	mL/ha

A 2.6.2 Study 190545: GF-4021 Vegetative Vigour Terrestrial Non Target Plants.

Comments of zRMS:	<p>The study was performed in line with OECD 227 with deviations described below.</p> <p>It was noted that a photo of representative plants from each plant species was not taken before the application to carrot and field bean but it was taken in the morning on 14 May 2020. It is not considered that this deviation has affected the integrity of the study.</p> <p>It was noted that the temperature exceeded the recommended range of 12-32°C on some occasions. The temperature deviations lasted approximately 3-9 hours depending on the day and species. The high temperature occurred during temporary heatwaves with strong winds. Safety measures prevents opening the vents in strong winds, which meant the temperature in the glasshouse rose above the required level. The control plants were healthy and grew well. It is not considered that this deviation has affected the integrity of the study.</p> <p>Further, it was noted that the relative humidity fell below the recommended range of 45-95% on some occasions. The deviations lasted approximately 3-15 hours depending on the day and species. The apparent low relative humidity readings were caused by the temperature and air pressure combination, which moved away from the dewpoint temperature, thus reducing the humidity. The relative humidity is dependent on the number of plants in the glasshouse and the amount of watering they receive, which can vary over a period of time. As the plants are watered via saucers, the relative humidity can be affected by the time of day they are watered and can potentially decrease before being re-watered. Consequently, on some occasions, the relative humidity can be below 70% ±25%. The control plants were healthy and grew well and for this study the low relative humidity was not to the detriment of the plants. It is not considered that this deviation has affected the integrity of the study.</p> <p>As all validity criteria were met and the control plants for which the above values were outside of the recommended range have not exhibited any adverse effects (0% phytotoxicity) related to too low relative humidity and too high temperature and all control plants for which the above values were outside of the recommended range survived. Therefore it is not expected that these deviations had a significant impact on the test results.</p> <p>Recovery rates for aminopyralid, halauxifen-methyl and picloram in the spray solution samples were within the range of 97-103 %, 91-97%, and 100-101%, respectively, therefore the endpoints can be expressed as nominal concentrations.</p> <p>The following validity criteria for the test were met:</p> <ul style="list-style-type: none"> the seedling emergence must be at least 70% (observed 100%), the control plants must not exhibit any phytotoxic effects (no effects in the study), the mean control plant survival must be at least 90% (observed 100%),
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	<ul style="list-style-type: none"> the environmental conditions must be identical for each of the species tested (yes). <p>Taking all of the above into account, the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>Lowest shoot fresh weight ER_{50} = 2.68 mL product/ha (tomato) Lowest phytotoxicity ER_{50} = 4.07 mL product/ha (tomato) Lowest survival ER_{50} = 95.6 mL product/ha (soybean)</p>
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Reference:	KCP 10.6.2/02
Report:	Bramby-Gunary, J; 2020b; GF-4021 Vegetative Vigour Terrestrial Non-Target Plants ; AgroChemex Ltd., Essex, CO11 2NF, United Kingdom.; Lab Study No. ACE-19-080; DAS Study No. 190545 ; 05 November 2020; Unpublished.
Guideline(s):	OECD Guideline 227 (2006)
Deviations:	See zRMS comments above
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study):	-

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-4021
Purity:	3.3 wt% aminopyralid (31.0 g/L), 1.08 wt% halauxifen-methyl (10 g/L) and 5.1 wt% picloram (48 g/L); nominally 32 g/L aminopyralid, 10 g/L halauxifen-methyl and 48 g/L picloram
Description (physical state):	Amber liquid. Emulsifiable concentrate (EC)
Lot/batch no.:	ENBK-170903-012 (TSN401447)

Test System

Monocotyledonous species:	onion, oat, and ryegrass
Dicotyledonous species:	sugar beet, oilseed rape, cucumber, carrot, soybean, sunflower, tomato and field bean
Study type:	Greenhouse study assessing Vegetative Vigour
Parameters measured:	Number of dead plants Foliar fresh weight Phytotoxicity rating system, if used: 0% No phytotoxicity 1 - 39% Slight phytotoxicity 40 - 69% Moderate phytotoxicity 70 - 99% Severe phytotoxicity 100% All plants dead
Growth conditions:	Temperature (range): 15.8 – 34.5°C Photoperiod: ≥16 hours Light intensity (range): 0.4 – 87.9 Klux Relative humidity: 23.1 – 87.2% Water regime and schedules: daily as required Water source/type: mains water

	Pest control method /fertilisation, if used: none / slow release fertiliser
Growth medium:	Soil type: sandy clay loam Details of nutrient medium, if used: 125 g slow release fertiliser (Osmocote® Pro) was incorporated into 30 litres of soil mix. For tomato, an additional 500 mL Miracle Gro Stock solution was added to 30 litres of soil mix. The Miracle Gro stock solution consisted of 1.25 mL Miracle Gro per Litre. For details of plant nutrients see Appendix 3. pH: 8.0
Test concentrations:	Nominal: 31.25, 62.5, 125, 250 and 500 mL GF-4021/ha (oat, ryegrass, oilseed rape and sunflower), 7.81, 15.63, 31.25, 62.5, 125, 250 and 500 mL GF-4021/ha (onion, sugar beet and cucumber) and 0.49, 0.98, 1.95, 3.91, 7.81, 15.63, 31.25, 62.5, 125 and 250 mL GF-4021/ha (carrot, soybean, tomato and field bean)
Analytical verification:	The spray solutions were analysed to determine the concentration of aminopyralid, halauxifen-methyl and picloram to verify the highest application rate using HPLC with ultraviolet (UV).
Test material application:	Method: Mardrive cabinet track sprayer with 8004E TeeJet even flat fan nozzle. Application interval: N/A Reference chemical (if used): N/A
Seeds:	Source: 1) Moles Seeds (UK) Ltd, Turkey Cock Lane, Stanway, Colchester, Essex, CO3 8PD, United Kingdom. (onion, cucumber, sunflower and tomato) 2) Senova Ltd, 49 North Road, Great Abington, Cambridge CB21 6AS, United Kingdom. (oat) 3) Walnes Seeds Ltd., Moat Farm, Moat Park, Earl Soham, Woodbridge, Suffolk IP13 7SR, United Kingdom. (ryegrass) 4) Lion Seeds, Maldon Road, Maldon, Essex, CM9 6SN, United Kingdom. (sugar beet) 5) Limagrain UK Ltd, Rothwell, Market Rasen Lincolnshire LN7 6DT, United Kingdom. (oilseed rape and field bean) 6) E. W. King & CO. Ltd., Kelvedon, CO5 9PG, United Kingdom. (carrot) 7) Soya UK, Longways House, Burnetts Lane, West End, Southampton, Hampshire, SO30 2HH, United Kingdom. (soybean) Method of seeding: Manually Prior seed treatment/sterilisation: None Number of plants per replicate pot: 5 (onion, oat and ryegrass), 3 (oilseed rape and carrot) and 1 (sugar beet, cucumber, soybean, sunflower, tomato and field bean) Growth stage at application: 12 – 14 (2 – 4 true leaves)
Number of control replicates:	4 (onion, oat and ryegrass), 7 (oilseed rape and carrot) and 20 (sugar beet, cucumber, soybean, sunflower, tomato and field bean)

Number of test concentration replicates: 4 (onion, oat and ryegrass), 7 (oilseed rape and carrot) and 20 (sugar beet, cucumber, soybean, sunflower, tomato and field bean)

Methodology

The methodology was based on OECD Guidelines for the Testing of Chemicals, Test No. 227: Terrestrial Plant Test: Vegetative Vigour Test, July 2006. The study was conducted to GLP standards.

Four species (oat, ryegrass, oilseed rape and sunflower) were exposed to a deionised water control and five test item concentrations (31.25, 62.5, 125, 250 and 500 mL GF-4021/ha), four species (carrot, soybean, tomato and field bean) were exposed to a deionised water control and ten test item concentrations (0.49, 0.98, 1.95, 3.91, 7.81, 15.63, 31.25, 62.5, 125 and 250 mL GF-4021/ha) and three species (onion, sugar beet and cucumber) were exposed to a deionised water control and seven test item concentrations (7.81, 15.63, 31.25, 62.5, 125, 250 and 500 mL GF-4021/ha). The test duration was 21 days after application.

Mortality and phytotoxicity were assessed weekly; biomass (fresh weight) were assessed at test termination. The 50% effect rates (ER_{50}) values were calculated from the data using final survival, final visual phytotoxicity (injury) and mean foliar fresh weight per surviving plant per replicate for each species. The values are expressed in millilitre GF-4021 per hectare (mL GF-4021/ha) for each species. The applications were made using a Mardrive cabinet track sprayer with 8004E TeeJet even flat fan nozzle.

RESULTS AND DISCUSSION

The ER_{50} values based on survival for onion, oat, ryegrass, sugar beet, oilseed rape, carrot, sunflower and tomato were greater than the highest concentration of GF-4021 tested. As there was no reduction or insignificant reduction (carrot, sugar beet and tomato) in the survival for these species it was not possible to calculate the ER_{50} . Therefore, in these cases, the ER_{50} value are reported as >250 mL GF-4021/ha (carrot and tomato) and > 500 mL GF-4021/ha (onion, oat, ryegrass, sugar beet, oilseed rape and sunflower).

The ER_{50} values based on phytotoxicity and fresh weight for oat were greater than the highest concentration of GF-4021 tested. As there was no phytotoxicity or reduction in fresh weight it was not possible to calculate the ER_{50} values. Therefore, in these cases, the ER_{50} values are reported as > 500 mL GF-4021/ha.

The ER_{50} values based on fresh weight for ryegrass and oilseed rape were greater than the highest concentration of GF-4021 tested. The concentration response of ryegrass to GF-4021 lead to a decrease in fresh weight of 28% at 500 mL GF-4021/ha. The ER_{50} value calculated is extrapolated beyond the highest concentration tested for this species (500 mL GF-4021/ha). For oilseed rape there was insignificant reduction in fresh weight, and it was not possible to calculate the ER_{50} . Therefore, in these cases, the ER_{50} value are reported as >500 mL GF-4021/ha.

Table 51: Observations Day 21 of % survival, % visual injury and shoot fresh weight (g): Monocotyledonous species

Treatment	Oat			Ryegrass		
	Survival	Visual injury	Shoot weight	Survival	Visual injury	Shoot weight
Control	100	0	11.3	100	0	8.12
31.25	100	0	12.0	100	0	8.52
62.5	100	0	13.3	100	0	7.87
125	100	0	12.5	100	0	8.25
250	100	0	12.8	100	5	7.90
500	100	0	11.9	100	50	5.82

Treatment	Onion		
	Survival	Visual injury	Shoot weight
Control	100	0	8.69
7.81	100	0	8.96
15.63	100	11	7.43
31.25	100	14	6.87
62.5	100	21	6.53
125	100	56	2.61
250	100	76	1.89
500	100	85	1.32

Table 52: Observations Day 21 of % survival, % visual injury and shoot fresh weight (g): Dicotyledonous species

Treatment	Oilseed rape			Sunflower		
	Survival	Visual injury	Shoot weight	Survival	Visual injury	Shoot weight
Control	100	0	48.0	100	0	68.6
31.25	100	0	48.7	100	31	64.0
62.5	100	10	51.5	100	42	54.8
125	100	31	48.1	100	62	34.5
250	100	39	47.8	100	77	13.2
500	100	46	45.6	100	88	7.70

Treatment	Soybean			Carrot			Tomato		
	Survival	Visual injury	Shoot weight	Survival	Visual injury	Shoot weight	Survival	Visual injury	Shoot weight
Control	100	0	12.8	100	0	14.0	100	0	111
0.49	100	2	12.7	100	0	13.3	100	11	98.8
0.98	100	8	11.2	100	0	13.8	100	22	83.1
1.95	100	29	10.5	100	0	15.0	100	41	64.0
3.91	100	43	9.48	100	1	12.6	100	51	44.4
7.81	100	53	9.01	100	25	10.7	100	62	28.6
15.63	100	61	7.93	95 86	64	6.44	100	73	16.3
31.25	100	65	5.73	95 86	77	2.20	100	76	11.3
62.5	95	79	4.44	81 43	84	1.07	95	84	6.22
125	15	99	3.06	90 71	92	0.800	95	90	3.80
250	0	100	-	81 43	95	0.668	80	95	2.01

– No result, all plants dead.

Treatment	Field bean		
	Survival	Visual injury	Shoot weight
Control	100	0	40.4
0.49	100	1	36.8
0.98	100	7	37.3
1.95	100	31	35.0
3.91	100	42	32.5
7.81	100	59	27.1
15.63	100	74	14.9
31.25	95	82	9.38
62.5	90	89	6.54
125	40	96	6.76
250	0	100	-

– No result, all plants dead.

Treatment	Onion			Sugar beet			Cucumber		
	Survival	Visual injury	Shoot weight	Survival	Visual injury	Shoot weight	Survival	Visual injury	Shoot weight
Control	100	0	8.69	100	0	68.1	100	0	104
7.81	100	0	8.96	100	1	70.9	100	45	81.3
15.63	100	11	7.43	100	27	62.4	100	53	68.7
31.25	100	14	6.87	100	50	45.8	100	57	57.3
62.5	100	21	6.53	100	65	24.6	100	61	48.9
125	100	56	2.61	100	81	9.81	100	74	28.5
250	100	76	1.89	100	87	6.10	70	89	15.7
500	100	85	1.32	95	91	4.39	0	100	-

– No result, all plants dead.

Table 53: Reported ER₅₀ values based on emergence, survival, phytotoxicity and shoot fresh weight (mL GF-4021/ha)

Species	Survival	Phytotoxicity	Shoot fresh weight
	ER ₅₀ (95% CL)	ER ₅₀ (95% CL)	ER ₅₀ (95% CL)
Onion	>500 (N/A)	121 (96.3 – 153)	93.0 (61.7 – 140)
Oat	>500 (N/A)	>500 (N/A)	>500 (N/A)
Ryegrass	>500 (N/A)	499 (494 – 505)	>500 (N/A)
Sugar beet	>500 (N/A)	35.0 (25.9 – 47.2)	45.0 (38.3 – 52.8)
Oilseed rape	>500 (N/A)	463 (197 – 1090)	>500 (N/A)
Cucumber	262 (262 – 262)	16.9 (8.64 – 31.4)	44.9 (37.6 – 53.3)
Carrot	>250 (N/A)	13.5 (10.8 – 16.8)	14.2 (12.1 – 16.6)
Soybean	95.6 (95.3 – 95.9)	7.47 (5.20 – 10.7)	24.4 (19.5 – 30.6)
Sunflower	>500 (N/A)	81.8 (70.3 – 94.8)	126 (109 – 144)
Tomato	>250 (N/A)	4.07 (3.25 – 5.11)	2.68 (2.47 – 2.92)
Field bean	112 (104 – 121)	5.30 (4.41 – 6.36)	12.8 (9.78 – 16.7)

N/A = Not applicable

Where 95% CL is reported, R₂ was >0.7

Regression models used:

Species	Survival	Phytotoxicity	Shoot fresh weight
Onion	N/A	3P Morgan-Mercer-Flodin	3P Log-Logistic
Oat	N/A	N/A	N/A
Ryegrass	N/A	3P Logistic	3P Cum Log-Normal (Probit)
Sugar beet	N/A	3P Morgan-Mercer-Flodin	3P Log-Logistic
Oilseed rape	N/A	3P Cum Log-Normal (Probit)	N/A
Cucumber	2P Cum Log-Normal (Probit)	3P Log-Gompertz	3P Log-Gompertz
Carrot	2P OECD Exponential #2	3P Log-Logistic	3P Cum Log-Normal (Probit)
Soybean	2P Cum Log-Normal (Probit)	3P Cum Log-Normal (Probit)	3P Log-Logistic
Sunflower	N/A	3P Log-Gompertz	3P Log-Logistic
Tomato	N/A	3P Morgan-Mercer-Flodin	3P Morgan-Mercer-Flodin
Field bean	2P Log-Gompertz	3P Cum Log-Normal (Probit)	3P Log-Logistic

N/A = Not applicable

CONCLUSION

Based on fresh weight and phytotoxicity the most sensitive species tested was tomato with an ER₅₀ value of 2.68 and 4.07 mL GF-4021/ha respectively. Based on survival the most sensitive species tested was soybean with an ER₅₀ values of 95.6 mL GF-4021/ha.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Tomato	<i>Lycopersicon esculentum</i>	GF-4021	N/A	Shoot fresh weight ER ₅₀	2.68	mL/ha

A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants

Not required to characterise the product in the current submission.

A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

No studies, other than those already evaluated during the EU Review of active substances halauxifen-methyl, picloram and aminopyralid, have been presented in support of this submission.

A 2.8 KCP 10.8 Monitoring data

Monitoring studies are not available for halauxifen-methyl, picloram and aminopyralid and are not considered necessary in light of the acceptable risk concluded for all non-target organisms from uses of GF-4021/ LaDiva.