

REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: GF-3969

Chemical active substances:

Rimsulfuron, 148.15 g/kg

Thifensulfuron methyl, 92.6 g/kg

Isoxadifen-ethyl, 111.1 g/kg

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorisation)

Applicant: Corteva/DuPont/DowAgroScience/Pioneer*

Submission date: February 2021

MS Finalisation date: December 2021 (initial Core Assessment)

August 2022 (final Core Assessment)

*Corteva Agriscience is new Legal Entity in most of EU countries and should be treated as an Applicant for GF-3969 registration. Information about Applicant for each country is provided in dRR Part A.

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Version history

When	What
February 2021	Submission of dRR by the Applicant
December 2021	Initial zRMS assessment The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are struck through and shaded for transparency .
August 2022	Final report (Core Assessment updated following the commenting period) Additional information/assessments included by the zRMS in the report in response to comments recieved from the cMS and the Applicant are highlighted in yellow. Information no longer relevant is struck through and shaded .

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9 Ecotoxicology (KCP 10)

Thifensulfuron methyl information belongs to FMC, and Corteva has Letter of Access.

Isoxadifen-ethyl as crop safener is not considered as an active substance, and consequently has not been subject to review on EU level for inclusion into Annex I of Directive 91/414/EEC or Regulation (EC) No 1107/2009. The ecotoxicological data of the safener isoxadifen-ethyl have been reviewed at Member State level by Germany (2002) and Greece (2016). The evaluation performed by Germany resulted in an evaluation report including a standard List of Endpoints. All exposure and risk assessments presented in this dRR are based on the country agreed endpoints, if not otherwise stated. Greece was the zRMS during the zonal review of tembotrione products which contained isoxadifen-ethyl as safener (dossier submission: October 2014). During the review, not all of the endpoints used for isoxadifen-ethyl could be found in the German national evaluation report. The studies which were not summarized in the German evaluation report for isoxadifen-ethyl but used in the ecotoxicological risk assessment of the above-mentioned formulated products were submitted by Bayer CropScience and evaluated by the zRMS (Greece).

Ecotoxicology endpoints for the active substances in GF-3969, rimsulfuron, and thifensulfuron methyl used in risk assessments are derived from the respective EFSA conclusions for these actives as indicated below.

For rimsulfuron: EFSA Scientific Report (2005) 45, 1-61. Conclusion regarding the peer review of the pesticide risk assessment of the active substance rimsulfuron. For thifensulfuron methyl: EFSA Journal 2015;13(7):4201. Conclusion on the peer review of the pesticide risk assessment of the active substance thifensulfuron methyl.

zRMS comments:

Evaluation was based on the active substance data as provided in the respective EFSA reports as well as on the basis of results of studies performed with the formulated product and submitted in support of this evaluation.

Thifensulfuron methyl is currently owned by FMC Corporation and a LoA has been issued indicating that the thifensulfuron methyl studies, data summaries and assessments owned and submitted by FMC or its affiliates may be referred to in the course of evaluation of GF-3969. It has to be, however, noted that the access is granted only to Corteva Agriscience Poland Sp. z o.o. and for this reason separate LoA has to be presented in case other subsidiary of Corteva Agriscience is applying for authorisation of GF-3969 in particular Member States.

As indicated by the Applicant above, GF-3969 contains safener, isoxadifen-ethyl, for which the risk assessment should be performed in a similar way as it is done for active compounds.

The toxicity data for isoxadifen-ethyl were evaluated at the national level by Germany (in 2002) and during zonal evaluations performed by Greece (in 2016). It should be, however, noted that in line with Regulation (EC) No 1107/2009, data for safener should be evaluated in line with requirements relevant for active substances in order to generate EU agreed endpoints. Such evaluation is, however, outside the scope of the product registration process and should be carried out at the EU level in order to derive uniform endpoints that may be used in evaluations performed for various formulations. As no EU agreed endpoints for isoxadifen-ethyl exist, no separate risk assessment has been performed for the safener by the zRMS. Instead, for purposes of authorisation of GF-3969, it was assumed that the toxicity endpoints derived from studies performed with the formulation include effects resulting from exposure to the safener, while the risk assessment based on exposure estimates and toxicity data for the formulated product covers potential risk resulting from exposure of non-target species to isoxadifen-ethyl. Respective risk assessment based on the toxicity data for isoxadifen-ethyl will be performed once EU agreed endpoints, derived in line with indications of current guidance documents, are available (these derived in 2002 are obsolete and would require complete re-evaluation, being outside the scope of the zonal assessment). It should be noted that this approach has been already accepted in the course of zonal evaluations of some formulations owned by the same Applicant (GF-3337 and GF-3313, both finalised in 2018).

Although the endpoints for isoxadifen-ethyl could not be confirmed, the approach in Applicants' risk assessment for this compound was checked and corrected when necessary. Nevertheless, the outcome is still not fully certain since the endpoints cannot be confirmed and for this reason the font colour has been changed to grey in parts of the report presenting data and calculations for the safener.

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use- No.	Member state(s)	Crop and/or situation (crop destination/ purpose of crop)	F, Fn, Fpn G, Gn, Gpn or [*]	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 of use a) per crop/ season b) per crop/ season	Min. interval between applications (days)	kg product/ha a) max. rate per appl. b) max. total rate per crop/season	g a.s./ha ^a 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																				
2-13	Zonal GAP envelope for CEU countries	Maize (ZEAMX) (silage & grain)	F	Annual monocotyledonous weeds (TTTMS), Annual dicotyledonous weeds (TTTDS), Perennial grass weeds (GGGPE)	Hydraulic sprayer overall	BBCH 11 to BBCH 18 Spring March-July	a) 1 b) 1	n.a. ^b	a) 0.135 b) 0.135	a) 32.5 (20 + 12.5) b) 32.5 (20 + 12.5)	100 / 400	n.a.	Safener: formulated product contains 111.1 g/kg isoxadifen-ethyl (max. 15 g/ha) Adjuvant: application with max. 0.2% DPX-KG691 or vegetable oil	A	A	R	A	A	A	R R.A. not finalised
15- 27	Zonal GAP envelope for CEU countries	Maize (ZEAMX) (silage & grain)	F	Annual monocotyledonous weeds (TTTMS), Annual dicotyledonous weeds (TTTDS), Perennial grass weeds (GGGPE)	Hydraulic sprayer overall	BBCH 11 to BBCH 18 Spring March-July	a) 2 b) 2	7	a) 0.135 b) 0.135	a) 32.5 (20 + 12.5) b) 32.5 (20 + 12.5)	100 / 400	n.a.	Safener: formulated product contains 111.1 g/kg isoxadifen-ethyl (max. 15 g/ha) Adjuvant: application with max. 0.2% DPX-KG691 or vegetable oil Split application possible without exceeding the total maximum of 135 g product/ha: 2 x 67.5 g product/ha for uses No 16, 17, 18, 20, 22, 23, 24, 25, 26, 27 85 + 50 g product/ha for uses No 15, 19, 21	A	A	R	A	A	A	R R.A. not finalised

- * 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
a Dose expressed as total g active substance (g rimsulfuron + g thifensulfuron methyl)
b n.a.- not applicable

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

zRMS comments:

Initially, the GAP table including detailed information on pests in particular cMS has been provided by the Applicant. However, pests are of no relevance for the ecotoxicological risk assessment and GAP table was thus shortened to provide critical GAP, which was considered in the risk assessment covering intended uses of GF-3969 in all concerned Member States. For detailed GAP for particular cMS, please refer to the Core Assessment, Part B, Section 0, where the use No indicated in column 1 were taken from.

9.1.1 Overall conclusions

zRMS comments:

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

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

Birds

Regulatory testing for birds has been conducted with rimsulfuron, thifensulfuron methyl and isoxadifen-ethyl in accordance with EU requirements. The risk to birds was assessed based on the maximum single application rate of 1×135 g GF-3969/ha as this is protective of all intended uses.

For each of the active substances, the calculated TER values exceeded the relevant acute and chronic trigger values at the screening step and Tier 1, and so acceptable risk can be concluded. The risk to birds from exposure via drinking water was assessed and an acceptable risk was concluded.

An assessment of the risks via secondary poisoning was not triggered for the active substances rimsulfuron and thifensulfuron methyl, as they have $\log K_{ow}$ values of <3 and the potential for bioaccumulation is considered to be low. For isoxadifen-ethyl the $\log K_{ow}$ of 3.8 exceeds the trigger value of 3, an assessment of the risk for secondary poisoning was conducted and shown to be acceptable.

As an acute study with birds is not available with the product GF-3969, therefore, acute combination toxicity assessment was conducted. None of the active substances was found to contribute to $>90\%$ of the mixture toxicity and, therefore, acute risk was assessed by deriving the TER between the predicted endpoint by the concentration addition model and the sum of application rates of active substances. The TER exceeded the relevant trigger value (10); therefore, acceptable risk was concluded.

The combined long-term risk was concluded to be low based on TER_{mix} exceeding the trigger of 5.

Calculations performed for isoxadifen-ethyl were presented for informative purposes only, since no EU agreed endpoints exist for this compound. In case the endpoints were confirmed at the EU level, acceptable acute and long-term risk from exposure to isoxadifen-ethyl would be concluded.

~~According to the Central Zone requirement, long term combination toxicity assessment was conducted. None of the active substances was found to contribute to $>90\%$ of the mixture toxicity and, therefore, long term risk was assessed by deriving the TER between the predicted endpoint by the concentration addition model and the sum of application rates of active substances. The TER exceeded the relevant trigger value (5); therefore, acceptable risk was concluded.~~

Mammals

Regulatory testing has been conducted with rimsulfuron, thifensulfuron methyl and isoxadifen-ethyl in accordance with EU requirements. The risk to mammals was assessed based on the maximum single application rate of 1×135 g GF-3969/ha as this is protective of all intended uses.

For each of the active substances, the calculated TER values exceeded the relevant acute and chronic trigger values at the screening step and Tier 1, and so acceptable risk can be concluded. The risk to mammals from exposure via drinking water was assessed and an acceptable risk was concluded.

An assessment of the risks via secondary poisoning was not triggered for the active substances rimsulfuron and thifensulfuron methyl, as they have log K_{ow} values of <3 the potential for bioaccumulation is considered to be low.

For isoxadifen-ethyl, the log K_{ow} of 3.8 exceeds the trigger value of 3. An assessment of the risk for secondary poisoning was conducted and shown to be acceptable.

An acute toxicity with GF-3969 has been conducted and reported the LD_{50} to be >2000 mg product/kg bw. The acute combination toxicity assessment was conducted. None of the active substances was found to contribute to >90% of the mixture toxicity and, therefore, acute risk was assessed by deriving the TER between the predicted endpoint by the concentration addition model and the sum of application rates of active substances. The TER exceeded the relevant trigger value (10), therefore, acceptable risk was concluded.

According to the Central Zone requirement, long-term combination toxicity assessment was conducted. None of the active substances was found to contribute to >90% of the mixture toxicity and, therefore, long-term risk was assessed by deriving the TER between the predicted endpoint by the concentration addition model and the sum of application rates of active substances. The TER exceeded the relevant trigger value (5), therefore, acceptable risk was concluded.

The combined long-term risk was concluded to be low based on TER_{mix} exceeding the trigger of 5.

Calculations performed for isoxadifen-ethyl were presented for informative purposes only, since no EU agreed endpoints exist for this compound. In case the endpoints were confirmed at the EU level, acceptable acute and long-term risk from exposure to isoxadifen-ethyl would be concluded.

~~No overt toxicity has been observed in any of the avian and mammalian studies relevant for the ecotoxicological risk assessment. In addition, low 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 applications of GF 3969 at rates up to and including 1×135 g/ha.~~

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

The maximum PEC_{sw} values resulted from the single application at a rate of 135 g GF-3969/ha (equivalent to a rate of 20 g rimsulfuron/ha, 12.5 g thifensulfuron methyl/ha and 15 g isoxadifen-ethyl/ha).

Based on this maximum exposure acceptable risk to all aquatic groups from isoxadifen-ethyl and its metabolites is shown at FOCUS Steps 1 and 2.

For rimsulfuron acceptable acute and chronic risk to fish, aquatic invertebrates and algae is shown at FOCUS Step 1.

For *Lemna gibba*, mitigation at FOCUS Step 4 is required to show acceptable risk for each of the uses. For the maximum application of 20 g rimsulfuron/ha, a 10-m buffer with 10 m vegetative filter strip is required to show acceptable risk in scenarios R1, R3 and R4. For remaining scenarios acceptable risk with no need for risk mitigation measures may be concluded.

An acceptable aquatic risk is concluded from the exposure to rimsulfuron metabolites at FOCUS Step 1 and 2.

For thifensulfuron methyl acceptable acute and chronic risk to fish, aquatic invertebrates, algae and sediment organisms is shown at FOCUS Step 1 and 2.

For aquatic plants a potential risk was triggered and so a refinement based on the agreed RMS geomean endpoint (from the review of confirmatory data) of 0.53 µg a.s./L was applied to the risk assessment. Acceptable risk could be concluded provided that following risk mitigation measures are respected, depending on the use pattern:

1. Single application at 1x135 g GF-3969/ha:

- scenarios D3, D4, D5, D6: 10 m unsprayed buffer zone to surface water bodies,
- scenario R2: 10 m VFS to surface water bodies,
- scenarios R1, R3 and R4: 20 m VFS (when based on indications of FOCUS L&M) or 10 m VFS (when VFSmod is used).

2. Split application at 2x67.5 g GF-3969/ha with 7 days interval:

- scenarios D3, D4, D5, D6: 10 m unsprayed buffer zone to surface water bodies,
- scenarios R1, R2, R3: 10 m VFS to surface water bodies,
- scenario R4: 20 m VFS (when based on indications of FOCUS L&M) or 10 m VFS (when VFSmod is used).

3. Split application at 85+50 g GF-3969/ha with 7 days interval:

- scenarios D3, D4, D5, D6: 10 m unsprayed buffer zone to surface water bodies,
- scenarios R1, R2, R4: 10 m VFS to surface water bodies,
- scenario R4: 20 m VFS (when based on indications of FOCUS L&M) or 10 m VFS (when VFSmod is used).

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

~~For the maximum single application rate of 1 × 12.5 g a.s./ha (equivalent to 1 × 135 g prod/ha) acceptable risk was shown with a 20 m buffer zone or a 10 m buffer with VFSmod. The same mitigation is required for the split application of 2 × 6.25 g a.s./ha (equivalent to 2 × 67.5 g prod/ha) to show acceptable risk. For the split application of 7.87 + 4.63 g a.s./ha (equivalent to 85 + 50 g prod/ha) acceptable risk is shown for all scenarios with a 10 m no-spray buffer zone with vegetated filter strip.~~

An acceptable aquatic risk is concluded from the exposure to thifensulfuron methyl metabolites at FOCUS Step 1 and 2.

~~Combined toxicity assessment for the active substances indicated the measured toxicity is comparable to predicted toxicity. For algae, potential antagonism (toxicity of the formulation is lower than expected) was identified however this can be explained by the fact that the algae endpoint for isoxadifen ethyl is a 'greater than' value.~~

~~The assessment of endpoints for fish, daphnia and algae met the requirement for calculated mixture toxicity to be used in the risk assessment of the product. *Lemna gibba* met the criteria for the measured product endpoints to be used in the risk assessment. Based on calculated endpoints, acceptable risk to fish, *Daphnia* and algae was concluded.~~

The combined toxicity assessment demonstrated that measured and estimated toxicity endpoints for *Lemna gibba* are comparable. For fish and *Daphnia magna* the formulated product was more toxic than predicted based on data for individual active substances and for this reason measured formulation endpoints were concluded to be relevant for the risk assessment purposes in case of these two groups of species.

For algae the estimated toxicity of the mixture was clearly lower than measured. Nevertheless, in case of algae the TU analysis demonstrated that thifensulfuron-methyl contributes at >90% to the toxicity of the mixture and hence no additional calculations were deemed necessary and risk assessment for this species based on active substance data was sufficient.

Bases on measured endpoints and calculated product PEC_{sw} values, an acceptable risk was concluded following the use of GF-3969 in maize at 135 g prod/ha with the inclusion of a ~~10~~ 20 m buffer zone. ~~This mitigation is in line with the required mitigation for thifensulfuron methyl.~~

9.1.1.3 Effects on bees (KCP 10.3.1)

Regulatory testing to assess the acute toxicity to bees has been conducted with rimsulfuron, thifensulfuron methyl, isoxadifen-ethyl and GF-3969 in accordance with EU requirements. HQ values for each of the active substances and product were calculated to be less than the trigger of 50, indicating acceptable risk to bees from acute oral and contact routes of exposure based on a single maximum application rate of 135 g GF-3969/ha to maize.

Since respective chronic and larvae toxicity studies performed with the formulation GF-3969 were provided by the Applicant during the commenting period, the risk assessment based on EFSA (2013) has been also performed. Acceptable acute oral and contact risk to adult bees as well as chronic risk to larvae from the intended uses of GF-3969 could be concluded already at the screening step. The chronic risk to adult bees was unacceptable at the screening step and Tier 1 evaluation was performed which resulted with acceptable chronic risk in field margin, adjacent crop and next crop scenarios. However, ETR values calculated for the treated crop and weeds scenarios were above the respective triggers indicating potentially unacceptable risk. This issue will have to be dealt with at the product authorisation by the cMS that consider indications of EFSA (2013) at the national level, since at the zonal level the risk assessment performed in line with EFSA (2013) is indicative only until the guidance is noted at the EU level.

~~Studies have been conducted with rimsulfuron to assess the chronic toxicity to adult bees and to assess acute and chronic toxicity to bee larva. The data from the chronic adult testing and larva testing were used in the risk assessment, the TERs were above the trigger of 1 indicating acceptable risk to adult bees and larvae based on a single maximum application rate of 135 g GF-3969/ha to maize.~~

~~Regulatory testing is being conducted with the product to assess the chronic toxicity to honey bee larvae and adults and the studies will be provided as soon as possible.~~

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

Regulatory testing has been conducted with the product. The Tier I laboratory studies showed acceptable in-field and off-field effects for *T. pyri* and *A. rhopalosiphi* from applications of GF-3969 according to the maximum exposure 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)

The risk to earthworms and other soil organisms was assessed using the toxicity exposure ratios (TERs) between the toxicity endpoints for GF-3969, rimsulfuron, thifensulfuron methyl, isoxadifen-ethyl and relevant metabolites, and the maximum PEC_{soil} or $PEC_{accumulation}$ resulting from the single application rate of 1×135 g product/ha. For each of the active substances and metabolites the ~~acute~~ and chronic TER values were greater than the trigger of 5 ~~and 10~~, indicating acceptable risk to non-target soil macro-organisms following use of GF-3969 according to the proposed use pattern. A low toxicity of the product to soil organisms was shown and acceptable risk concluded based on maximum predicted exposure.

The risk of GF-3969, the active substances and relevant metabolites to soil micro-organisms was evaluated by comparison of the reported concentrations with effects <25% derived from laboratory tests, with maximum initial PEC_{soil} or $PEC_{accumulation}$ based on the highest single application rate of 135 g product/ha. No significant effects of >25% effect were reported at soil concentrations where

exceeded the relevant PEC_{soil} values, indicating that the risk to soil micro-organisms is acceptable following the use of GF-3969 according to the proposed use pattern.

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

Regulatory testing has been conducted with the product, GF-3969 to assess effects on vegetative vigour and seedling emergence. The seedling emergence study was accepted by the zRMS with no concerns, but the vegetative vigour study was agreed after exclusion of control replicates of oilseed rape and sorghum which exhibited phytotoxic effects and recalculation of endpoints for these two species. The risk assessment was performed using deterministic and probabilistic approach. Overall, acceptable risk to non-target terrestrial plants could be concluded from the intended uses of GF-3969, provided that following risk mitigation measures are respected:

1. Deterministic 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
- 90% drift reduction.

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

~~invalidated due to phytotoxic effects observed in control replicates and their potential impact on growth parameters of control plants at the test termination and in consequence on the endpoints calculated for the test item groups.~~

~~Since no other data exist, the risk assessment for non target plants could not be finalised and no final conclusion may be taken.~~

~~Based on the probabilistic risk assessment for vegetative vigour effects, taking into account the 5th percentile ER_{50} derived from the SSD for effects on vegetative vigour, an acceptable risk to terrestrial non target plants can be concluded following uses of GF 3969 with~~

- ~~• 1 m buffer with 75% drift reducing technology,~~
- ~~• 5 m buffer with no drift reducing technology~~

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

Not relevant.

9.1.2 Grouping of intended uses for risk assessment

The grouping of the intended uses to support application has been done based on a risk envelope approach (according to SANCO/11244/2011).

Use	Application rate (g a.s./ha)	Application method	Number of applications	Minimum application interval (days)	Application timing
Maize	20 g rimsulfuron 12.5 g thifensulfuron methyl 15 g isoxadifen-ethyl	Hydraulic sprayer overall	1 application	N/A	BBCH 11-18
Maize	10/10 g rimsulfuron 6.25/6.25 g thifensulfuron methyl 7.5/7.5 g isoxadifen-ethyl	Hydraulic sprayer overall	2 applications	7	BBCH 11-18
Maize	12.59/7.41 g rimsulfuron 7.87/4.63 g thifensulfuron methyl 9.44/5.56 g isoxadifen-ethyl	Hydraulic sprayer overall	2 applications	7	BBCH 11-18

In this assessment, concentrations of rimsulfuron and thifensulfuron methyl, the active substances contained in GF-3969, in various environmental compartments, are predicted following the proposed use pattern for GF-3969. The predicted environmental concentrations (PEC values) in soil, surface water, sediment, groundwater, and air are provided. The long-term concentrations are based on results obtained for the active substance contained in the formulation.

zRMS comments:

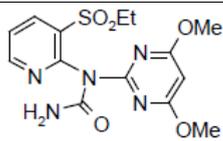
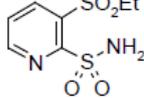
Grouping of intended uses provided in table above is agreed by the zRMS. For split application at 2x67.5 g product/ha additional information on the active substance application rate at second treatment has been provided for clarity and in order to comply with information provided for split application at 85+50 g product/ha.

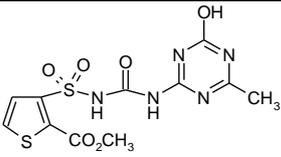
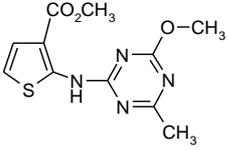
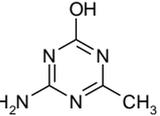
9.1.3 Consideration of metabolites

A list of metabolites found in environmental compartments is provided below. The need for conducting a metabolite-specific risk assessment in the context of the evaluation of GF-3969 is indicated in the table below for a re-evaluation of relevance.

Rimsulfuron

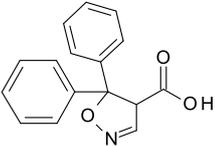
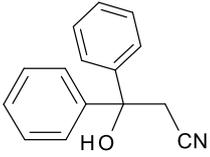
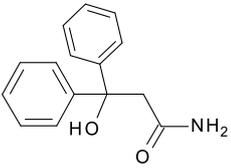
Table 9.1-2: Metabolites of rimsulfuron potentially relevant for exposure assessment

Metabolite	Molar weight (g/mol)	Chemical structure	Maximum observed occurrence in compartments (%)	Risk assessment required due to
IN-70941	367.4		Soil: 54.5% aerobic Total water/sediment system: 87.2% Air: 0%	PEC _{soil} , PEC _{sw/sed}
IN-70942	324.36		Soil: 23.5% aerobic; Total water/sediment system: Hydrolysis in aquatic systems: 83.8% (pH 7) Air: 0%	PEC _{soil} , PEC _{sw/sed}
IN-E9260	250.3		Soil: 18.9% Total water/sediment system: Photolysis in aquatic systems: 16.2% Air: 0%	PEC _{soil} , PEC _{sw/sed}

Metabolite	Molar weight (g/mol)	Chemical structure	Maximum observed occurrence in compartments (%)	Risk assessment required due to
IN-L9226	373.4		Soil: 18.5% aerobic Total Water/sediment system: 7.8% (water); 7.2% (sediment)	PEC _{soil} , PEC _{sw/seed}
IN-D8858	280.3		Aqueous photolysis: 15.3%	PEC _{sw/seed}
IN-B5528	126.1		Hydrolysis: 25.3% (pH 4), not formed at pH 7	PEC _{sw/seed}

Isxadifen-ethyl (safener)

Table 9.1-4: Metabolites of isxadifen-ethyl potentially relevant for exposure assessment

Metabolite	Molar mass	Chemical structure	Maximum observed occurrence in compartments	Risk assessment required due to
Isxadifen-ethyl AE F129431	267.28		Soil: 92.8% (aerobic), 81.6% (anaerobic) Soil (photolysis): 90.9% Abiotic hydrolysis: 98.5% Water: 52.4-87.1% considered for modelling: 86.0% Sediment: n.d.- 26.5% considered for modelling: 25.1% Water/sediment: 93.5%	PEC _{soil} , PEC _{sw/seed}
AE C637375	223.27		Soil: 6.8% (aerobic), 88.2% (anaerobic) Water: 6.3-13.6% considered for modelling: 11.8% Sediment: 15.9-37.2% considered for modelling: 33.8% Water/sediment: 40.1%	PEC _{sw/seed}
AE C642961	241.28		Soil: 3.1% (aerobic), 3.8% (anaerobic) Water: 5.7-11.2% considered for modelling: 9.2% Sediment: n.d.-17.5% considered for modelling: 16.8% Water/sediment: 20.9%	PEC _{sw/seed}

zRMS comments:

Information regarding metabolites of rimsulfuron and thifensulfuron-methyl is in general in line with EU agreed endpoints reported in EFSA Scientific Report (2005) 45 and EFSA Journal 2015;13(7):4201, respectively. Some corrections were introduced by the zRMS so information in tables above is fully in line with data reported in the list of endpoints.

No EU agreed data exist for the safener, isxadifen-ethyl, and for this reason validation of information provided in Table 9.1-4 against EU agreed endpoints was not possible. Nevertheless, data provided by the Applicant for isxadifen-ethyl have been retained for informative purposes, with font colour changed to grey in order to easily distinguish validated from non-validated data.

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with rimsulfuron, thifensulfuron methyl and isoxadifen-ethyl. 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 birds of GF-3969 were not evaluated as part of the EU assessment of rimsulfuron and thifensulfuron methyl. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

Species	Substance	Exposure System	Results	Reference
<i>Colinus virginianus</i>	Rimsulfuron	Oral 1 d, Acute	LD₅₀ >2250 mg a.s./kg bw	EFSA 2005 ¹ Grimes, J., Jaber, M., 1988 (HLO 797-88)
<i>Anas platyrhynchos</i>	Rimsulfuron	Oral 1 d, Acute	LD ₅₀ >2250 mg a.s./kg bw	EFSA 2005 (Author not available, 1990, HLO 363-90)
Short-term dietary toxicity to birds				
<i>Colinus virginianus</i>	Rimsulfuron	Dietary 8 d, Short-term	LC ₅₀ >5620 mg a.s./kg feed NOEC = 5620 mg a.s./kg feed	EFSA 2005 Grimes, J., Jaber, M., 1989a (HLO 16-89)
<i>Anas platyrhynchos</i>	Rimsulfuron	Dietary 8 d, Short-term	LC ₅₀ >5620 mg a.s./kg feed NOEC = 5620 mg a.s./kg feed	EFSA 2005 Grimes, J., Jaber, M., 1989b (HLO 17-89)
<i>Anas platyrhynchos</i>	Rimsulfuron	Dietary 8 d, Short-term	LD ₅₀ >1610 mg a.s./kg bw/d	EFSA 2005 Author not available, 1990 (HLO 363-90)
Chronic and reproductive toxicity to birds				
<i>Colinus virginianus</i>	Rimsulfuron	Dietary Reproductive toxicity	NOAEL = 1250 mg a.s./kg bw/d (reproduction)	EFSA 2005 Beavers, J.B. <i>et al.</i> , 1994 (HLO 257-94)
<i>Anas platyrhynchos</i>	Rimsulfuron	Dietary Reproductive toxicity	NOAEL = 1250 mg a.s./kg bw/d (reproduction)	EFSA 2005 Frey, L.T. <i>et al.</i> , 1996 (AMR 3553-95)
<i>Colinus virginianus</i>	Rimsulfuron	Dietary Reproductive toxicity	NOAED = 142 mg a.s./kg bw/d (reproduction)	EFSA 2005 Beavers, J.B. <i>et al.</i> , 1994 (HLO 257-94)

Bold values are used in the 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 rimsulfuron, the extrapolated LD₅₀/10 is 377.6 mg a.s./kg bw/d which is not lower than the reproductive measured NOAED 142 mg a.s./kg bw/d, therefore, the NOAED is used in the long-term risk assessment.

¹ European Food Safety Authority; Conclusion on the peer review of the pesticide risk assessment of the active substance rimsulfuron. EFSA Journal 2005; 45, 1-61. Available online: www.efsa.europa.eu/efsajournal.htm

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

Species	Substance	Exposure System	Results	Reference
<i>Anas platyrhynchos</i>	Thifensulfuron methyl	Oral 1 d, Acute	LD₅₀ = 4739 mg a.s./kg bw (extrapolated) LD ₅₀ >2510 mg a.s./kg bw	EFSA 2015 ² (HLO 125-84)
<i>Colinus virginianus</i>	Thifensulfuron methyl	Dietary 8 d, Short-term	LDD ₅₀ >1524 mg a.s./kg bw/d LC ₅₀ >5620 mg a.s./kg feed	EFSA 2015 (HLO 31-84)
<i>Anas platyrhynchos</i>	Thifensulfuron methyl	Dietary 8 d, Short-term	LDD ₅₀ >1306 mg a.s./kg bw/d LC ₅₀ >5620 mg a.s./kg feed	EFSA 2015 (HLO 30-84)
<i>Anas platyrhynchos</i>	Thifensulfuron methyl	Dietary Reproductive toxicity	NOAEL = 172 mg a.s./kg bw/d (reproduction) NOAEC = 1250 mg a.s./kg feed	EFSA 2015 (HLO 410-94)
<i>Colinus virginianus</i>	Thifensulfuron methyl	Dietary Reproductive toxicity	NOAEL = 23 mg a.s./kg bw/d (reproduction) NOAEC = 250 mg a.s./kg feed	EFSA 2015 (HLO 411-94)

Bold values are used in the risk assessment.

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.

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 thifensulfuron methyl, the LD₅₀/10 is 473.9 mg a.s./kg bw/d which is not lower than the reproductive measured NOAEL 23 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 – isoxadifen-ethyl

Species	Substance	Exposure System	Results	Reference
<i>Colinus virginianus</i>	Isoxadifen-ethyl	Oral 1 d, Acute	LD₅₀ >3776 mg a.s./kg bw (extrapolated) NOLED ≥2000 mg a.s./kg bw	Zonal evaluation by zRMS Greece (2016)
<i>Anas platyrhynchos</i>	Isoxadifen-ethyl	Oral 1 d, Acute	LD ₅₀ >3776 mg a.s./kg bw (extrapolated) NOLED ≥2000 mg a.s./kg bw	Zonal evaluation by zRMS Greece (2016)
Short-term dietary toxicity to birds				
<i>Colinus virginianus</i>	Isoxadifen-ethyl	Dietary 5 d, Short-term	NOLEC ≥5000 ppm NOLED ≥980 g a.s./kg bw	Zonal evaluation by zRMS Greece (2016)
<i>Anas platyrhynchos</i>	Isoxadifen-ethyl	Dietary 5 d, Short-term	NOLEC ≥5000 ppm NOLED ≥1675 g a.s./kg bw	Zonal evaluation by zRMS Greece (2016)
Chronic and reproductive toxicity to birds				
<i>Anas platyrhynchos</i>	Isoxadifen-ethyl	Dietary, 21 weeks Reproductive toxicity	NOEC = 200 ppm NOEL = 22.4 mg a.s./kg bw	Zonal evaluation by zRMS Greece (2016)

Bold values are used in the risk assessment. NOLED = no observed lethal effect dose.

² European Food Safety Authority; Conclusion on the peer review of the pesticide risk assessment of the active substance thifensulfuron-methyl. EFSA Journal 2015;13(7):4201, 144 pp. doi:10.2903/j.efsa.2015.4201. Available online: www.efsa.europa.eu/efsajournal.htm

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. Therefore, the endpoint for isoxadifen-ethyl was extrapolated from >2000 mg a.s./kg bw to 3776 mg a.s./kg bw by applying a factor of 1.888 (10 birds were tested at the limit dose and no mortalities occurred).

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 isoxadifen-ethyl, the LD₅₀/10 is 377.6 mg a.s./kg bw/d which is not lower than the reproductive measured NOEL 22.4 mg a.s./kg bw/d, therefore, the NOAED is used in the long-term risk assessment.

zRMS comments:

Avian toxicity data for rimsulfuron and thifensulfuron-methyl are in line with EU agreed endpoints reported in EFSA Scientific Report (2005) 45 and EFSA Journal 2015;13(7):4201, respectively.

No EU agreed data exist for the safener, isoxadifen-ethyl, and for this reason validation of information provided in Table 9.2-3 against EU agreed endpoints was not possible. Nevertheless, data provided by the Applicant for isoxadifen-ethyl have been retained for informative purposes, with font colour changed to grey in order to easily distinguish validated from non-validated data.

9.2.1.1 Justification for new endpoints

No new endpoints. The effects of GF-3969 on birds has not been measured experimentally to minimise vertebrate testing. A predicted toxicity value for the formulation has been derived based on the toxicity data available on the active substances. The combined risk assessment is presented in the following section.

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/2009/1438).

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group 1× application to maize at 135 g product/ha also covers the risk for birds from all other intended uses.

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

The results of the acute and reproductive screening risk assessments are summarised in the following tables.

The acute 'daily dietary dose' (DDD) is calculated by multiplying the shortcut value (SV) based on the 90th percentile residues by the application rate in kg a.s./ha and the multiple application factor for 90th percentile residue data (MAF₉₀).

$$\text{DDD} = \text{application rate (kg a.s./ha)} \times \text{MAF}_{90} \times \text{SV}$$

The long-term 'daily dietary dose' (DDD) is calculated by multiplying the shortcut value (SV) based on the mean residues by the application rate in kg a.s./ha, the appropriate multiple application factor (MAF_M), and a time weighted average residue exposure (f_{TWA}). The f_{TWA} based upon a default DT₅₀ of 10 days is 0.53, as given in EFSA/2009/1438.

$$\text{DDD} = \text{application rate (kg a.s./ha)} \times \text{SV} \times f_{\text{TWA}} \times \text{MAF}_{\text{M}}$$

The TER_a and TER_{lt} values are calculated by dividing the acute and chronic toxicity endpoint by the respective daily dietary dose. The results of the acute and reproductive screening risk assessments are summarised in the following tables.

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.

Table 9.2-4: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of GF-3969 in maize – rimsulfuron

Intended use		Maize				
Active substance		Rimsulfuron				
Application rate (g/ha)		1 × 20 g a.s./ha				
Acute toxicity (mg/kg bw)		>2250				
TER criterion		10				
Crop scenario	Indicator species for screening	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a	
Screening	Small omnivorous bird	158.8	1	3.18	708	
Reprod. toxicity (mg/kg bw/d)		142				
TER criterion		5				
Crop scenario	Indicator species for screening	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}	
Screening	Small omnivorous bird	64.8	1 × 0.53	0.69	207	

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 9.2-5: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of GF-3969 in maize – thifensulfuron methyl

Intended use		Maize				
Active substance/product		Thifensulfuron methyl				
Application rate (g/ha)		1 × 12.5 g a.s./ha				
Acute toxicity (mg/kg bw)		4739				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a	
Screening	Small omnivorous bird	158.8	1	1.99	2381	
Reprod. toxicity (mg/kg bw/d)		23				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}	
Screening	Small omnivorous bird	64.8	1 × 0.53	0.43	54	

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

Table 9.2-6: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of GF-3969 in maize – isoxadifen-ethyl

Intended use		Maize				
Active substance		Isoxadifen-ethyl				
Application rate (g/ha)		1 × 15 g a.s./ha				
Acute toxicity (mg/kg bw)		3776				
TER criterion		10				
Crop scenario	Indicator species for screening	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a	
Screening	Small omnivorous bird	158.8	1	2.38	1587	
Reprod. toxicity (mg/kg bw/d)		22.4				
TER criterion		5				
Crop scenario	Indicator species for screening	SV_m	MAF_m TWA ×	DDD_m (mg/kg bw/d)	TER_{lt}	
Screening	Small omnivorous bird	64.8	1 × 0.53	0.52	44	

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

zRMS comments:

The risk assessment performed for rimsulfuron and thifensulfuron-methyl in tables above is agreed by the zRMS. Evaluation was performed considering single application of both compound, covering also split applications. On the basis of performed calculations acceptable acute and long-term dietary risk from exposure of birds to both active compounds may be concluded.

In absence of the EU agreed avian toxicity data for isoxadifen-ethyl, validation of calculations presented in Table 9.2-6 was not possible. Nevertheless, performed calculations have been retained for informative purposes, with font colour changed to grey in order to easily distinguish validated from non-validated data. In case the endpoints were confirmed at the EU level, acceptable risk from exposure to isoxadifen-ethyl would be concluded.

Combination effects of rimsulfuron, thifensulfuron methyl and isoxadifen-ethyl in GF-3969

An acute oral toxicity study for birds exposed to GF-3969 is not available, so 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) = fraction of active substance in the mixture (sum must be 1)

LD₅₀ (a.s._i) = LD₅₀ for acute toxicity of active substances

The values used for the calculation of acute combination toxicity effects are the following:

Table 9.2-7: Combination toxicity endpoints of rimsulfuron, thifensulfuron methyl and isoxadifen-ethyl calculated from active substances toxicity endpoints of birds

	Rimsulfuron	Thifensulfuron methyl	Isoxadifen-ethyl
Content in the formulation GF-3969 (% w/w)	14.82%	9.26%	11.11%
Fraction in mixture	42.11%	26.31%	31.57%
LD ₅₀ of a.s. [mg/kg bw]	>2250	4739	3776 ^a
Fraction / LD ₅₀	0.00019	0.00006	0.00008
Σ (Fraction / LD ₅₀)	0.00032		
1/ sum = predicted LD ₅₀ (mix)	3064		
Contribution of the active to predicted toxicity	57.36%	17.02%	25.62%

a Extrapolated value

According to EFSA/2009/1438, Appendix B, when an active substance is found to contribute to $\geq 90\%$ of the toxicity according to the combination toxicity assessment the risk assessment can be performed for the most toxic active substance alone. In this case, none of the active substance contribute to $>90\%$ of the toxicity and, therefore, the calculated mixture toxicity is used in the risk assessment with the exposure estimate as the sum of the application rates of the 3 actives (0.0475 kg/ha).

~~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 9.2-8: Combination toxicity endpoints of rimsulfuron, thifensulfuron methyl and isoxadifen-ethyl calculated from active substances toxicity endpoints of birds**~~

	Rimsulfuron	Thifensulfuron methyl	Isoxadifen-ethyl
Content in the formulation GF-3969 (% w/w)	14.82%	9.26%	11.11%
Fraction in mixture	42.11%	26.31%	31.57%
NOED of a.s. [mg/kg bw]	142.00	23	22.40
Fraction / NOED	0.00297	0.01144	0.01409
Σ (Fraction / NOED)	0.0285		
1/ sum = predicted NOED (mix)	35.09		
Contribution of the active to predicted toxicity	10.41%	40.14%	49.45%

~~According to EFSA/2009/1438, Appendix B, when none of the active substances are found to contribute to $\geq 90\%$ of the toxicity according to the combination toxicity assessment, the risk assessment should be performed based on the predicted NOEL (mix) and the sum of the application rates of the 3 actives (0.0475 kg/ha).~~

Table 9.2-9: First-tier assessment of the long-term/reproductive risk for birds due to the use of GF-3969 in maize – combination assessment

Intended use		Maize				
Active substance		Total of rimsulfuron, thifensulfuron methyl and isoxadifen-ethyl				
Application rate (g/ha)		1 × 47.5 g a.s./ha ^a				
Acute toxicity (mg/kg bw)		3064 (predicted)				
TER criterion		10				
Crop scenario	Indicator species for screening	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a	
Screening	Small omnivorous bird	158.8	1	7.54	406	
Reprod. toxicity (mg/kg bw/d)		35.09 (predicted)				
TER criterion		5				
Crop scenario	Indicator species for screening	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{tt}	
Screening	Small omnivorous bird	64.8	1 × 0.53	1.63	22	

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

a sum of application rates of rimsulfuron, thifensulfuron methyl and isoxadifen-ethyl.

All TERs (acute and long-term) exceed the relevant trigger values for rimsulfuron, thifensulfuron methyl, isoxadifen-ethyl and the combination of all the active substances; therefore, an acceptable risk from uses of GF-3969 in maize is concluded.

zRMS comments:

Since no EU agreed data exist for isoxadifen-ethyl, validation of combined toxicity assessment performed above was not possible. Nevertheless, performed calculations of the acute combined toxicity were retained for illustrative purposes, with font colour changed to grey in order to easily distinguish validated from non-validated data.

In case of the combined risk assessment for the active compounds, the LD_{50mix} of 2811 mg/kg bw may be calculated considering the endpoints of >2250 and 4739 mg/kg bw for rimsulfuron and thifensulfuron-methyl, respectively and their fraction in the formulation (0.62 and 0.38). The acute combined risk assessment for the active compounds is provided below.

Intended use		Maize				
Active substance		Total of rimsulfuron and thifensulfuron methyl				
Application rate (g/ha)		1 × 32.5 g a.s./ha (sum of a.s.)				
Acute toxicity (mg/kg bw)		2811 (predicted on the basis of acute toxicity of rimsulfuron and thifensulfuron-methyl)				
TER criterion		10				
Crop scenario	Indicator species for screening	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a	
Screening	Small omnivorous bird	158.8	1	5.2	541	

With regard to the long-term risk it should be noted that it is not appropriate to calculate the surrogate endpoint based on long-term toxicity of particular compounds, since they may be based on different parameters. Instead, the combined long-term risk assessment should be performed by calculation of the TER_{mix}. Alternatively, the long-term risk assessment may be performed using the lowest available long-term endpoint together with the application rate expressed as the sum of active compounds. The calculations performed by the Applicant above has been struck through as being not correct and TER_{mix} approach has been presented below.

Crop scenario and/or indicator species		TER _{LT} rimsulfuron	TER _{LT} thifensulfuron-methyl	TER _{LTmix}	Trigger	
Reproductive (screening step)						
Maize	Small omnivorous bird	207	54	42.8	5	
Overall, based on the above calculations, acceptable acute and long-term combined dietary risk to birds may be concluded from the intended uses of GF-3969.						
In case endpoints for isoxadifen-ethyl were confirmed at the EU level, following TER _{LTmix} would be calculated:						
Crop scenario and/or indicator species		TER _{LT} rimsulfuron	TER _{LT} thifensulfuron-methyl	TER _{LT} isoxadifen-ethyl	TER _{LTmix}	Trigger
Reproductive (screening step)						
Maize	Small omnivorous bird	207	54	44	21.7	5
Above calculations indicate that in case endpoints for isoxadifen-ethyl were confirmed at the EU level, acceptable acute and long-term combined dietary risk to birds would be concluded from the exposure to active compounds and the safener.						

9.2.2.2 Higher-tier risk assessment

Not required, since an acceptable risk was demonstrated in the first-tier risk assessment.

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-3969 is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not have to be considered.

Puddle scenario

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

The effective application rate is calculated according to the following formula:

$$AR_{eff} = AR \times MAF_m$$

Where:

- AR is the application rate (g a.s./ha).
- MAF_m is the Multiple Application Factor based on the DT₅₀ in soil (single first order kinetics, geometric mean). In case of single applications, MAF_m = 1.

The ratio of effective application rate to relevant endpoint was calculated according to the following formula:

$$HQ = \frac{AR(g/ha)}{LD_{50}(mg\ a.s./kg\ bw/day)}$$

Rimsulfuron

With a $K_{(f)oc}$ of 63 (range 19-63 L/kg, EFSA Scientific Report (2005) 45, 1-61), rimsulfuron belongs to the group of less sorptive substances.

Effective application rate (g/ha)	=	1 × 20 g a.s./ha			Trigger
Acute toxicity (mg/kg bw)	=	>2250	quotient =	0.008	50
Reprod. toxicity (mg/kg bw/d)	=	142	quotient =	0.14	50

Thifensulfuron methyl

With a $K_{(f)oc}$ of 9 (range 3.1-86 L/kg, EFSA Journal 2015;13(7):4201), thifensulfuron methyl belongs to the group of less sorptive substances.

Effective application rate (g/ha)	=	1 × 12.5 g a.s./ha			Trigger
Acute toxicity (mg/kg bw)	=	4739	quotient =	0.0026	50
Reprod. toxicity (mg/kg bw/d)	=	23	quotient =	0.54	50

Isoxadifen-ethyl

With a $K_{(f)oc}$ of 727 (Zonal evaluation by zRMS Greece (2016)) isoxadifen-ethyl belongs to the group of more sorptive substances.

Effective application rate (g/ha)	=	1 × 15 g a.s./ha			Trigger
Acute toxicity (mg/kg bw)	=	3776	quotient =	0.004	3000
Reprod. toxicity (mg/kg bw/d)	=	22.4	quotient =	0.7	3000

Since the ratios of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) do not exceed the critical value of 50 or 3000 for any of the active substances, a quantitative risk assessment (calculation of TER values) due to uptake of contaminated drinking water is not necessary as a low risk from uptake via drinking water can be concluded.

zRMS comments:

Drinking water risk assessment performed above for rimsulfuron and thifensulfuron-methyl is agreed by the zRMS.

In absence of the EU agreed avian toxicity data for isoxadifen-ethyl, validation of drinking water risk assessment for this compound was not possible. Nevertheless, calculations provided above have been retained for informative purposes, with font colour changed to grey in order to easily distinguish validated from non-validated data. In case the endpoints were confirmed at the EU level, acceptable risk from exposure via drinking water would be concluded.

No calculations were provided by the Applicant for the pertinent soil metabolites of both active compounds. However, the risk would be acceptable since the maximum ratio for metabolites based on the worst case assumptions (10 times toxicity of the parent and parent exposure) would be <50 (worst case trigger assumed, covering also risk from less sorptive metabolites) for the acute and long-term risk. Hence, no further evaluation has been performed.

9.2.2.1 Effects of secondary poisoning

The log K_{ow} values of rimsulfuron and thifensulfuron methyl are -1.46 (at pH 7) and -1.65 (at pH 7), respectively; thus, they do not exceed the trigger value of 3. A risk assessment for effects due to

secondary poisoning is not required for rimsulfuron and thifensulfuron methyl as the K_{ow} values indicate a low potential for bioaccumulation.

As the $\log K_{ow}$ of isoxadifen-ethyl ($\log K_{ow} = 3.8$) exceeds the trigger value of 3, an assessment of the risk for secondary poisoning is required. However, to note, isoxadifen-ethyl is rapidly decomposed in environmental matrices, resulting in products of high polarity. The half-life of isoxadifen-ethyl ranges from <0.1 days to 3.6 days in soil, or from 0.2 to 1.5 days in water/sediment systems. The abiotic hydrolysis DT_{50} at pH 7 was 2.3 days. The predominant breakdown product is the carboxylic acid AE F129431, $\log P_{ow}$ of which ranges from -0.18 (pH 4) to -1.77 (pH 10). Under neutral to alkaline conditions of pH AE F129431 is quantitatively deprotonated and thus in an ionic form. Consequently, a negative value can be estimated for the logarithmic value of the octanol/water coefficient. Prolonged exposure to the parent compound at relevant environmental pH values is therefore unlikely, and based on the characteristics of the degradation products accumulation of AE F129431, AE C637375 and AE C642961 will not occur. Therefore, a fish bioaccumulation study with either isoxadifen-ethyl or its metabolites is not justified, and the risk of secondary poisoning is deemed very low.

To further quantify the risk, a calculation is nonetheless provided for risk assessment for earthworm and fish-eating birds via secondary poisoning has been provided based on a calculated BCF for fish and earthworms.

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 isoxadifen-ethyl via bioaccumulation in earthworms (secondary poisoning) for the intended use in maize.

Parameter	Isoxadifen-ethyl	Comments
PEC _{soil} (21d TWA) (mg/kg soil)	0.002	See Part B, Section 8
Log K_{ow} / K_{ow}	3.8 / 6309.6	Zonal evaluation by zRMS Greece (2016)
K_{oc}	727	Zonal evaluation by zRMS Greece (2016)
f_{oc}	0.02	Default value, EFSA Journal 2009; 7(12):1438
BCF _{worm}	52.13	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times K_{ow}) / f_{oc} \times K_{oc}$
PEC _{worm}	0.10426	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.1095	$DDD = PEC_{worm} \times 1.046$
NOEL (mg/kg bw/d)	22.4	Zonal evaluation by zRMS Greece (2016)
TER _t	205	

The TER_t for the assessment of the risk for earthworm-eating birds due to isoxadifen-ethyl exposure via bioaccumulation in earthworms greatly exceeds the relevant trigger TER value of 5, indicating acceptable risk to birds following applications of isoxadifen-ethyl to maize.

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 isoxadifen-ethyl via bioaccumulation in fish (secondary poisoning) for the intended use in maize

Parameter	Isoxadifen-ethyl	Comments
PEC _{sw} (21 day TWA) (mg/L)	0.000006	See Part B, Section 8
BCF _{fish}	149.4	Calculated based the measured log K _{ow} = 3.8 and SMILES code C1(C(OCC)=O)=NOC(C1)(c1ccccc1)c1ccccc1 using EPIWeb 4.1, BCFBAF version 3.01.
BMF	N/A	Biomagnification factor (relevant for BCF ≥2000)
PEC _{fish}	0.00090	PEC _{fish} = PEC _{water} × BCF _{fish} × TWA
Daily dietary dose (mg/kg bw/d)	0.00014	DDD = PEC _{fish} × 0.159
NOEL (mg/kg bw/d)	22.4	Zonal evaluation by zRMS Greece (2016)
TER _{it}	160000	

The TER_{it} for the assessment of the risk for fish-eating birds due to isoxadifen-ethyl exposure via bioaccumulation in fish does not fall below the relevant trigger TER value of 5, indicating acceptable risk to birds following applications of isoxadifen-ethyl in maize.

This assessment confirmed the low expected risk of secondary poisoning from isoxadifen-ethyl based on the rapid degradation of the substance and metabolites.

zRMS comments:

The evaluation of the risk of secondary poisoning was not triggered for rimsulfuron, thifensulfuron-methyl and their relevant metabolites due to log Pow values being al <3.

In absence of the EU agreed avian toxicity data for isoxadifen-ethyl, validation of the evaluation of the risk of secondary poisoning for this compound was not possible. Nevertheless, calculations provided above have been retained for informative purposes, with font colour changed to grey in order to easily distinguish validated from non-validated data. In case the endpoints were confirmed at the EU level, acceptable risk of secondary poisoning would be concluded.

9.2.2.2 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 for birds has been conducted with rimsulfuron, thifensulfuron methyl and isoxadifen-ethyl in accordance with EU requirements. The risk to birds was assessed based on the maximum single application rate of 1 × 135 g GF-3969/ha as this is protective of all intended uses.

For each of the active substances, the calculated TER values exceeded the relevant acute and chronic trigger values at the screening step and Tier 1, and so acceptable risk can be concluded. The risk to birds from exposure via drinking water was assessed and an acceptable risk was concluded.

An assessment of the risks via secondary poisoning was not triggered for the active substances rimsulfuron and thifensulfuron methyl, as they have log K_{ow} values of <3 and the potential for bioaccumulation is considered to be low.

For isoxadifen-ethyl, as the log K_{ow} of 3.8 exceeds the trigger value of 3, an assessment of the risk for secondary poisoning was conducted and shown to be acceptable.

As an acute study with birds is not available with the product GF-3969; therefore, acute combination toxicity assessment was conducted. None of the active substances was found to contribute to >90% of the mixture toxicity and, therefore, acute risk was assessed by deriving the TER between the predicted endpoint by the concentration addition model and the sum of application rates of active substances. The TER exceeded the relevant trigger value (10); therefore, acceptable risk was concluded.

The combined long-term risk was concluded to be low based on TER_{mix} exceeding the trigger of 5.

Calculations performed for isoxadifen-ethyl were presented for informative purposes only, since no EU agreed endpoints exist for this compound. In case the endpoints were confirmed at the EU level, acceptable acute and long-term risk from exposure to isoxadifen-ethyl would be concluded.

~~According to the Central Zone requirement, long term combination toxicity assessment was conducted. None of the active substances was found to contribute to >90% of the mixture toxicity and, therefore, long term risk was assessed by deriving the TER between the predicted endpoint by the concentration addition model and the sum of application rates of active substances. The TER exceeded the relevant trigger value (5); therefore, acceptable risk was concluded.~~

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 rimsulfuron, thifensulfuron methyl, isoxadifen-ethyl and their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

The acute oral toxicity of GF-3969 to mammals has been assessed in a study with the rat. Full details of this study are provided in the Core, Part B, Section 6.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process. Relevant justifications are provided below.

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

Species	Substance	Exposure System	Results	Reference
Rat	Rimsulfuron	Oral 1 d, Acute	LD₅₀ = 5000 mg a.s./kg bw/d	EFSA 2005
Rat	Rimsulfuron	Long-term	NOAED = 11.8 mg a.s./kg bw/d	EFSA 2005
Rat	Rimsulfuron	Dietary Reproductive toxicity Two-generation study	NOAEL = 3000 mg a.s./kg feed	EFSA 2005

Bold values are used in the risk assessment.

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

Species	Substance	Exposure System	Results	Reference
Rat	Thifensulfuron methyl	Oral 1 d, Acute	LD₅₀ >5000 mg a.s./kg bw/d	EFSA 2015
Rat	IN-L9225	Oral 1 d, Acute	LD ₅₀ >2000 mg met./kg bw	EFSA 2015
Rat	IN-A4098	Oral 1 d, Acute	LD ₅₀ >2000 mg met./kg bw (males) LD ₅₀ = 1000 mg met./kg bw (females)	EFSA 2015
Rat	IN-W8268	Oral 1 d, Acute	LD ₅₀ >2000 mg met./kg bw	EFSA 2015
Rat	CHA 8730 (TSM)	Oral 1 d, Acute	LD ₅₀ >2000 mg product/kg bw	EFSA 2015
Rat	FH-009 (TSM)	Oral 1 d, Acute	LD ₅₀ >5000 mg product/kg bw	EFSA 2015
Rat	Thifensulfuron methyl	Long-term	NOAEL = 1.3 mg a.s./kg bw/d[#]	EFSA 2015
Rat	Thifensulfuron methyl	Long-term	NOAEL = 43 mg a.s./kg bw/d[#]	EFSA 2015

Bold values are used in the risk assessment.

The NOAEL of 1.3 mg/kg bw per day was used in the screening step assessment as it was used in the human risk assessment to set the ADI. This NOAEL was further refined at first tier to 43 mg/kg bw per day. Should a higher tier assessment be required in the future then the ecological relevance of this NOAEL should be considered further.

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

Species	Substance	Exposure System	Results	Reference
Rat	Isoxadifen-ethyl	Oral 1 d Acute	LD₅₀ = 1740 mg a.s./kg bw/d^a	Zonal evaluation by zRMS Greece (2016)
Rat	Isoxadifen-ethyl	Dietary Reproductive toxicity Two-generation study	NOAEC = 200 mg a.s./kg feed NOAED_{mean} = 16.4 mg a.s./kg bw/d^a	Zonal evaluation by zRMS Greece (2016)

Bold values are used in the risk assessment.

a Combined for male and female rats.

Table 9.3-4: Endpoints and effect values relevant for the risk assessment for mammals – GF-3969

Species	Substance	Exposure System	Results	Reference
Rat	GF-3969	Oral 1 d, Acute	LD ₅₀ >2000 mg product/kg bw	Fallers, M.N., 2018 (DuPont-49958)

zRMS comments:

Mammalian toxicity data for rimsulfuron and thifensulfuron-methyl are in line with EU agreed endpoints reported in EFSA Scientific Report (2005) 45, 1-61 and EFSA Journal 2015;13(7):4201, respectively.

No EU agreed data exist for the safener, isoxadifen-ethyl, and for this reason validation of information provided in Table 9.3-3 against EU agreed endpoints was not possible. Nevertheless, data provided by the Applicant for isoxadifen-ethyl have been retained for informative purposes, with font colour changed to grey in order to easily distinguish validated from non-validated data.

9.3.1.1 Justification for new endpoints

A new study to assess the acute toxicity of the product GF-3969 has been conducted and is provided in the Core, Part B, Section 6.

zRMS comments:

The study on acute toxicity of GF-3969 to mammals has been agreed in the course of evaluation performed in area of Section B6. For details, please refer to the Core Assessment, Part B, Section 6.

9.3.2 Risk assessment for spray applications

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group for 1 × 135 g product/ha covers the risk for mammals from all other intended split application uses (see Section 0).

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

The results of the acute and reproductive screening risk assessments are summarised in the following tables.

The acute ‘daily dietary dose’ (DDD) is calculated by multiplying the shortcut value (SV) based on the 90th percentile residues by the application rate in kg a.s./ha and the multiple application factor for 90th percentile residue data (MAF₉₀).

$$\text{DDD} = \text{application rate (kg a.s./ha)} \times \text{MAF}_{90} \times \text{SV}$$

The long-term ‘daily dietary dose’ (DDD) is calculated by multiplying the shortcut value (SV) based on the mean residues by the application rate in kg a.s./ha, the appropriate multiple application factor (MAF_M), and a time weighted average residue exposure (f_{TWA}). The f_{TWA} based upon a default DT₅₀ of 10 days is 0.53, as given in EFSA/2009/1438.

$$\text{DDD} = \text{application rate (kg a.s./ha)} \times \text{SV} \times f_{\text{TWA}} \times \text{MAF}_M$$

The TER_a and TER_{lt} values are calculated by dividing the acute and chronic toxicity endpoint by the respective daily dietary dose. The results of the acute and reproductive screening risk assessments are summarised in the following tables.

There is no requirement for the calculation of TER_{st} (short-term) for mammals 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.

Table 9.3-5: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of GF-3969 in maize– rimsulfuron

Intended use	Maize				
Active substance/product	Rimsulfuron				
Application rate (g/ha)	1 × 20				
Acute toxicity (mg/kg bw)	5000				
TER criterion	10				
Crop scenario	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Growth stage					
Screening	Small herbivorous mammal	136.4	1	2.73	1833
Reprod. toxicity (mg/kg bw/d)	11.8				
TER criterion	5				
Crop scenario	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Growth stage					
Screening	Small herbivorous mammal	72.3	1 × 0.53	0.77	15

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

Table 9.3-6: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of GF-3969 in maize – thifensulfuron methyl

Intended use		Maize				
Active substance/product		Thifensulfuron methyl				
Application rate (g/ha)		1 × 12.5				
Acute toxicity (mg/kg bw)		5000				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a	
Screening	Small herbivorous mammal	136.4	1	1.71	2933	
Reprod. toxicity (mg/kg bw/d)		43 1.3 [#]				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{tt}	
Screening	Small herbivorous mammal	72.3	1 × 0.53	0.48	89.7 3	
Reprod. toxicity (mg/kg bw/d)		43 [#]				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{tt}	
Maize, BBCH 10 – 19	Small herbivorous mammal 'shrew' ²	4.2	1 × 0.53	0.03	1545	
Maize, BBCH 10 – 29	Small herbivorous mammal 'vole' ²	72.3	1 × 0.53	0.48	90	
Maize, BBCH 10 – 29	Small herbivorous mammal 'mouse' ²	7.8	1 × 0.53	0.05	832	

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 NOAEL of 1.3 mg/kg bw per day was used in the screening step assessment as it was used in the human risk assessment to set the ADI. This NOAEL was further refined at first tier to 43 mg/kg bw per day (EFSA, 2015).

Based on the lowest reported NOAEL of 1.3 mg/kg bw/day a potential chronic risk to mammals is triggered at the screening step. However as noted in the EFSA conclusion, this value has been refined to more biologically relevant endpoint of 43 mg/kg bw/day. The Tier 1 assessment using this endpoint indicates acceptable risk.

Table 9.3-7: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of GF-3969 in maize – isoxadifen-ethyl

Intended use		Maize				
Active substance/product		Isoxadifen-ethyl				
Application rate (g/ha)		1 × 15 g a.s./ha				
Acute toxicity (mg/kg bw)		1740				
TER criterion		10				
Crop scenario	Indicator species for screening	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Growth stage						
Screening	Small herbivorous mammal	136.4	1	2.04	852	
Reprod. toxicity (mg/kg bw/d)		16.4				
TER criterion		5				
Crop scenario	Indicator species for screening	SV _m	MAF _m TWA	× DDD _m (mg/kg bw/d)	TER _{lt}	
Growth stage						
Screening	Small herbivorous mammal	72.3	1 × 0.53	0.57	29	

zRMS comments:

The risk assessment performed for rimsulfuron and thifensulfuron-methyl in tables above is in general agreed by the zRMS. Evaluation was performed considering single application of both compound, covering also split applications. It is noted that different endpoints were used for thifensulfuron-methyl in the screening and Tier 1 evaluation. However, in line with the current approach single endpoint should be used at all steps of the assessment. Since at the EU level NOAEL of 43 mg a.s./kg/dw/d was considered to be ecotoxicologically relevant, this endpoint should have been used also at the screening step. Respective corrections has been introduced in Table 9.3-6 above.

On the basis of performed calculations acceptable acute and long-term dietary risk from exposure of birds to both active compounds may be concluded.

In absence of the EU agreed mammalian toxicity data for isoxadifen-ethyl, validation of calculations presented in Table 9.3-7 was not possible. Nevertheless, performed calculations have been retained for informative purposes, with font colour changed to grey in order to easily distinguish validated from non-validated data. In case the endpoints were confirmed at the EU level, acceptable risk from exposure to isoxadifen-ethyl would be concluded.

GF-3969 combined effects risk assessment

An acute oral toxicity study for mammals has been conducted with GF-3969. The LD₅₀ is reported to be >2000 mg product/bw.

The acute toxicity to mammals has also 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) = fraction of active substance in the mixture (sum must be 1)

LD₅₀ (a.s._i) = LD₅₀ for acute toxicity of active substances

The values used for the calculation of acute combination toxicity effects are the following:

Table 9.3-8: Combination toxicity endpoints of rimsulfuron and thifensulfuron methyl and safener isoxadifen-ethyl calculated from active substances toxicity endpoints of mammals

	Rimsulfuron	Thifensulfuron methyl	Isoxadifen-ethyl
Content in the formulation GF-3969 (% w/w)	14.82%	9.26%	11.11%
Fraction in mixture	42.11%	26.31%	31.57%
LD ₅₀ of a.s. [mg/kg bw]	> 5000	>5000	1740
Fraction / LD ₅₀	0.000084	0.000053	0.000181
Σ (Fraction / LD ₅₀)	0.000318		
1/ sum = predicted LD ₅₀ (mix)	3125 3142		
Contribution of the active to predicted toxicity	26.46%	16.53%	57.00%

According to EFSA/2009/1438, Appendix B, when an active substance is found to contribute to $\geq 90\%$ of the toxicity according to the combination toxicity assessment the risk assessment can be performed for the most toxic active substance alone. The predicted LD₅₀ of 3142 mg product/kg bw is comparable to the measured LD₅₀ of >2000 mg product/kg bw.

In this case, none of the active substances were contributing >90% of the toxicity, therefore, a risk assessment is conducted based on the mixture toxicity endpoint. As the measured endpoint for GF-3969 is lower than the predicted, the endpoint of >2000 mg product/kg bw is applied to the risk assessment and compared to the maximum application rate of 135 g product/ha.

~~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 9.3-9: Combination toxicity endpoints of rimsulfuron and thifensulfuron methyl and safener isoxadifen-ethyl calculated from active substances toxicity endpoints of mammals**~~

	Rimsulfuron	Thifensulfuron methyl	Isoxadifen-ethyl
Content in the formulation GF-3969 (% w/w)	14.82%	9.26%	11.11%
Fraction in mixture	42.11%	26.31%	31.57%
LD ₅₀ of a.s. [mg/kg bw]	11.8	43 [#]	16.40
Fraction / LD ₅₀	0.035690	0.202418	0.019251
Σ (Fraction / LD ₅₀)	0.2573		
1/ sum = predicted LD ₅₀ (mix)	16.38		
Contribution of the active to predicted toxicity	13.87%	78.65%	7.48%

~~[#] The NOAEL of 1.3 mg/kg bw per day was used in the screening step assessment as it was used in the human risk assessment to set the ADI. This NOAEL was further refined at first tier to 43 mg/kg bw per day and therefore used in the combination toxicity assessment.~~

~~According to EFSA/2009/1438, Appendix B, as none of the active substances are found to contribute to $\geq 90\%$ of the toxicity according to the combination toxicity assessment, the risk assessment should be performed based on the predicted NOEL(mix) and the sum of the application rates of the 3 actives.~~

Table 9.3-10: Screening assessment of the long-term/reproductive risk for mammals due to the use of GF-3969 in maize

Intended use		Maize			
Active substance/product		Combination of rimsulfuron, thifensulfuron methyl and isoxadifen-ethyl			
Application rate (g/ha)		1 × 135 g product/ha			
Acute toxicity (mg/kg bw)		>2000 (measured)			
TER criterion		10			
Crop scenario	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Growth stage					
Screening	Small herbivorous mammal	136.4	1	18.4	109
Reprod. toxicity (mg/kg bw/d)		16.38 (predicted)			
TER criterion		5			
Crop scenario	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{tt}
Growth stage					
Screening	Small herbivorous mammal	72.3	1 × 0.53	1.63	10

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

a sum of the application rates of rimsulfuron, thifensulfuron methyl and isoxadifen-ethyl.

Acute ~~All~~ TERs (~~acute and long term~~) exceed the relevant trigger values for rimsulfuron, thifensulfuron methyl, isoxadifen-ethyl and the combination of all the active substances, therefore, an acceptable risk from uses of GF-3969 in maize is concluded.

zRMS comments:

Since no EU agreed data exist for isoxadifen-ethyl, validation of combined toxicity assessment performed above was not possible. Nevertheless, performed calculations of the acute combined toxicity were retained for illustrative purposes, with font colour changed to grey in order to easily distinguish validated from non-validated data.

In case of the combined risk assessment for the active compounds, the LD_{50mix} of 5000 mg/kg bw may be calculated considering the endpoints of 5000 mg/kg bw for both, rimsulfuron and thifensulfuron-methyl and their fraction in the formulation (0.62 and 0.38). This surrogate endpoint is higher than measured LD50 od >2000 mg product/kg bw and for this reason evaluation presented in Table 9.3-10 above is sufficient. It should be noted that it covers also exposure from the safener.

With regard to the long-term risk it should be noted that it is not appropriate to calculate the surrogate endpoint based on long-term toxicity of particular compounds, since they may be based on different parameters. Instead, the combined long-term risk assessment should be performed by calculation of the TER_{mix}. Alternatively, the long-term risk assessment may be performed using the lowest available long-term endpoint together with the application rate expressed as the sum of active compounds. The calculations performed by the Applicant above has been struck through as being not correct and TER_{mix} approach has been presented below.

Crop scenario and/or indicator species		TER _{LT} rimsulfuron	TER _{LT} thifensulfuron-methyl	TER _{LTmix}	Trigger
Reproductive (screening step)					
Maize	Small herbivorous mammal	15	89.7	12.9	5

Overall, based on the above calculations, acceptable acute and long-term combined dietary risk to mammals may be concluded from the intended uses of GF-3969.

In case endpoints for isoxadifen-ethyl were confirmed at the EU level, following TER_{LTmix} would be calculated:

Crop scenario and/or indicator species		TER _{LT} rimsulfuron	TER _{LT} thifensulfuron- methyl	TER _{LT} isoxadifen- ethyl	TER _L Tmix	Trigger
Reproductive (screening step)						
Maize	Small herbivorous mammal <small>Small omnivorous bird</small>	15	89.7	29	8.9	5
Above calculations indicate that in case endpoints for isoxadifen-ethyl were confirmed at the EU level, acceptable acute and long-term combined dietary risk to mammals would be concluded from the exposure to active compounds and the safener.						

9.3.2.2 Higher-tier risk assessment

Not required, since an acceptable risk was demonstrated in the screening and first-tier risk assessment.

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

For mammals, the puddle scenario is relevant. 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).

The effective application rate is calculated according to the following formula:

$$AR_{eff} = AR \times MAF_m$$

Where:

- AR is the application rate (g a.s./ha).
- MAF_m is the Multiple Application Factor based on the DT₅₀ in soil (single first order kinetics, geometric mean). In case of single applications, MAF_m = 1.

The ratio of effective application rate to relevant endpoint was calculated according to the following formula:

$$HQ = \frac{AR(g/ha)}{LD_{50}(mg\ a.s./kg\ bw/day)}$$

Rimsulfuron

With a $K_{(f)oc}$ of 63 (range 19-63 L/kg, EFSA Scientific Report (2005) 45, 1-61) rimsulfuron belongs to the group of less sorptive substances.

Effective application rate (g/ha)	=	1 × 20 g a.s./ha			Trigger
Acute toxicity (mg/kg bw)	=	5000	quotient =	0.004	50
Reprod. toxicity (mg/kg bw/d)	=	11.8	quotient =	1.69	50

Thifensulfuron methyl

With a $K_{(f)oc}$ of 9 (range 3.1-86 L/kg, EFSA Journal 2015;13(7):4201) thifensulfuron methyl belongs to the group of less sorptive substances.

Effective application rate (g/ha)	=	1 × 12.5 g a.s./ha			Trigger
Acute toxicity (mg/kg bw)	=	5000	quotient =	0.0025	50
Reprod. toxicity (mg/kg bw/d)	=	43 13	quotient =	0.29 9.61	50

Isoxadifen-ethyl

With a $K_{(f)oc}$ of 727 (Zonal evaluation by zRMS Greece (2016)) isoxadifen-ethyl belongs to the group of more sorptive substances.

Effective application rate (g/ha)	=	1 × 15 g a.s./ha			Trigger
Acute toxicity (mg/kg bw)	=	3776	quotient =	0.004	3000
Reprod. toxicity (mg/kg bw/d)	=	22.4	quotient =	0.7	3000

Since the ratios of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) do not exceed the critical value of 50 or 3000 for any of the active substances, a quantitative risk assessment (calculation of TER values) due to uptake of contaminated drinking water is not necessary as a low risk from uptake via drinking water can be concluded.

zRMS comments:

Drinking water risk assessment performed above for rimsulfuron and thifensulfuron-methyl is in general agreed by the zRMS. However, the long-term ratio for thifensulfuron-methyl has been corrected by the zRMS in order to take into account endpoint considered in the dietary risk assessment.

In absence of the EU agreed avian toxicity data for isoxadifen-ethyl, validation of drinking water risk assessment for this compound was not possible. Nevertheless, calculations provided above have been retained for informative purposes, with font colour changed to grey in order to easily distinguish validated from non-validated data. In case the endpoints were confirmed at the EU level, acceptable risk from exposure via drinking water would be concluded.

No calculations were provided by the Applicant for the pertinent soil metabolites of both active compounds. However, the risk would be acceptable since the maximum ratio for metabolites based on the worst case assumptions (10 times toxicity of the parent and parent exposure) would be <50 (worst case trigger assumed, covering also risk from less sorptive metabolites) for the acute and long-term risk. Hence, no further evaluation has been performed.

9.3.2.1 Effects of secondary poisoning

The log K_{ow} values of rimsulfuron and thifensulfuron methyl are -1.46 (at pH 7) and -1.65 (at pH 7) respectively, thus do not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required for rimsulfuron and thifensulfuron methyl as the K_{ow} values indicate a low potential for bioaccumulation.

As the log K_{ow} of isoxadifen-ethyl (log K_{ow} = 3.8) exceeds the trigger value of 3, an assessment of the risk for secondary poisoning is required. However, to note, isoxadifen-ethyl is rapidly decomposed in environmental matrices, resulting in products of high polarity. The half-life of isoxadifen-ethyl ranges from <0.1 days to 3.6 days in soil, or from 0.2 to 1.5 days in water/sediment systems. The abiotic hydrolysis DT_{50} at pH 7 was 2.3 days. The predominant breakdown product is the carboxylic acid AE F129431, log P_{ow} of which ranges from -0.18 (pH 4) to -1.77 (pH 10). Under neutral to alkaline conditions of pH AE F129431 is quantitatively deprotonated and thus in an ionic form. Consequently, a negative value can be estimated for the logarithmic value of the octanol/water coefficient. Prolonged exposure to the parent compound at relevant environmental pH values is therefore unlikely, and based

on the characteristics of the degradation products accumulation of AE F129431, AE C637375 and AE C642961 will not occur. Therefore, a fish bioaccumulation study with either isoxadifen-ethyl or its metabolites is not justified, and the risk of secondary poisoning is deemed very low.

To further quantify the risk, a calculation is nonetheless provided for risk assessment for earthworm and fish-eating mammals via secondary poisoning has been provided based on a calculated BCF for fish and earthworms.

Table 9.3-11: Assessment of the risk for earthworm-eating mammals due to exposure to isoxadifen-ethyl via bioaccumulation in earthworms (secondary poisoning) for the intended use in maize

Parameter	Isoxadifen-ethyl	Comments
PEC _{soil} (21d TWA) (mg/kg soil)	0.002	See Part B, Section 8
log K _{ow} / K _{ow}	3.8 / 6309.6	Zonal evaluation by zRMS Greece (2016)
K _{oc}	727	Zonal evaluation by zRMS Greece (2016)
F _{oc}	0.02	Default, EFSA Journal 2009; 7(12):1438
BCF _{worm}	52.13	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times K_{ow}) / f_{oc} \times K_{oc}$
PEC _{worm}	0.10426	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.133	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	16.4	Zonal evaluation by zRMS Greece (2016)
TER _{it}	123.3	

The TER_{it} for the assessment of the risk for earthworm-eating mammals due to isoxadifen-ethyl exposure via bioaccumulation in earthworms greatly exceeds the relevant trigger TER value of 5, indicating acceptable risk to mammals following applications of isoxadifen-ethyl to maize.

Risk assessment for fish-eating mammals via secondary poisoning

According to EFSA aquatic guidance document (2013)³ 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.

Bioaccumulation in fish is estimated based on predicted concentrations in surface water.

Table 9.3-12: Assessment of the risk for fish-eating mammals due to exposure to isoxadifen-ethyl via bioaccumulation in fish (secondary poisoning) for the intended use in maize

Parameter	Isoxadifen-ethyl	Comments
FOCUS Step PEC _{sw} (21 day TWA) (mg/L)	0.000006	See Part B, Section 8
BCF _{fish}	149.4	Calculated based the measured log K _{ow} = 3.8 and SMILES code C1(C(OCC)=O)=NOC(C1)(c1ccccc1)c1ccccc1 using EPIWeb 4.1, BCFBAF version 3.01.
BMF	N/A	biomagnification factor (relevant for BCF ≥2000)
PEC _{fish}	0.00090	$PEC_{fish} = PEC_{water} \times BCF_{fish} \times TWA$
Daily dietary dose (mg/kg bw/d)	0.00013	$DDD = PEC_{fish} \times 0.138$
NOEL (mg/kg bw/d)	16.4	Zonal evaluation by zRMS Greece (2016)
TER _{it}	126154	

³ EFSA guidance on tiered risk assessment for edge of field surface waters (EFSA PPR Panel 2013)

The TER_{it} for the assessment of the risk for fish-eating mammals due to isoxadifen-ethyl exposure via bioaccumulation in fish does not fall below the relevant trigger TER value of 5, indicating low risk to mammals following applications of isoxadifen-ethyl to maize.

zRMS comments:

The evaluation of the risk of secondary poisoning was not triggered for rimsulfuron, thifensulfuron-methyl and their relevant metabolites due to log Pow values being <3 .

In absence of the EU agreed avian toxicity data for isoxadifen-ethyl, validation of the evaluation of the risk of secondary poisoning for this compound was not possible. Nevertheless, calculations provided above have been retained for informative purposes only, with font colour changed to grey in order to easily distinguish validated from non-validated data. In case the endpoints were confirmed at the EU level, acceptable risk of secondary poisoning would be concluded.

9.3.2.2 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 rimsulfuron, thifensulfuron methyl and isoxadifen-ethyl in accordance with EU requirements. The risk to mammals was assessed based on the maximum single application rate of 1×135 g GF-3969/ha as this is protective of all intended uses.

For each of the active substances, the calculated TER values exceeded the relevant acute and chronic trigger values at the screening step and Tier 1, and so acceptable risk can be concluded. The risk to mammals from exposure via drinking water was assessed and an acceptable risk was concluded.

An assessment of the risks via secondary poisoning was not triggered for the active substances rimsulfuron and thifensulfuron methyl, as they have log K_{ow} values of <3 and, the potential for bioaccumulation is considered to be low.

For isoxadifen-ethyl, the log K_{ow} of 3.8 exceeds the trigger value of 3. An assessment of the risk for secondary poisoning was conducted and shown to be acceptable.

An acute toxicity with GF-3969 has been conducted and reported the LD_{50} to be >2000 mg product/kg bw. The acute combination toxicity assessment was conducted. None of the active substances was found to contribute to $>90\%$ of the mixture toxicity and, therefore, acute risk was assessed by deriving the TER between the predicted endpoint by the concentration addition model and the sum of application rates of active substances. The TER exceeded the relevant trigger value (10), therefore, acceptable risk was concluded.

The combined long-term risk was concluded to be low based on TER_{mix} exceeding the trigger of 5.

Calculations performed for isoxadifen-ethyl were presented for informative purposes only, since no EU agreed endpoints exist for this compound. In case the endpoints were confirmed at the EU level, acceptable acute and long-term risk from exposure to isoxadifen-ethyl would be concluded.

~~According to the Central Zone requirement, long term combination toxicity assessment was conducted. None of the active substances was found to contribute to $>90\%$ of the mixture toxicity and, therefore, long term risk was assessed by deriving the TER between the predicted endpoint by the concentration addition model and the sum of application rates of active substances. The TER exceeded the relevant trigger value (5), therefore, acceptable risk was concluded.~~

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) 283/2013⁴ and 284/2013⁵), 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 active substance rimsulfuron, thifensulfuron methyl and isoxadifen ethyl. 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, 2012⁶; Weltje *et al.*, 2013⁷). 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 0 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 3969 at rates up to and including 1×135 g/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, 2012⁸). 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.

⁴-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.

⁵-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.

⁶-Fryday S. and Thompson H. (2009): Literature reviews on ecotoxicology of chemicals with a special focus on plant protection products. Lot I. Exposure of reptiles to plant protection products. EFSA (CFT/EFSA/PPR/2008/01).

⁷-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

⁸-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, low 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 applications of GF 3969 at rates up to and including 1×135 g/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 rimsulfuron, thifensulfuron methyl, isoxadifen-ethyl and their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on aquatic organisms of GF-3969 were not evaluated as part of the EU assessments of rimsulfuron and thifensulfuron methyl. 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 – rimsulfuron and relevant metabolites

Species	Substance	Exposure System	Results	Reference
Acute toxicity to fish				
<i>Lepomis macrochirus</i>	Rimsulfuron	96 h, s	LC ₅₀ >390 mg a.s./L _{mm}	EFSA 2005 Hutton, D.G., 1990 (HLR 352-89, Revision No. 1)
<i>Oncorhynchus mykiss</i>	Rimsulfuron	96 h, s	LC ₅₀ >390 mg a.s./L _{mm}	EFSA 2005 Hutton, D.G., 1989a (HLR 351-89)
<i>Oncorhynchus mykiss</i>	IN-70941	96 h, s	LC ₅₀ >110 mg met./L _{mm}	EFSA 2005 Grube, P.W., 1998 (HL-1997-00909)
<i>Oncorhynchus mykiss</i>	IN-70942	96 h, s	LC ₅₀ = 180 mg met./L _{mm}	EFSA 2005 Kreamer, G.C., 1993 (HLR 85-93)
<i>Oncorhynchus mykiss</i>	IN-E9260	96 h, s	LC ₅₀ >314 mg met./L _{mm}	EFSA 2005 Pottinger, T.G., 1992 (FT 16)
Chronic toxicity to fish				
<i>Oncorhynchus mykiss</i>	Rimsulfuron	21 d	NOEC = 125 mg a.s./L _{nom}	EFSA 2005 WAT2002-85 (HLR 672-91, Revision No. 1)
<i>Oncorhynchus mykiss</i>	Rimsulfuron	90 d (ELS), f	NOEC = 125 mg a.s./L _{nom}	EFSA 2005 Kreamer, G.C., 1994 (HLR 507-94)
Acute toxicity to aquatic invertebrates				
<i>Daphnia magna</i>	Rimsulfuron	48 h, s	EC ₅₀ >360 mg a.s./L _{mm}	EFSA 2005 Hutton, D.G., 1989b (HLR 350-89)
<i>Daphnia magna</i>	IN-70941	48 h, s	EC ₅₀ = 95 mg met./L _{mm}	EFSA 2005 Pierson, K.B., 1991a (HLR 111-91, Revision No. 1)
<i>Daphnia magna</i>	IN-70942	48 h, s	EC ₅₀ = 178 mg met./L _{mm}	EFSA 2005 Pierson, K.B., 1991b (HLR 113-91, Revision No. 1)
<i>Daphnia magna</i>	IN-E9260	48 h, s	EC ₅₀ = 184 mg met./L _{mm}	EFSA 2005 Hewitt, D.P., 1992 (DTA 16)
Chronic toxicity to aquatic invertebrates				
<i>Daphnia magna</i>	Rimsulfuron	21 d, ss	NOEC = 1 mg a.s./L _{mm}	EFSA 2005 Baer, K.N., 1990 (HLR 95-90, Revision No. 1)

Species	Substance	Exposure System	Results	Reference
Toxicity to aquatic sediment dwelling invertebrates				
<i>Chironomus riparius</i>	IN-70942	28 d, spiked sediment	NOEC >200 µg met./kg sed	EFSA 2005 WAT2003-681 (DuPont-12329)
Toxicity to algae				
<i>Selenastrum capricornutum</i>	Rimsulfuron	72 h, s	EC₅₀ (biomass) = 1.2 mg a.s./L_{mm}	EFSA 2005 Douglas, M.T., Halls, R.W.S., 1990 (DPT 171(w)/90466)
<i>Selenastrum capricornutum</i>	IN-70941	72 h, s	EC₅₀ (biomass) >8.9 mg met./L_{mm}	EFSA 2005 Sloman, T.L., Leva, S.E., 1997 (AMR 4572-97)
<i>Selenastrum capricornutum</i>	IN-70942	72 h, s	EC₅₀ (biomass) >10 mg met./L_{mm}	EFSA 2005 Sloman, T.L., 2000a (DuPont-3743)
<i>Scenedesmus subspicatus</i>	IN-E9260	72 h, s	EC₅₀ (biomass) >100 mg met./L_{mm}	EFSA 2005 Sloman, T.L., 2000c (DuPont-3781)
Toxicity to aquatic macrophytes				
<i>Lemna minor</i>	Rimsulfuron	14 d, s	EC₅₀ (fronds) = 0.0046 mg a.s./L_{mm}	EFSA 2005 Douglas, M.T., Halls, R.W.S., 1992 (DPT 186(e)/920708)
<i>Myriophyllum spicatum</i>	Rimsulfuron	14 d, ss	E _r C ₅₀ (shoot length) = 0.0051 mg a.s./L _{mm}	EFSA Journal 2018;16(5):5258
<i>Myriophyllum spicatum</i>	Rimsulfuron	14 d, ss	E _r C ₅₀ (shoot length) = 0.00484 mg a.s./L _{mm}	EFSA Journal 2018;16(5):5258
<i>Lemna gibba</i>	IN-70942	14 d, s	EC₅₀ (fronds) >0.02 mg met./L_{mm}	EFSA 2005 Sloman, T.L., 1996 (AMR 4060-96)
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
Bold endpoints are used in the risk assessment

zRMS comments:

Endpoints presented in Table 9.5-2 are EU agreed endpoints reported in EFSA Scientific Report (2005) 45, 1-61.

Although the new LoEP (EFSA Journal 2018;16(5):5258) is not applicable yet, the endpoints for *Myriophyllum spicatum* derived in the course of the EU renewal of rimsulfuron were included by the zRMS in the Table 9.5-1 above in order to demonstrate that the second aquatic macrophyte species is not more sensitive and the endpoints for *Lemna gibba* available from the first EU review is sufficiently protective to be used in the risk assessment.

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

Species	Substance	Exposure System	Results	Reference
Acute toxicity to fish				
<i>Oncorhynchus mykiss</i>	Thifensulfuron methyl 50SG	96 h, s	LC ₅₀ >120 mg product/L _{nom} LC₅₀ >56.4 mg a.s./L_{mm}	EFSA 2015 Not available, 2001 (DuPont-11440)
<i>Oncorhynchus mykiss</i>	IN-L9225	96 h, s	LC₅₀ >120 mg met./L_{mm}	EFSA 2015 Not available, 2001 (DuPont-5622)

Species	Substance	Exposure System	Results	Reference
<i>Oncorhynchus mykiss</i>	IN-JZ789	96 h, s	LC ₅₀ >0.94 mg met./L _{mm}	EFSA 2015 Not available, 1991 (DuPont-1655)
<i>Oncorhynchus mykiss</i>	IN-V7160	96 h, s	LC ₅₀ >1.0 mg met./L _{mm}	EFSA 2015 Not available, 1999 (DuPont-3561)
<i>Oncorhynchus mykiss</i>	IN-A4098	96 h, s	LC ₅₀ >200 mg met./L _{nom}	EFSA 2015 Not available, 1984 (Ciba 87 26)
<i>Oncorhynchus mykiss</i>	IN-A4098	96 h, s	LC ₅₀ >0.93 mg met./L _{mm}	EFSA 2015 Not available, 1999 (DuPont-3559)
<i>Oncorhynchus mykiss</i>	IN-W8268	96 h, s	LC ₅₀ >115 mg met./L _{mm}	EFSA 2015 Not available, 2000 (DuPont-4683)
Chronic toxicity to fish				
<i>Oncorhynchus mykiss</i>	Thifensulfuron methyl	ELS	NOEC = 10.6 mg/L	Confirmatory data submitted by FMC Gerke, A., 2010 (DuPont-28722)***
Acute toxicity to invertebrates				
<i>Daphnia magna</i>	Thifensulfuron methyl	48 hr	EC ₅₀ >120 mg a.s./L	Confirmatory data submitted by FMC Brougher, D.S., Lockard, L., Gallagher, S.P., 2017 (DuPont-46007, Revision No. 1)***
<i>Chironomus riparius</i>	Thifensulfuron methyl	48 h, s	EC ₅₀ >100 mg a.s./L _{nom}	EFSA 2015 Juckeland, D, 2012 (11 10 48 045 W)
<i>Daphnia magna</i>	IN-L9225	48 h, s	EC ₅₀ >130 mg met./L _{mm}	EFSA 2015 Samel, A., 2001 (DuPont-5621)
<i>Daphnia magna</i>	IN-L9223	48 h, s	EC ₅₀ >1.2 mg met./L _{mm}	EFSA 2015 Samel, A., 1999 (DuPont-3216)
<i>Daphnia magna</i>	IN-JZ789	48 h, s	EC ₅₀ >1.1 mg met./L _{mm}	EFSA 2015 Hoke, R., 1999 (DuPont-1654)
<i>Daphnia magna</i>	IN-V7160	48 h, s	EC ₅₀ >1.3 mg met./L _{mm}	EFSA 2015 Hoke, R.A., 2001 (DuPont-4507)
<i>Daphnia magna</i>	IN-A4098	48 h, s	EC ₅₀ >99 mg met./L _{mm}	EFSA 2015 Samel, A., 1999 (DuPont-3247)
<i>Daphnia magna</i>	IN-W8268	48 h, s	EC ₅₀ >125 mg met./L _{mm}	EFSA 2015 Samel, A., 2000 (DuPont-4682)
<i>Daphnia magna</i>	Thifensulfuron methyl 50 SG	48 h, s	EC ₅₀ >120 mg product/L _{nom} EC ₅₀ >60.7 mg a.s./L _{nom}	EFSA 2015 Hoke, R.A., 2003 (DuPont-11439)
Chronic toxicity to invertebrates				
<i>Daphnia magna</i>	Thifensulfuron methyl	21 d, ss	NOEC = 99 mg/L	Confirmatory data submitted by FMC Hutton, D.G., 1989 (HLR 70-89)***
<i>Daphnia magna</i>	IN-L9223	21 d, ss	NOEC(reproduction) = 13 mg met./L _{mm}	EFSA 2015 Samel, A., 2000 (DuPont-4487)

Species	Substance	Exposure System	Results	Reference
<i>Daphnia magna</i>	IN-V7160	21 d, ss	NOEC(adult body length) = 11 mg met./L_{mm}	EFSA 2015 Hoke, R., 2001 (DuPont-4507)
<i>Daphnia magna</i>	IN-A4098	21 d, ss	NOEC(reproduction) = 32 mg met./L_{nom}	EFSA 2015 Grade, R., Wydra, V., Moll, M., 2006 (168 MEM)
Toxicity to algae				
<i>Pseudokirchneriella subcapitata</i>	Thifensulfuron methyl	72 hr	E_bC₅₀ = 0.027 mg a.s./L	Confirmatory data submitted by FMC Arnie, J.R., Lockard, L., Martin, K.H., Porch, J.R., 2017 (DuPont-46004, Revision No. 1)***
<i>Pseudokirchneriella subcapitata</i>	IN-L9225	72 h, s	E_rC₅₀ = 36.5 mg met./L_{nom} E _b C ₅₀ (cell density) = 33.4 mg met./L _{nom}	EFSA 2015 Sloman, T., 2001 (DuPont-5620)
<i>Pseudokirchneriella subcapitata</i>	IN-L9223	72 h, s	EC₅₀ >1.3 mg met./L_{mm}	EFSA 2015 Sloman, T., 1999 (DuPont-3012)
<i>Pseudokirchneriella subcapitata</i>	IN-JZ789	72 h, s	EC₅₀ >1.28 mg met./L_{mm}	EFSA 2015 Sloman, T., 1999 (DuPont-2850)
<i>Pseudokirchneriella subcapitata</i>	IN-V7160	72 h, s	EC₅₀ >11 mg met./L_{mm}	EFSA 2015 Sloman, T., 1999 (DuPont-3190)
<i>Pseudokirchneriella subcapitata</i>	IN-A4098	72 h, s	E _b C ₅₀ >10 mg met./L _{nom} E_rC₅₀ >10 mg met./L_{nom}	EFSA 2015
<i>Pseudokirchneriella subcapitata</i>	IN-W8268	72 h, s	E _b C ₅₀ (Cell density) = 29.9 mg met./L _{nom} E_rC₅₀ = 31.6 mg met./L_{nom}	EFSA 2015 Sloman, T., 2000 (DuPont-4680)
<i>Pseudokirchneriella subcapitata</i>	IN-L9226	72 h, s	EC ₅₀ (yield, biomass and growth rate) >89 mg met./L_{nom}	EFSA 2015 Vinken & Wydra, 2007 (51 TIM)
<i>Pseudokirchneriella subcapitata</i>	IN-A5546	72 h, s	E _b C ₅₀ = 48 mg met./L _{mm} E_rC₅₀ >110 mg met./L_{mm}	EFSA 2015 Hoberg, J.R., 2007 (DuPont-21528)
<i>Pseudokirchneriella subcapitata</i>	2-acid-3-triuret	72 h, s	E _y C ₅₀ >100 mg met./L _{nom} E_rC₅₀ >100 mg met./L_{nom}	EFSA 2015 Falk, S., 2012 (S12-01019)
<i>Pseudokirchneriella subcapitata</i>	IN-D8858	72 h, s	E _y C ₅₀ >0.045 mg met./L _{nom} E_rC₅₀ >0.045 mg met./L_{nom}	Confirmatory data submitted by FMC Arnie, J.R., Zhang, L., Porch, J.R., Martin, K.H., 2016 (DuPont-42163, Revision No. 1)***
Toxicity to macrophytes				
<i>Lemna gibba</i>	Thifensulfuron methyl	14 d, s	E _y C ₅₀ (frond count) = 0.00066 mg/L E_rC₅₀ (frond count) = 0.0011 mg/L	Arnie et al., 2015, DuPont-44981 (study agreed by zRMS (HU) in the course of the zonal evaluation of DPX-Q9B30 50 SG, also evaluated as a part of confirmatory data Applicant access via the LoA)
<i>Ceratophyllum demersum</i>	Thifensulfuron methyl	14 d, s	E _r C ₅₀ = 32.15 mg a.s./L _{mm}	EFSA 2015** Hoberg, J., 2011
<i>Elodea canadensis</i>	Thifensulfuron methyl	14 d, s	E _r C ₅₀ = 0.0217 mg a.s./L _{mm}	EFSA 2015** Hoberg, J., 2011

Species	Substance	Exposure System	Results	Reference
<i>Myriophyllum aquaticum</i>	Thifensulfuron methyl	14 d, s	ErC ₅₀ = 0.1871 mg a.s./L _{mm}	EFSA 2015** Hoberg, J., 2011
<i>Vallisneria americana</i>	Thifensulfuron methyl	14 d, s	ErC ₅₀ = 0.00023 mg a.s./L_{mm} NOEC = 0.00011 mg a.s./L _{mm}	EFSA 2015** Hoberg, J., 2011
<i>Lemna gibba</i>	IN-L9225	14 d, s	EC ₅₀ = 36.76 mg met./L _{mm} ErC ₅₀ (frond count) = 82.2 mg met./L_{mm}	EFSA 2015 Boeri, R., 2001 (DuPont-4654)
<i>Lemna gibba</i>	IN-L9223	14 d, s	EC ₅₀ (frond count and biomass) >172.1 mg met./L _{nom} ErC ₅₀ (frond count) > 172.1 mg met./L_{nom}	EFSA 2015 Sloman, T., 2001 (DuPont-5618)
<i>Lemna gibba</i>	IN-JZ789	14 d, s	EC ₅₀ (frond count and biomass) >100 mg met./L _{nom} ErC ₅₀ (frond count) > 100 mg met./L_{nom}	EFSA 2015 Sloman, T., 2001 (DuPont-5617)
<i>Lemna gibba</i>	IN-V7160	14 d, s	EC ₅₀ (frond count and biomass) >100 mg met./L _{nom} ErC ₅₀ (frond count) > 100 mg met./L_{nom}	EFSA 2015 Sloman, T., 2001 (DuPont-5619)
<i>Lemna gibba</i>	IN-A4098	7 d, s	ErC ₅₀ > 100 mg met./L_{nom} EbC ₅₀ >100 mg met./L _{nom}	EFSA 2015 Sowing, P., 2002 (CE01/072)
<i>Lemna gibba</i>	IN-W8268	14 d, s	EbC ₅₀ >100 mg met./L _{nom} EC ₅₀ = 39.5 mg met./L _{nom} ErC ₅₀ > 100 mg met./L_{nom}	EFSA 2015 Sloman, T., 2000 (DuPont-4681)
<i>Lemna gibba</i>	IN-L9226	7 d, s	EyC ₅₀ = 0.17 mg met./L _{mm} ErC ₅₀ = 0.31 mg met./L_{mm}	EFSA 2015 Vinken & Wydra 2007 (54 TIM)
<i>Lemna gibba</i>	IN-A5546	7 d, s	EC ₅₀ (yield, biomass, and growth rate) > 40.4 mg met./L_{mm}	EFSA 2015 Sloman, T., 2006 (DuPont-19856)
<i>Lemna gibba</i>	2-acid-3-triuret	7 d, s	EyC ₅₀ >100 mg met./L _{nom} ErC ₅₀ > 100 mg met./L_{nom}	EFSA 2015 Weber, 2012 (S1201020)
<i>Lemna gibba</i>	IN-B5528	7 d, s	EyC ₅₀ >119.52 mg met./L _{nom} ErC ₅₀ > 119.52 mg met./L_{nom} EbC ₅₀ >119.52 mg met./L _{nom}	EFSA 2015 Chandrasehar, 2010 (DuPont-29481)
<i>Lemna gibba</i>	IN-D8858	7 d, s	EC ₅₀ >0.044 mg met/L	Confirmatory data submitted by FMC Arnie, J.R., Zhang, L., Porch, J.R., Martin, K.H., 2016 (DuPont-42164, Revision No. 1)***
<i>Lemna gibba</i>	Thifensulfuron methyl 50 SG	7 d, s	EyC ₅₀ = 0.0014 mg product/L _{nom} EyC ₅₀ = 0.00071 mg a.s./L _{nom} ErC ₅₀ = 0.0026 mg product/L _{nom} ErC ₅₀ = 0.0013 mg a.s./L_{nom}	EFSA 2015
Higher-tier studies (micro- or mesocosm studies)				
Variable exposure studies				

Species	Substance	Exposure System	Results	Reference
<i>Lemna gibba</i>	Thifensulfuron methyl	7 d, variable exposure duration	E _y C ₅₀ (12 hr exposure) = 0.149 mg a.s./L _{nom} E _r C ₅₀ (12 hr exposure) = 0.632 mg a.s./L _{nom} E _y C ₅₀ (24 hr exposure) = 0.0149 mg a.s./L _{nom} E _r C ₅₀ (24 hr exposure) >0.198 mg a.s./L _{nom} E _y C ₅₀ (48 hr exposure) = 0.0035 mg a.s./L _{nom} E _r C ₅₀ (48 hr exposure) >0.0593 mg a.s./L _{nom} E _y C ₅₀ (96 hr exposure) = 0.00045 mg a.s./L _{nom} E _r C ₅₀ (96 hr exposure) = 0.0032 mg a.s./L _{nom}	EFSA 2015*
<i>Lemna gibba</i>	Thifensulfuron methyl	16 d (9 d period at lower temperature)	E _y C ₅₀ = 0.068 mg a.s./L _{mm} E _r C ₅₀ >0.447 mg a.s./L _{mm}	EFSA 2015*

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

* These studies were considered to be valid however they were not considered to be suitable for use in a refined risk assessment, please refer to the RAR for further information.

** Endpoints from these studies were not deemed to be appropriate for use in a higher tier risk assessment but were used in a qualitative way together with the E_rC₅₀ proposed by the RMS for *Vallisneria americana*.

*** Summarised in 0.

zRMS comments:

Majority of endpoints presented in Table 9.5-2 are EU agreed endpoints reported in EFSA Journal 2015;13(7):4201.

Long-term endpoint for fish (thifensulfuron-methyl), acute endpoint for *Daphnia magna* (thifensulfuron-methyl), chronic endpoint for algae (thifensulfuron-methyl and metabolite IN-D8858) and chronic endpoint for *Lemna gibba* (metabolite IN-D8858) were agreed by the RMS (UK) in the course of the evaluation of the confirmatory data (for details, please refer to EFSA Supporting publication 2020:EN-1627).

It is also noted that study on toxicity of thifensulfuron-methyl to *Lemna gibba* (Arnie et al., 2015, DuPont-44981) was agreed by the zRMS (HU) during the zonal assessment of formulation DPX-Q9B30 50 SG and during evaluation of confirmatory data for thifensulfuron methyl in 2019. Initially it was concluded that since the study is co-owned by DuPont (as indicated in the Core Assessment for DPX-Q9B30 50 SG) and all studies submitted for GF-3969 also belong to DuPont, its results may be ~~were~~ included by the zRMS of GF-3969 in Table 9.5-2 above. However, during the commenting period the Applicant clarified that the study by Arnie et al. (2015, DuPont-44981) is no longer owned by DuPont since thifensulfuron-methyl was sold to FMC following merger of DuPont and Dow to form Corteva. Nevertheless, at least one of subsidiaries of the Applicant for GF-3969 (specifically Corteva Agriscience Poland Sp. z o.o.) has access to thifensulfuron methyl data granted by the data owner (FMC Corporation) and for this reason the endpoints derived from the study by Arnie et al. (2015) were used in the risk assessment below.

It is noted that two additional studies with *Myriophyllum spicatum* were agreed by the RMS in the course of evaluation of confirmatory data for thifensulfuron methyl (one standard test the other test with variable exposure duration). However, derived endpoints were higher comparing to already EU agreed endpoint for *Vallisneria americana* reported in EFSA (2015) and still considered relevant for the Tier 1 risk assessment. Taking this into account, the endpoints from the new studies with *M. spicatum* were not included in Table 9.5-2 above as being not relevant for the risk assessment.

Table 9.5-4: Endpoints and effect values relevant for the risk assessment for aquatic organisms – isoxadifen-ethyl safener

Species	Substance	Exposure System	Results	Reference
Acute toxicity to fish				
<i>Lepomis macrochirus</i>	Isoxadifen-ethyl	96 h, f	LC ₅₀ = 0.22 mg a.s./L _{mm}	Evaluation from Germany (2002)
<i>Oncorhynchus mykiss</i>	Isoxadifen-ethyl	96 h, f	LC ₅₀ = 0.34 mg a.s./L _{mm}	Evaluation from Germany (2002)
<i>Oncorhynchus mykiss</i>	Isoxadifen-ethyl	96 h, s	LC ₅₀ >100 mg a.s./L _{mm}	Evaluation from Germany (2002)
<i>Oncorhynchus mykiss</i>	AE F129431	96 h, s	LC ₅₀ >100 mg met/L _{nom}	Zonal evaluation by zRMS Greece (2016)
<i>Oncorhynchus mykiss</i>	AE C637375	96 h, ss	LC ₅₀ >15.2 mg met/L _{mm}	Zonal evaluation by zRMS Greece (2016)
<i>Oncorhynchus mykiss</i>	AE C642961	96 h, ss	LC ₅₀ >10 mg a.s./L _{nom}	Zonal evaluation by zRMS Greece (2016)
Acute toxicity to aquatic invertebrates				
<i>Daphnia magna</i>	Isoxadifen-ethyl	48 h, f	EC ₅₀ >0.51 mg a.s./L _{mm}	Evaluation from Germany (2002)
<i>Daphnia magna</i>	Isoxadifen-ethyl	48 h, s	EC ₅₀ >150 mg a.s./L _{mm}	Evaluation from Germany (2002)
<i>Daphnia magna</i>	AE F129431	48 h, s	EC ₅₀ >150 mg met/L _{nom}	Zonal evaluation by zRMS Greece (2016)
<i>Daphnia magna</i>	AE C637375	48 h, ss	EC ₅₀ >28 mg met./L _{mm}	Zonal evaluation by zRMS Greece (2016)
<i>Daphnia magna</i>	AE C642961	48 h, ss	EC ₅₀ >7.0 mg met./L _{mm}	Zonal evaluation by zRMS Greece (2016)
Acute toxicity to algae				
<i>Pseudokirchneriella subcapitata</i>	Isoxadifen-ethyl	72 h, s	EC ₅₀ (biomass) >100 mg a.s./L _{mm} EC ₅₀ (growth) >100 mg a.s./L _{mm}	Evaluation from Germany (2002)
<i>Pseudokirchneriella subcapitata</i>	Isoxadifen-ethyl	72 h, s	EC ₅₀ (biomass) >1.26 mg a.s./L _{im}	Zonal evaluation by zRMS Greece (2016)
<i>Pseudokirchneriella subcapitata</i>	AE F129431	96 h, s	E _r /bC ₅₀ >100 mg met/L _{nom}	Zonal evaluation by zRMS Greece (2016)
<i>Pseudokirchneriella subcapitata</i>	AE C637375	96 h, s	E _r C ₅₀ >39.7 mg met/L _{mm}	Zonal evaluation by zRMS Greece (2016)
<i>Pseudokirchneriella subcapitata</i>	AE C642961	96 h, s	E _r /bC ₅₀ >10 mg met/L _{mm}	Zonal evaluation by zRMS Greece (2016)
Toxicity to aquatic macrophytes				
<i>Lemna gibba</i>	Isoxadifen-ethyl	7 d, ss	E _r C ₅₀ >1.48 mg a.s./L	Zonal evaluation by zRMS Greece (2016)

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

zRMS comments:

No EU agreed data exist for the safener, isoxadifen-ethyl, and for this reason validation of information provided in Table 9.5-3 against EU agreed endpoints was not possible. Nevertheless, data provided by the Applicant for isoxadifen-ethyl have been retained for informative purposes, with font colour changed to grey in order to easily distinguish validated from non-validated data.

Table 9.5-5: Endpoints and effect values relevant for the risk assessment for aquatic organisms – GF-3969

Species	Substance	Exposure System	Results	Reference ^a
Acute toxicity to fish				
<i>Oncorhynchus mykiss</i>	GF-3969 plus DPX-KG691	96 h, s	LC₅₀ = 6.78 mg product/L_{nom}	xxxxxxx, 2019 (DuPont-49948, Revision No. 1)
Acute toxicity to aquatic invertebrates				
<i>Daphnia magna</i>	GF-3969 plus DPX-KG691	48 h, s	EC₅₀ = 11.6 mg product/L_{nom}	Goudie, O., 2019 (DuPont-49949, Revision No. 1)
Toxicity to algae				
<i>Pseudokirchneriella subcapitata</i>	GF-3969 plus DPX-KG691	72 h, s	E_rC₅₀ = 3.25 mg product/L_{nom} E _b C ₅₀ = 0.510 mg product/L _{nom} E _y C ₅₀ = 0.532 mg product/L _{nom}	Hoover, E., 2019 (DuPont-49943)
Toxicity to aquatic macrophytes				
<i>Lemna gibba</i>	GF-3969 plus DPX-KG691	7 d, s	E_rC₅₀ (growth rate) = 0.00411 mg product/L_{mm} E _y C ₅₀ (yield) = 0.00228 mg product/L _{mm} E _r C ₅₀ (biomass growth rate) >0.00958 mg product/L _{mm} E _y C ₅₀ (biomass yield) = 0.00376 mg product/L _{mm}	Bergfield, A., 2019 (DuPont-49944)
<i>Lemna gibba</i>	GF-3969 plus crop oil (Codacide)	7 d, s	E _r C ₅₀ (growth rate) = 0.00291 mg product/L _{mm} E _y C ₅₀ (yield) = 0.000940 mg product/L _{mm} E _r C ₅₀ (biomass growth rate) = 0.00853 mg product/L _{mm} E _y C ₅₀ (biomass yield) = 0.00204 mg product/L _{mm}	Goudie, O., 2019 (DuPont-49978)
Higher-tier studies (micro- or mesocosm studies)				
Not required				

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

a Summarised in Appendix 2.

Studies have been conducted to assess the toxicity of GF-3969 plus **adjuvant surfactant** DPX-KG691 to fish, aquatic invertebrates and *Lemna gibba*. As GF-3969 is also intended to be used with Codacide **adjuvant surfactant**, a study has also been conducted with *Lemna gibba* as this is shown to be the most sensitive organism. The study indicated a comparable toxicity between the two **adjuvants surfactants** with the formulation (E_rC₅₀ values reported to be 0.00853 mg product/L and >0.00958 mg product/L for Codacide and DPX-KG691, respectively).

zRMS comments:

Studies on toxicity of GF-3969 used with two **adjuvants surfactants** to aquatic organisms were agreed by the zRMS and the endpoints reported in Table 9.5-4 above are confirmed. For summaries of the studies and details of the evaluation, please refer to Appendix 2.

It is noted that influence of **adjuvant surfactant** Codacide on toxicity of GF-3969 was investigated only for *Lemna gibba*. This is agreed by the zRMS since data available for particular active substances indicate that aquatic macrophytes are the most sensitive group of aquatic species.

The Applicant proposed to use in the risk assessment results of the study performed with **adjuvant surfactant** DPX-KG691, however obtained results indicate higher toxicity of the mixture of GF-3969 with Codacide to *Lemna gibba* and for this reason endpoint from study by Goudie (2019) should be used for the risk assessment purposes.

9.5.1.1 Justification for new endpoints

Isoxadifen-ethyl metabolites

The approach for metabolite risk assessment refers to Part 10.2.4 decision scheme of the new aquatic guidance document (EFSA Journal 2013;11(7):3290). The decision scheme is followed step by step.

Step 1: Is the exposure to the metabolite in the toxicity test with the a.s. measured and adequate for assessing the potential effect of the metabolite?

None of the studies with the active substance is adequate for assessing the potential effect of the metabolites. => step 3.

Step 3: Is it clear that the toxophore has been lost from the molecule?

No information is available to demonstrate that the toxophore is lost. => step 4.

Step 4: Identify the species or taxonomic group determining the lowest tier 1 $RAC_{sw,ac}$ for the parent compound. Is the acute metabolite $L(E)C_{50} > 10$ times the a.s. $L(E)C_{50}$ (on a molar basis)?

For isoxadifen-ethyl the lowest tier 1 $RAC_{sw,ac}$ is determined by fish (2.2 µg/L). The metabolites (*i.e.* AE F129431, AE C637375 and AE C642961) are acutely more than 10x less toxic to fish than the parent (on a molar basis). => step 6

Step 6: Assume that the toxicity of the metabolite is equal to the toxicity of the a.s. for all first tier taxonomic groups.

Thus, for AE F129431, AE C637375 and AE C642961 parent endpoints are used in the risk assessment, where no test data are available, as it is the case for aquatic plants.

zRMS comments:

No EU agreed data exist for the safener, isoxadifen-ethyl, and for this reason validation of information provided above against EU agreed endpoints was not possible. Nevertheless, information provided by the Applicant above has been retained for informative purposes, with font colour changed to grey in order to easily distinguish validated from non-validated data.

Combination effects of rimsulfuron, thifensulfuron methyl and isoxadifen-ethyl in GF-3969

Even though toxicity studies for aquatic plants are available for GF-3969, the dose additivity principle has been used to derive the theoretical acute LC/EC_{50} of GF-3969 to fish, *Daphnia*, algae and *Lemna* according to the following equation (EFSA Journal 2013; 11(7):3290, Equation 13):

$$EC_{50,mix-CA} = \left(\sum_i \frac{X(a.s.i)}{EC_{50,(a.s.i)}} \right)^{-1}$$

where: $X(a.s.i)$ is the fraction of the active substance i in the formulation (with $\sum \times(a.s.i)=1$);
 $EC_{50}(a.s.i)$ is the acute toxicity for the active substance i .

According to the EFSA Aquatic Guidance (EFSA, 2013) measured and calculated mixture toxicity should be compared to determine synergistic, additive or antagonistic effects of the formulation. In the following text, the concentration addition (CA) model is used as proposed by EFSA. To determine the respective formulation effect, EFSA proposed to calculate the model deviation ratio (MDR), which divides the calculated mixture toxicity (LC_{50}/EC_{50} mix-CA) by the measured mixture toxicity (LC_{50}/EC_{50} PPP).

Ecotoxicity studies are biological test systems which underlie a certain natural biological variability when repeating a study. Hence, a threshold has to be defined when an increased/decreased mixture

toxicity effect cannot be seen as only additive any longer. EFSA proposes a factor of 5, i.e. if the MDR is between 0.2 and 5 the observed and calculated mixture toxicities are considered in agreement.

Considering the lowest EC_{50} values determined for rimsulfuron, thifensulfuron methyl and isoxadifen-ethyl their nominal concentrations in GF-3969 the resulting $EC_{50, \text{mix-CA}}$ value for GF-3969 were calculated and shown below.

Table 9.5-6: Mixture toxicity assessment for GF-3969

Organism	Fraction considering density % (w/w) ^a			LC ₅₀ /EC ₅₀ (mg/L)			LC/EC _x (mg/L)		MDR ^d
	Rimsulfuron	Thifensulfuron methyl	Isoxadifen-ethyl	Rimsulfuron	Thifensulfuron methyl	Isoxadifen-ethyl	PPP ^b	Mix-ca ^c	
Fish	14.8	9.3	11.1	>390	56.4	0.22	2.386	0.694	0.29
<i>Daphnia</i>				>360	>120	>0.51	4.082	1.601	0.39
Algae				1.2	0.027	>1.26	1.14	0.097	0.09
<i>Lemna gibba</i>				0.0046	0.0013	>1.48	0.001	0.003	2.35

a Product bulk density 1.0 g/cm³

b Measured mixture toxicity, EC_{xPPP} based on GF-3969

c Calculated mixture toxicity, EC_{xmix-CA}

d MDR = EC_{xmix-CA}/EC_{xPPP}

Based on the endpoints for fish, *Daphnia magna* and *Lemna gibba*, the MDR values are between 0.2 and 5, indicating that the formulation does not cause an unexpected increased toxicity compared to the active substances for these organisms. No synergisms or additional toxicity occur due to the co-formulations. For algae the MDR is <0.2 and indicates a potential antagonism (toxicity of the formulation is lower than expected) however this can be explained by the fact that the algae endpoint for isoxadifen-ethyl is a 'greater than' value. According to section 10.3.4 of the EFSA Guidance on Tiered Risk Assessment for Plant Protection Products for Aquatic Organisms in Edge-of-Field Surface Waters (EFSA Journal 2013; 11(7): 3290) when the MDR is 0.2-5, the measured toxicity values for the product can be used in the risk assessment for the formulated product. As the apparent antagonism for algae can be explained by the use of 'greater than' values for the active substances data in the assessment, it is also appropriate to use measured data for the risk assessment of the formulation to algae.

A comparison of the mixture toxicity of the measured ($EC_{50,mix-PPP}$) and the theoretical calculated ($EC_{50,mix-CA}$) can be calculated based on the relative proportions of the active substances in the formulation (PEC_{mix}) using the following equations:

$$\text{Equation 16: } PEC_{mix} = \sum_{i=1}^n PEC_i \quad \text{Equation 19: } p_i = \frac{PEC_i}{PEC_{mix}}$$

and

The PEC_{sw} values for the relevant FOCUS scenarios are summarised in the table below for each active substance in GF-3969. The concentration of the individual active is calculated based on the total concentration (PEC_{mix}) of active substances in the product.

Table 9.5-7: PEC_i and PEC_{mix}

FOCUS Step	Rimsulfuron		Thifensulfuron methyl		Isoxadifen-ethyl		PEC_{mix}
	PEC_{sw}	Pi PEC^a	PEC_{sw}	Pi PEC^a	PEC_{sw}	Pi PEC^a	
	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	
Step 2	0.001790	0.814	0.000270	0.123	0.000138	0.063	0.002198

a Relative proportions of the individual mixture components in the environment (pi PEC)

Secondly, the formula below is used to see if the mixtures are similar or not.

$$EC_{50,mix-CA} \text{ (a.s. in PPP)} / EC_{50,mix-CA} \text{ (a.s. in } PEC_{mix})$$

If the mixtures are similar, then the result is between 0.8-1.2 and a direct comparison of PEC_{mix} with the EC_{xPPP} is feasible. If not, the measured data cannot be used directly for calculating the ETR.

The data used in the calculations are summarised in the tables below.

Table 9.5-8: EC_{mix} based on PEC_{mix}

Organism	$EC_{50, mix-CA}$ (a.s. in PPP) $EC_{50, mix-CA}$ (a.s. in PEC_{mix})	Trigger met? 0.8-1.2
Fish	0.201	No
<i>Daphnia</i>	0.204	No
Algae	0.510	No
<i>Lemna gibba</i>	0.923	yes

The results summarised in the table above indicate that the comparison between the mixture based on PPP and the mixture based on PEC_{mix} are between 0.8-1.2 at FOCUS Step 2 PEC values for *Lemna gibba*; therefore, the next step is to conduct the mixture risk assessment based on a measured mixture toxicity using the equation ($ETR_{mix} = PEC_{mix} / EC_x PPP$ (a.s. based)). The risk assessment is provided in the following section.

For fish, *Daphnia magna* and algae the ratio is below 0.8 and so the risk assessment is based on calculated toxicity and PEC_{mix} is provided (refer to sections below).

zRMS comments:

Since no EU agreed data exist for isoxadifen-ethyl, validation of combined toxicity assessment performed above was not possible. Nevertheless, performed calculations were retained for illustrative purposes, with font colour changed to grey in order to easily distinguish validated from non-validated data.

The combined risk assessment performed with consideration of the EU agreed toxicity data for particular active substances together with results of the studies performed with GF-3969 is presented below.

Comparison of the measured and estimated toxicity of the formulation presents the below table.

Species	Fraction of a.s. in formulation		LC ₅₀ /EC ₅₀ [mg/L]		EC _x [mg a.s./L]		MDR ^{c)}
	Rimsulfuron	Thifensulfuron-methyl	Rimsulfuron	Thifensulfuron-methyl	PPP ^{a)}	Mix-ca ^{b)}	
Fish	0.62	0.38	>390	>56.4	1.63	120.1	73.6
<i>D. magna</i>			>360	>120	2.79	204.5	73.3
Algae			1.2	0.027	0.78	0.07	0.09
<i>Lemna gibba</i>			0.0046	0.0011	0.0007	0.0021	3.0

a) measured mixture toxicity, based on sum of active substances, see Table 9.5-3

b) calculated mixture toxicity, EC_{mix}-CA

c) MDR = EC_{mix-CA}/EC_{PPP}

Above calculations demonstrated that MDR for *Lemna gibba* only is between 0.2 and 5, indicating comparable measured and estimated mixture toxicity and for this reason the combined risk assessment for this species may be based on the measured data.

For fish and *Daphnia* the MDR values are far above 5 showing that the measured mixture toxicity is higher than predicted based on active substance data, while for algae MDR is below 0.2 demonstrating that the measured mixture toxicity is lower comparing to this predicted based on the active substance endpoints. Taking this into account the combined risk assessment for fish and *Daphnia* should be based on the measured mixture toxicity, while for algae - on the predicted mixture toxicity.

In case of algae it was additionally checked if one of the active compounds contributes to >90% of the calculated mixture toxicity.

Species	Substance	Fraction in PPP	Toxicity [mg/L]	Toxic Unit (TU)	% of total TU
Algae	Rimsulfuron	0.62	1.2	0.5167	3.54
	Thifensulfuron-methyl	0.38	0.0046	14.0740	96.46
				Σ14.5907	

Based on above calculations it may be concluded that the toxicity of the mixture to algae may be explained at >90% by presence of thifensulfuron-methyl. Taking this into account, no combined risk assessment is required for this species and evaluation based on individual active substance is sufficient to address the risk.

EFSA (2013) indicates that before the measured mixture toxicity data are used in the risk assessment it should be checked if the mixture composition giving the measured toxicity is similar to the mixture composition at the PEC_{mix} in terms of the relative proportions of the individual active compounds. It is, however, noted that no clear guidance is given for situation when the mixture composition at PEC_{mix} is not similar to the mixture composition giving the measured toxicity. In fact it is only indicated that in such case the measured toxicity data cannot be used in the risk assessment, which should rather be performed with estimated mixture toxicity compared with PEC_{mix}. Nevertheless, the zRMS is of the opinion that in case of clearly higher toxicity of the formulated product risk assessment based on estimated toxicity data may be not sufficiently protective, which will be the case for GF-3969 due to very low toxicity of individual active substances to fish and algae and clearly higher measured toxicity of the product resulting - most probably - from presence of the safener (for which no EU agreed endpoints are available), **adjuvants** ~~surfactants~~ or other co-formulants and not from the synergistic effects of the active compounds. Taking into account that the risk assessment based on the measured formulation data needs to be performed anyway in order to address the risk from the safener and **adjuvants** ~~surfactants~~, the comparison of the mixture composition giving the measured endpoints with mixture composition at PEC_{mix} was not performed. Instead, the risk assessment based on measured formulation toxicity expressed in terms of the formulated product compared with PEC for the formulation was performed. This approach is not ideal, however this is the only way to address the risk from the safener and **adjuvants** ~~surfactants~~.

9.5.2 Risk assessment

Rimsulfuron and metabolites

For rimsulfuron and metabolites, the maximum PEC_{sw} values resulted from the single application at a rate of 135 g GF-3969/ha (equivalent to 1×20 g rimsulfuron/ha) to maize and so this rate is considered in the aquatic risk assessment as it is protective of all intended uses.

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 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC <1) for rimsulfuron for each organism group based on maximum FOCUS Steps 1, 2 and 3 calculations for the use of GF-3969 in maize (1 × 135 g GF-3969/ha and split applications at 2x67.5 GF-3969/ha and 85+50 g GF-3969/ha)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic plants
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Selenastrum capricornutum</i>	<i>Lemna minor</i>
Endpoint (µg/L)		LC ₅₀	NOEC	EC ₅₀	NOEC	E _r C ₅₀	E _r C ₅₀
AF		>390000	125000	>360000	1000	1200	4.60
RAC (µg/L)		100	10	100	10	10	10
FOCUS Scenario	PEC _{sw} (µg/L)	PEC/RAC Ratio					
Step 1							
	6.458	<0.01	<0.01	<0.01	0.06	0.05	14
Step 2^a							
N-Europe	0.949	<0.01	<0.01	<0.01	0.01	0.01	2.1
S-Europe	1.779	<0.01	<0.01	<0.01	0.02	0.01	3.9
Step 3 application scenario							
D3/ditch	0.1153	<0.01	<0.01	<0.01	<0.01	<0.01	0.25
D4/pond	0.027 0.0261	<0.01	<0.01	<0.01	<0.01	<0.01	0.06
D4/stream	0.096	<0.01	<0.01	<0.01	<0.01	<0.01	0.21
D5/pond	0.0224	<0.01	<0.01	<0.01	<0.01	<0.01	0.05
D5/stream	0.103	<0.01	<0.01	<0.01	<0.01	<0.01	0.22
D6/ditch	0.106	<0.01	<0.01	<0.01	<0.01	<0.01	0.23
R1/pond	0.0277	<0.01	<0.01	<0.01	<0.01	<0.01	0.06
R1/stream	0.604	<0.01	<0.01	<0.01	0.01	0.01	1.30
R2/stream	0.170 0.159	<0.01	<0.01	<0.01	<0.01	<0.01	0.37 0.35
R3/stream	0.836	<0.01	<0.01	<0.01	0.01	0.01	1.8
R4/stream	0.840	<0.01	<0.01	<0.01	0.01	0.01	1.8

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

^a Max from Mar-May and Jun-Sep.

For the intended use in maize, for several relevant scenarios (R1, R3 and R4 stream) the calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic plants at FOCUS Steps 1-3 scenarios. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{sw} considering reduced exposure of surface water bodies. The scenarios which triggered a risk (R1 stream, R3 stream and R4 stream) have run-off as the main route of entry and so buffer zones and filter strips are considered in the mitigation.

Table 9.5-10: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC <1) for rimsulfuron based on maximum FOCUS Step 4 calculations and toxicity data for aquatic plants with mitigation for the use of GF-3969 in maize

Intended use		Maize
Active substance		Rimsulfuron
Application rate (g a.s./ha)		1 × 20 g
Nozzle reduction	No-spray buffer (m)	10
	Vegetated filter strip (m)	10
None	R1/stream	0.274
None	R3/stream	0.377
None	R4/stream	0.382
RAC (µg/L)		
0.46		PEC/RAC ratio
None	R1/stream	0.60
None	R3/stream	0.82
None	R4/stream	0.83

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration

For the intended application of GF-3969 to maize acceptable risk to aquatic organisms from exposure to rimsulfuron is shown with a 10 m buffer zone and vegetated filter strip.

zRMS comments:

The risk assessment for rimsulfuron is in general agreed by the zRMS. It is, however, noted that it was performed with consideration of the surface water exposure resulting from single application of GF-3969 and split applications were not considered although they give higher PEC_{sw} in some scenarios. Taking this into account, respective corrections were made in Table 9.5-8 in order to cover all intended uses of GF-3969 in the Central Zone. Single application of maximum rate gave highest Step 4 PEC_{sw} and no corrections in Table 9.5-9 were necessary.

Overall, based on the performed calculations acceptable risk to aquatic species may be concluded, provided that 10 m vegetated filter strip to surface water bodies is respected in scenarios R1, R3 and R4. In remaining scenarios no risk mitigation measures are necessary.

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

Please note also that additional calculations may be required by the Member States that do not accept surface water exposure calculated according to FOCUS recommendations.

Metabolites of rimsulfuron

Table 9.5-11: Aquatic organisms: acceptability of risk (PEC/RAC <1) for IN-70941 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GF-3969 in maize

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Selenastrum capricornutum</i>
Endpoint (µg/L)		LC ₅₀ >110000	EC ₅₀ 95000	E _r C ₅₀ >8900
AF		100	100	10
RAC (µg/L)		>1100	950	>890
FOCUS Scenario	PEC _{gl-max} (µg/L) ^a	PEC/RAC Ratio		
Step 1				
	7.754	<0.01	<0.01	<0.01
Step 2^b				
N-Europe	1.170	<0.01	<0.01	<0.01
S-Europe	2.221	<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.

a Worst case global maximum PEC_{sw} for alkaline and acidic IN-70941.

b Max from Mar-May and Jun-Sep.

Table 9.5-12: Aquatic organisms: acceptability of risk (PEC/RAC <1) for IN-70942 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GF-3969 in maize

Group		Fish acute	Inverteb. acute	Algae	Aquatic plants		Sed. dwell. prolonged
Test species		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Selenastrum capricornutum</i>	<i>Lemna gibba</i>		<i>Chironomus riparius</i>
Endpoint (µg/L)		LC ₅₀ 180000	EC ₅₀ 178000	E _r C ₅₀ >10000	E _r C ₅₀ >20		NOEC >200 µg a.s./kg
AF		100	100	10	10		10
RAC (µg/L)		1800	1780	>1000	>2.0		>20 µg a.s./kg
FOCUS Scenario	PEC _{gl-max} (µg/L)	PEC/RAC Ratio				PEC _{gl-max} (µg/kg)	PEC/RAC Ratio
Step 1							
	4.389	<0.01	<0.01	<0.01	2.19	8.289	0.41
Step 2^a							
N-Europe	0.666	<0.01	<0.01	<0.01	0.33	1.272	0.06
S-Europe	1.243	<0.01	<0.01	<0.01	0.62	2.385	0.12

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

a Max from Mar-May and Jun-Sep.

Table 9.5-13: Aquatic organisms: acceptability of risk (PEC/RAC <1) for IN-E9260 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GF-3969 in maize

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Selenastrum subcapitatus</i>
Endpoint (µg/L)		LC ₅₀ >314000	EC ₅₀ 184000	E _r C ₅₀ >100000
AF		100	100	10
RAC (µg/L)		>3140.00	1840	>10000
FOCUS Scenario	PEC _{gl-max} (µg/L)	PEC/RAC Ratio		
Step 1				
	1.333	<0.01	<0.01	<0.01
Step 2^a				
N-Europe	0.203	<0.01	<0.01	<0.01
S-Europe	0.388	<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.

a Max from Mar-May and Jun-Sep.

An acceptable aquatic risk is concluded from the exposure to rimsulfuron metabolites at FOCUS Step 1 and 2 following the uses of GF-3969 in maize.

zRMS comments:

The risk assessment for rimsulfuron metabolites is agreed by the zRMS. Performed calculations cover all intended uses of GF-3969 in the Central Zone (including split applications). Acceptable risk may be concluded with no need for risk mitigation measures.

It is noted that no EU agreed toxicity data are reported in EFSA Scientific Report (2005) 45 for metabolites IN-J0290 and IN-JF999. However, information available from the EU renewal of rimsulfuron indicate that these two compounds are of low toxicity to aquatic species with endpoints close of exceeding 100 mg/L and for this reason no unacceptable risk from these metabolites is expected when GF-3969 is used according to recommendations.

Please note also that additional calculations may be required by the Member States that do not accept surface water exposure calculated according to FOCUS recommendations.

Thifensulfuron-methyl and metabolites

For thifensulfuron methyl and metabolites the maximum PEC_{sw} values resulted from the single application at a rate of 135 g GF-3969/ha to maize (12.5 g thifensulfuron methyl/ha) and so this rate is considered in the aquatic risk assessment as it is protective of all intended uses. The PEC_{sw} values which resulted from the split applications were comparable to the highest single application rate and so do not affect the mitigation required.

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 9.5-14: Aquatic organisms: acceptability of risk (PEC/RAC <1) for thifensulfuron methyl for each organism group based on maximum FOCUS Steps 1, 2 and 3 calculations for the use of GF-3969 in maize (1 × 135 g GF-3969/ha and split applications at 2x67.5 GF-3969/ha and 85+50 g GF-3969/ha)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. Acute Prolonged	Aquatic plants	
Test species		<i>Oncorhynchus mykiss</i> ^a	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Chironomus riparius</i>	<i>Lemna gibba</i> ^a	<i>Vallisneria americana</i>
Endpoint (µg/L)		LC ₅₀ >56400	NOEC 10600	EC ₅₀ >120000	NOEC 99000	E _b C ₅₀ 27	EC ₅₀ NOEC 100000	E _r C ₅₀ 1.1 1.3	E _r C ₅₀ 0.23
AF		100	10	100	10	10	100 40	10	10
RAC (µg/L)		>564	1060	1200	9900	2.7	1000 10000	0.11 0.13	0.023
FOCUS Scenario	PEC (µg/L) ^{gl-max}	PEC/RAC Ratio							
Step 1									
	4.23	0.01	<0.01	0.06	<0.01	1.6	<0.01	38 33	184
Step 2*									
N-Europe	0.19	<0.01	<0.01	<0.01	<0.01	0.07	<0.01	1.7 1.5	8.0
S-Europe	0.27	<0.01	<0.01	<0.01	<0.01	0.1	<0.01	2.5 2.1	12
Step 3 - application scenario									
D3/ditch	0.07	<0.01	<0.01	<0.01	<0.01	0.03	<0.01	0.64 0.54	2.9
D4/pond	0.003	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.03 0.02	0.12
D4/stream	0.06	<0.01	<0.01	<0.01	<0.01	0.02	<0.01	0.55 0.46	2.4
D5/pond	0.003	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.03 0.02	0.12
D5/stream	0.06	<0.01	<0.01	<0.01	<0.01	0.37	<0.01	0.55 0.46	2.6
D6/ditch	0.07	<0.01	<0.01	<0.01	<0.01	0.03	<0.01	0.64 0.54	2.9
R1/pond	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.09 0.08	0.37
R1/stream	0.15	<0.01	<0.01	<0.01	<0.01	0.37	<0.01	1.4 1.2	6.6
R2/stream	0.06	<0.01	<0.01	<0.01	<0.01	0.02	<0.01	0.55 0.46	2.6
R3/stream	0.19	<0.01	<0.01	<0.01	<0.01	0.07	<0.01	1.7 1.5	8.1
R4/stream	0.17	<0.01	<0.01	<0.01	<0.01	0.06	<0.01	1.5 1.3	7.6

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

* Max from Mar-May and Jun-Sep.

a Endpoint taken from study with Thifensulfuron methyl 50SG.

For the intended use in maize, for several relevant scenarios (D3, D4, D5, D6, R1, R2, R3 and R4) the calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic plants at FOCUS Steps 1-3 scenarios. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{sw} considering reduced exposure of surface water bodies.

Table 9.5-15: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC <1) for thifensulfuron methyl based on maximum FOCUS Step 4 calculations and toxicity data for aquatic plants with mitigation for the use of GF-3969 in maize

Active substance		Thifensulfuron methyl		
Application rate (g a.s./ha)		1 × 12.50 g		
Nozzle reduction	No-spray buffer (m)	10	20	10
	Vegetated filter strip (m)	10	20	10 VFSMod
None	D3/ditch	0.01	-	
	D4/stream	0.01 0.002	-	
	D5/stream	0.01	-	
	D6/ditch	0.01		
	R1/stream	0.069	0.036	0.01
	R2/stream	0.014	-	
	R3/stream	0.085	0.044	0.014
	R4/stream	0.079	0.041	0.010
RAC (µg/L)		PEC/RAC		
0.023				
None	D3/ditch	0.43	-	-
	D4/stream	0.09	-	-
	D5/stream	0.43	-	-
	D6/ditch	0.43	-	-
	R1/stream	3.01	1.60	0.43
	R2/stream	0.43	-	-
	R3/stream	3.68	1.91	0.61
	R4/stream	3.43	1.78	0.43

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

For the intended application of GF-3969 to maize acceptable risk to aquatic organisms from exposure to thifensulfuron methyl is shown with a 10 m no-spray buffer zone with vegetated filter strip for scenarios D3, D4, D5, D6 and R2. For scenarios R1 stream, R3 stream and R4 stream a 10 m buffer with VFSmod is required.

Refined risk assessment

In the published outcome of the consultation of member states and EFSA on the confirmatory data provided for thifensulfuron methyl (EFSA 2019)⁹, the RMS (UK) supported the use of a geomean endpoint of 0.53 µg a.s./L for the aquatic plant risk assessment based on the most sensitive monocot species. The RMS noted ‘the RMS considers that there are enough studies and parameters measured to justify a geometric mean approach and the proposed endpoint is protective’.

Based on the geomean endpoint of 0.53 µg a.s./L and the assessment factor of 10, relevant for aquatic plant endpoints, the refined RAC of 0.053 µg a.s./L is applied to the risk assessment below.

⁹ EFSA technical report: Outcome of the consultation with member states, the applicant and EFSA on the pesticide risk assessment for thifensulfuron-methyl in light of confirmatory data. April 2019.

Table 9.5-16: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC <1) for thifensulfuron methyl based on refined RAC and FOCUS Step 4 calculations with mitigation for 1 × 12.5 g a.s./ha

Active substance		Thifensulfuron methyl		
Application rate (g a.s./ha)		1 × 12.50 g		
Nozzle reduction	No-spray buffer (m)	10	20	10
	Vegetated filter strip (m)	10	20	10 VFSSMod
None	D3/ditch	0.01	-	-
	D4/stream	0.002	-	-
	D5/stream	0.01	-	-
	D6/ditch	0.01	-	-
	R1/stream	0.069	0.036	0.010
	R2/stream	0.014	-	-
	R3/stream	0.085	0.044	0.014
	R4/stream	0.079	0.041	0.010
RAC (µg/L)		PEC/RAC		
0.053				
None	D3/ditch	0.18	-	-
	D4/stream	0.04	-	-
	D5/stream	0.18	-	-
	D6/ditch	0.18	-	-
	R1/stream	1.30	0.70	0.19
	R2/stream	0.26	-	-
	R3/stream	1.89	1.10	0.26
	R4/stream	1.49	0.77	0.19

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

Based on the refined assessment acceptable risk to aquatic organisms from exposure to thifensulfuron methyl is shown with a 10 m no-spray buffer zone with vegetated filter strip for scenarios D3, D4, D5, D6 and R2. For scenarios R1 stream, R3 stream and R4 stream a 20 m buffer or 10 m buffer with VFSSmod is required.

To ensure appropriate mitigation is also applied to the multiple applications considered in the GAP, the risk assessment is also presented for 2 × 6.25 g thifensulfuron methyl/ha and 7.87 + 4.64 g thifensulfuron methyl/ha.

Table 9.5-17: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC <1) for thifensulfuron methyl based on refined RAC and FOCUS Step 4 calculations with mitigation for 2 × 6.25 g a.s./ha

Active substance		Thifensulfuron methyl		
Application rate (g a.s./ha)		2 × 6.25 g		
Nozzle reduction	No-spray buffer (m)	10	20	10
	Vegetated filter strip (m)	10	20	10 VFSMod
None	D3/ditch	0.005	-	-
	D4/stream	0.005	-	-
	D5/stream	0.006	-	-
	D6/ditch	0.005	-	-
	R1/stream	0.047	-	-
	R2/stream	0.006	-	-
	R3/stream	0.043	-	-
	R4/stream	0.072	0.037	0.004
RAC (µg/L)		PEC/RAC		
0.053				
None	D3/ditch	0.09	-	-
	D4/stream	0.09	-	-
	D5/stream	0.11	-	-
	D6/ditch	0.09	-	-
	R1/stream	0.89	-	-
	R2/stream	0.11	-	-
	R3/stream	0.81	-	-
	R4/stream	1.36	0.70	0.08

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

Based on the refined assessment acceptable risk to aquatic organisms from exposure to thifensulfuron methyl is shown for application of 2 × 6.25 g a.s./ha with a 10 m no-spray buffer zone with vegetated filter strip for scenarios D3, D4, D5, D6, R1, R2 and R3 stream. For R4 stream a 20 m buffer or 10 m buffer with VFSmod is required.

Table 9.5-18: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC <1) for thifensulfuron methyl based on refined RAC and FOCUS Step 4 calculations with mitigation for split application 7.87 + 4.63 g a.s./ha

Active substance		Thifensulfuron methyl		
Application rate (g a.s./ha)		7.87 + 4.63g		
Nozzle reduction	No-spray buffer (m)	10	20	10
	Vegetated filter strip (m)	10	20	10 VFSMod
None	D3/ditch	0.006	-	-
	D4/stream	0.006	-	-
	D5/stream	0.007	-	-
	D6/ditch	0.006	-	-
	R1/stream	0.046	-	-
	R2/stream	0.007	-	-
	R3/stream	0.053	0.028	0.007
	R4/stream	0.052	-	-
RAC (µg/L)		PEC/RAC		
0.053				
None	D3/ditch	0.11	-	-
	D4/stream	0.11	-	-
	D5/stream	0.13	-	-
	D6/ditch	0.11	-	-
	R1/stream	0.87	-	-
	R2/stream	0.13	-	-
	R3/stream	1.0	0.53	0.13
	R4/stream	0.98	-	-

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

Based on the refined assessment acceptable risk to aquatic organisms from exposure to thifensulfuron methyl is shown for application of 7.87 + 4.63 g a.s./ha acceptable risk is shown for all scenarios with a 10 m no-spray buffer zone with vegetated filter strip.

zRMS comments:

The risk assessment for thifensulfuron is in general agreed by the zRMS with minor correction of the *Lemna gibba* endpoint for calculations based on Step 1-3 PEC_{SW}, which had, however, no significant impact on the outcome of these calculations. All calculations based on Tier 1 data were performed with consideration of the maximum surface water exposure calculated for all intended uses if GF-3969 (including split applications).

Tier 1 refinement of the risk was performed with consideration of an endpoint for the most sensitive species of aquatic macrophytes, covering the risk for all other species. Acceptable risk to aquatic species could be concluded in majority of scenarios, provided that respective risk mitigation measures are considered (10 m unsprayed buffer zone or 10 m vegetated filter strip). However, in scenarios R1, R3 and R4 the risk could not be resolved for Step 4 simulations performed with consideration of indications of FOCUS work group on landscape and mitigation factors and acceptable risk with assumption of 10 meters vegetated filter strip could be concluded only when VFSmod was used in surface water exposure assessment.

Since not all Member States accept use of VFSmod, the Applicant performed Tier 2A refinement, based on geometric mean endpoint for aquatic species (0.53 µg a.s./L), calculated by the RMS (UK) in the course of the evaluation of confirmatory data for thifensulfuron-methyl. Although no clear conclusion regarding use of this endpoint may be found in EFSA Supporting publication 2020:EN-1627, analysis of the comments submitted by particular Member States and EFSA indicates that this approach has been agreed. It should be also noted that toxicity data for several aquatic macrophyte species are available and in line with EFSA aquatic guidance (2013) calculation of the geometric mean endpoint is a relevant option for refinement. The geometric mean of 0.53 µg/L is still protective for all other aquatic species.

When geometric mean EC₅₀ for aquatic macrophytes is considered, following risk mitigation measures are required, depending on the use pattern:

1. Single application at 1x135 g GF-3969/ha:
 - scenarios D3, D4, D5, D6: 10 m unsprayed buffer zone to surface water bodies,
 - scenario R2: 10 m VFS to surface water bodies,
 - scenarios R1, R3 and R4: 20 m VFS (when based on indications of FOCUS L&M) or 10 m VFS (when VFSmod is used).
2. Split application at 2x67.5 g GF-3969/ha with 7 days interval:
 - scenarios D3, D4, D5, D6: 10 m unsprayed buffer zone to surface water bodies,
 - scenarios R1, R2, R3: 10 m VFS to surface water bodies,
 - scenario R4: 20 m VFS (when based on indications of FOCUS L&M) or 10 m VFS (when VFSmod is used).
3. Split application at 85+50 g GF-3969/ha with 7 days interval:
 - scenarios D3, D4, D5, D6: 10 m unsprayed buffer zone to surface water bodies,
 - scenarios R1, R2, R4: 10 m VFS to surface water bodies,
 - scenario R4: 20 m VFS (when based on indications of FOCUS L&M) or 10 m VFS (when VFSmod is used).

Concerned Member States must decide on relevant risk mitigation in their countries.

Please note also that additional calculations may be required by the Member States that do not accept surface water exposure calculated according to FOCUS recommendations.

Metabolites of thifensulfuron methyl

Table 9.5-19: Aquatic organisms: acceptability of risk (PEC/RAC <1) for IN-L9225 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GF-3969 in maize (1 × 135 g GF-3969/ha)

Group		Fish acute	Inverteb. acute	Algae	Aquatic plants
Test species		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Lemna gibba</i>
Endpoint (µg/L)		LC ₅₀ >120000	EC ₅₀ >130000	E _r C ₅₀ 36500	E _r C ₅₀ 82200
AF		100	100	10	10
RAC (µg/L)		1200	1300	3650	8220
FOCUS Scenario	PEC (µg/L) ^{gl-max}	PEC/RAC Ratio			
Step 1					
	8.45	0.01	0.01	<0.01	<0.01
Step 2^a					
N-Europe	0.823	<0.01	<0.01	<0.01	<0.01
S-Europe	1.53	<0.01	<0.01	<0.01	<0.01

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

a Max from Mar-May and Jun-Sep.

Table 9.5-20: Aquatic organisms: acceptability of risk (PEC/RAC <1) for IN-JZ789 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GF-3969 in maize (1 × 135 g GF-3969/ha)

Group		Fish acute	Inverteb. acute	Algae	Aquatic plants
Test species		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Lemna gibba</i>
Endpoint (µg/L)		LC ₅₀ >940	EC ₅₀ >1100	E _r C ₅₀ >1280	E _r C ₅₀ 100000
AF		100	100	10	10
RAC (µg/L)		9.4	11	128	10000
FOCUS Scenario	PEC (µg/L) ^{gl-max}	PEC/RAC Ratio			
Step 1					
	8.45	0.90	0.77	0.07	<0.01
Step 2^a					
N-Europe	0.823	0.09	0.14	0.01	<0.01
S-Europe	1.53	0.16	0.14	0.01	<0.01

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

a Max from Mar-May and Jun-Sep.

Table 9.5-21: Aquatic organisms: acceptability of risk (PEC/RAC <1) for IN-V7160 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GF-3969 in maize (1 × 135 g GF-3969/ha)

Group		Fish acute	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic plants
Test species		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Lemna gibba</i>
Endpoint (µg/L)		LC ₅₀ 1000	EC ₅₀ >1300	NOEC 11000	E _r C ₅₀ >11000	E _r C ₅₀ >100000
AF		100	100	10	10	10
RAC (µg/L)		10	13	1100	1100	10000
FOCUS Scenario	PEC ^{gl-max} (µg/L)	PEC/RAC Ratio				
Step 1						
	8.45	0.84	0.65	0.01	0.01	<0.01
Step 2^a						
N-Europe	0.823	0.08	0.06	<0.01	<0.01	<0.01
S-Europe	1.53	0.15	0.12	<0.01	<0.01	<0.01

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

a Max from Mar-May and Jun-Sep.

Table 9.5-22: Aquatic organisms: acceptability of risk (PEC/RAC <1) for IN-A4098 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GF-3969 in maize (1 × 135 g GF-3969/ha)

Group		Fish acute	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic plants
Test species		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Lemna gibba</i>
Endpoint (µg/L)		LC ₅₀ >930	EC ₅₀ >99000	NOEC 32000	E _r C ₅₀ >10000	E _r C ₅₀ >10000
AF		100	100	10	10	10
RAC (µg/L)		9.3	990	3200	1000	1000
FOCUS Scenario	PEC ^{gl-max} (µg/L)	PEC/RAC Ratio				
Step 1						
	8.45	0.91	0.01	<0.01	0.01	0.01
Step 2^a						
N-Europe	0.823	0.09	<0.01	<0.01	<0.01	<0.01
S-Europe	1.53	0.16	<0.01	<0.01	<0.01	<0.01

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

a Max from Mar-May and Jun-Sep.

Table 9.5-23: Aquatic organisms: acceptability of risk (PEC/RAC <1) for IN-W8268 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GF-3969 in maize (1 × 135 g GF-3969/ha)

Group		Fish acute	Inverteb. acute	Algae	Aquatic plants
Test species		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Lemna gibba</i>
Endpoint (µg/L)		LC ₅₀ >115000	EC ₅₀ >125000	E _r C ₅₀ >31600	E _r C ₅₀ >100000
AF		100	100	10	10
RAC (µg/L)		1150	1250	3160	10000
FOCUS Scenario	PEC ^{gl-max} (µg/L)	PEC/RAC Ratio			
Step 1					
	8.45	0.01	0.01	<0.01	<0.01
Step 2^a					
N-Europe	0.823	<0.01	<0.01	<0.01	<0.01
S-Europe	1.53	<0.01	<0.01	<0.01	<0.01

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration;
a Max from Mar-May and Jun-Sep.

Table 9.5-24: Aquatic organisms: acceptability of risk (PEC/RAC <1) for IN-L9223 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GF-3969 in maize (1 × 135 g GF-3969/ha)

Group		Inverteb. acute	Inverteb. prolonged	Algae	Aquatic plants
Test species		<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Lemna gibba</i>
Endpoint (µg/L)		EC ₅₀ >1200	NOEC 13000	E _r C ₅₀ >1300	E _r C ₅₀ >172100
AF		100	10	10	10
RAC (µg/L)		12	1300	130	17210
FOCUS Scenario	PEC _{gl-max} (µg/L)				
Step 1					
	8.45	0.70	0.01	0.06	<0.01
Step 2^a					
N-Europe	0.823	0.07	<0.01	0.01	<0.01
S-Europe	1.53	0.13	<0.01	0.01	<0.01

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration;
a Max from Mar-May and Jun-Sep.

Table 9.5-25: Aquatic organisms: acceptability of risk (PEC/RAC <1) for IN-L9226 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GF-3969 in maize (1 × 135 g GF-3969/ha)

Group		Algae	Aquatic plants
Test species		<i>Pseudokirchneriella subcapitata</i>	<i>Lemna gibba</i>
Endpoint (µg/L)		EC ₅₀ >89000	E _r C ₅₀ 310
AF		10	10
RAC (µg/L)		8900	31
FOCUS Scenario	PEC _{gl-max} (µg/L)		
Step 1			
	8.45	<0.01	0.27
Step 2^a			
N-Europe	0.823	<0.01	0.03
S-Europe	1.53	<0.01	0.05

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration;
a Max from Mar-May and Jun-Sep.

Table 9.5-26: Aquatic organisms: acceptability of risk (PEC/RAC <1) for IN-A5546 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GF-3969 in maize (1 × 135 g GF-3969/ha)

Group		Algae	Aquatic plants
Test species		<i>Pseudokirchneriella subcapitata</i>	<i>Lemna gibba</i>
Endpoint (µg/L)		E _r C ₅₀ >110000	E _r C ₅₀ >40400
AF		10	10
RAC (µg/L)		11000	4040
FOCUS Scenario	PEC _{gl-max} (µg/L)		
Step 1			
	8.45	<0.01	<0.01
Step 2^a			
N-Europe	0.823	<0.01	<0.01
S-Europe	1.53	<0.01	<0.01

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration;
a Max from Mar-May and Jun-Sep.

Table 9.5-27: Aquatic organisms: acceptability of risk (PEC/RAC <1) for 2-acid-3-triuret for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GF-3969 in maize (1 × 135 g GF-3969/ha)

Group		Algae	Aquatic plants
Test species		<i>Pseudokirchneriella subcapitata</i>	<i>Lemna gibba</i>
Endpoint (µg/L)		E _r C ₅₀ >100000	E _r C ₅₀ >100000
AF		10	10
RAC (µg/L)		10000	10000
FOCUS Scenario	PEC _{gl-max} (µg/L)		
Step 1			
	8.45	0.00	0.00
Step 2^a			
N-Europe	0.823	0.00	0.00
S-Europe	1.53	0.00	0.00

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration;
a Max from Mar-May and Jun-Sep.

Table 9.5-28: Aquatic organisms: acceptability of risk (PEC/RAC <1) for IN-B5528 based on FOCUS Steps 1 and 2 calculations for the use of GF-3969 in maize (1 × 135 g GF-3969/ha)

Group			Aquatic plants
Test species			<i>Lemna gibba</i>
Endpoint (µg/L)			E _r C ₅₀ >119520
AF			10
RAC (µg/L)			11952
FOCUS Scenario	PEC _{gl-max} (µg/L)		
Step 1			
	8.45		<0.01
Step 2^a			
N-Europe	0.823		<0.01
S-Europe	1.53		<0.01

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration;
a Max from Mar-May and Jun-Sep.

Table 9.5-29: Aquatic organisms: acceptability of risk (PEC/RAC <1) for IN-D8858 based on FOCUS Steps 1 and 2 calculations for the use of GF-3969 in maize (1 × 135 g GF-3969/ha)

Group		Algae	Aquatic plants
Test species		<i>Pseudokirchneriella subcapitata</i>	<i>Lemna gibba</i>
Endpoint (µg/L)		E _r C ₅₀ >45	E _r C ₅₀ >44
AF		10	10
RAC (µg/L)		4.5	4.4
FOCUS Scenario	PEC _{gl-max} (µg/L)		
Step 1			
	8.45	1.9	1.9
Step 2^a			
N-Europe	0.823	0.18	0.2
S-Europe	1.53	0.34	0.35

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration;
a Max from Mar-May and Jun-Sep.

An acceptable aquatic risk is concluded from the exposure to thifensulfuron methyl metabolites at FOCUS Step 1 and 2 following the uses of GF-3969 in maize.

zRMS comments:

The risk assessment for thifensulfuron-methyl metabolites is agreed by the zRMS. Performed calculations cover all intended uses of GF-3969 in the Central Zone (including split applications). Acceptable risk may be concluded with no need for risk mitigation measures.

Isoxadifen-ethyl and metabolites

For isoxadifen-ethyl and metabolites the maximum PEC_{sw} values resulted from the single application at a rate of 135 g GF-3969/ha to maize and so this rate is considered in the aquatic risk assessment as it is protective of all intended uses.

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 9.5-30: Aquatic organisms: acceptability of risk (PEC/RAC <1) for isoxadifen-ethyl for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GF-3969 in maize

Group		Fish acute	Inverteb. acute	Algae	Macrophytes
Test species		<i>Lepomis macrochirus</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Lemna gibba</i>
Endpoint (µg/L)		LC ₅₀ 220	E _r C ₅₀ >510	E _b C ₅₀ >126	E _b C ₅₀ >1480
AF		100	100	10	10
RAC (µg/L)		2.2	5.10	126	>148
FOCUS Scenario	PEC _{gl-max} (µg/L)				
Step 1					
	2.68	1.22	0.52	0.02	0.02
Step 2^a					
N-Europe	0.138	0.06	0.03	<0.01	<0.01
S-Europe	0.138	0.06	0.03	<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.

^a Max from Mar-May and Jun-Sep.

Table 9.5-31: Aquatic organisms: acceptability of risk (PEC/RAC <1) for AE F129431-for each organism group based on FOCUS Step 1 calculations for the use of GF-3969 in maize

Group		Fish acute	Inverteb. acute	Algae	Macrophytes
Test species		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Lemna gibba</i>
Endpoint (µg/L)		LC ₅₀	EC ₅₀	ErC ₅₀	ErC ₅₀
AF		>100000	>150000	>100000	>1480 ^a
RAC (µg/L)		100	100	10	10
FOCUS Scenario	PEC (µg/L) ^{gl-max}	1000	1500	>10000	>148
PEC/RAC Ratio					
Step 1					
	7.63	0.01	0.01	<0.01	0.05

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

a Based on active substance endpoint

Table 9.5-32: Aquatic organisms: acceptability of risk (PEC/RAC <1) for AE C637375 for each organism group based on FOCUS Step 1 calculations for the use of GF-3969 in maize

Group		Fish acute	Inverteb. acute	Algae	Macrophytes
Test species		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Lemna gibba</i>
Endpoint (µg/L)		LC ₅₀	EC ₅₀	ErC ₅₀	ErC ₅₀
AF		>15200	>28000	>39700	>1480 ^a
RAC (µg/L)		100	100	10	10
FOCUS Scenario	PEC (µg/L) ^{gl-max}	>152	>280	>3970	>148
PEC/RAC Ratio					
Step 1					
	1.36	0.01	<0.01	<0.01	0.009

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

a Based on active substance endpoint

Table 9.5-33: Aquatic organisms: acceptability of risk (PEC/RAC <1) for AE C642961 for each organism group based on FOCUS Step 1 calculations for the use of GF-3969 in maize

Group		Fish acute	Inverteb. acute	Algae	Macrophytes
Test species		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Lemna gibba</i>
Endpoint (µg/L)		LC ₅₀	EC ₅₀	ErC ₅₀	ErC ₅₀
AF		>10000	>7000	>10000	>1480 ^a
RAC (µg/L)		100	100	10	10
FOCUS Scenario	PEC (µg/L) ^{gl-max}	>100	>70	>1000	>148
PEC/RAC Ratio					
Step 1					
	0.87	0.01	0.01	<0.01	0.0059

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

a Based on active substance endpoint

An acceptable aquatic risk was concluded from the exposure to isoxadifen-ethyl and its metabolites following the uses of GF-3969 in maize.

zRMS comments:

In absence of the EU agreed aquatic toxicity data for isoxadifen-ethyl, validation of calculations presented in tables above was not possible. Nevertheless, performed calculations have been retained for informative purposes, with font colour changed to grey in order to easily distinguish validated from non-validated data. In case the endpoints were confirmed at the EU level, acceptable risk from exposure to isoxadifen-ethyl would be concluded.

Combination effects of rimsulfuron, thifensulfuron methyl and isoxadifen-ethyl in GF-3969

The assessment of endpoints for fish, daphnia and algae met the requirement for calculated mixture toxicity to be used in the risk assessment of the product. *Lemna gibba* met the criteria for the measured product endpoints to be used in the risk assessment.

For a mixture risk assessment based on calculated mixture toxicity, the Exposure-Toxicity Ratio (ETR) is calculated by dividing PEC_{mix} by the calculated mixture toxicity assuming concentration addition according to the EFSA Aquatic Guidance (EFSA 2013). For the combined risk assessment of rimsulfuron, thifensulfuron methyl and isoxadifen-ethyl the ETR was calculated for fish, aquatic invertebrate, algae and aquatic plants using the following equation as given in the EFSA Aquatic Guidance (EFSA 2013):

Equation 1:
$$ETR_{mix-CA} = \frac{PEC_{mix}}{ECx_{mix-CA}}$$

For fish and *Daphnia*, if $ETR_{mix-CA} < \text{trigger of } 0.01 = \text{low risk}$

For algae and aquatic plants, if $ETR_{mix-CA} < \text{trigger of } 0.1 = \text{low risk}$

In the following tables the combined risk assessment is based on maximum FOCUS Step 2 PEC_{sw} values.

Table 9.5-34: Combined risk assessment of rimsulfuron, thifensulfuron methyl and isoxadifen-ethyl for acceptability of risk to fish, aquatic invertebrate, algae and aquatic macrophytes ($ETR_{mix} < 1$) based on maximum FOCUS Step 2 values calculations for the use of GF-3969 in maize

Organism	FOCUS Step	PEC (mg/L) mix^a	Mixture toxicity of the product (a.s. based) (mg/L)	ETR_{mix}	Trigger
Fish	FOCUS Step 2	0.002198	0.691 ^b	0.003	0.01
<i>Daphnia</i>			1.606 ^b	0.0014	
Algae			0.097 ^b	0.023	0.1
<i>Lemna gibba</i>			0.001	2.2	0.1

ETR_{mix} values above the relevant trigger are shown in bold.

a Total exposure concentration of the mixture (a.s. based);

b Calculated mixture toxicity (a.s. in PEC_{mix}) ($ECx_{mix-CA} = 1/\sum (pi PEC/ECx i)$) [mg a.s./L]

The risk assessment for fish, *Daphnia* and algae indicates a low risk (ETR_{mix} is < 0.001 for fish and *Daphnia* and < 0.1 for algae) based on the maximum potential exposure at FOCUS Step 2.

For *Lemna gibba*, a potential risk is indicated and so further refinement can be considered at FOCUS Steps 3 and 4.

In the following table, the risk combined risk to *Lemna gibba* is assessed further using FOCUS Step 4 10 m buffer PEC_{sw} values for rimsulfuron, 10 m buffer with VFSmod for thifensulfuron methyl and Step 3 is applied for isoxadifen-ethyl. The maximum PEC_{sw} values resulted from R1 stream, R3 stream and R4 stream and so these are considered in the risk assessment as they are protective of all scenarios.

This mitigation is consistent with the mitigation required for the risk assessment of the individual active substances.

Table 9.5-35: Combined risk assessment of rimsulfuron, thifensulfuron methyl and isoxadifen-ethyl for acceptability of risk to fish, aquatic invertebrate, algae and aquatic macrophytes (ETR_{mix} <1) based on maximum FOCUS Step 3 and 4 values calculations for the use of GF-3969 in maize

Organism	Scenario	PEC mix ^a (mg/L)	Mixture toxicity of the product (a.s. based) (mg/L)	ETR _{mix}	Trigger
<i>Lemna gibba</i>	R1/stream	0.000667	0.001	0.667	0.1
	R3/stream	0.000468	0.001	0.468	0.1
	R4/stream	0.000446	0.001	0.446	0.1

ETR_{mix} values above the relevant trigger are shown in bold.

a Total exposure concentration of the mixture (a.s. based).

The ETR_{mix} for *Lemna gibba* exceeds the trigger of 0.1 when including the mitigation applied for the active substances. The PEC_{mix} provides a worst-case assessment of exposure as it considers combined exposure from each of the active substances at the same time.

However as the MDR analysis confirmed there is no enhanced toxicity from the formulation in comparison to the active substances, there should be no increased risk from the formulation than from the individual active substances. As acceptable risk has been shown for each of the active substances with appropriate mitigation and so acceptable risk from the formulation to *Lemna gibba* can also be concluded.

PEC_{sw} values have also been calculated for the product based on spray drift. Full details of the calculations are provided in the Core, Part B, Section 8.

The maximum predicted exposure resulted from the single application at 1 × 135 g product/ha, and so these PEC_{sw} values are used in the risk assessment as they are protective of all intended uses. The product PEC_{sw} values are compared with the measured product effect concentrations (L/EC₅₀ values) in the following table.

Table 9.5-36: Aquatic organisms: acceptability of risk (PEC/RAC <1) for GF-3969 for each organism group based on drift calculations for the use at rate of 1 × 135 g/ha in maize

Group		Fish acute	Inverteb. acute	Algae	Macrophytes
Test species		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Lemna gibba</i>
Endpoint (µg/L)		LC ₅₀ 6780	EC ₅₀ 11600	E _r C ₅₀ 3250	E _r C ₅₀ 2.91 4.11
AF		100	100	10	10
RAC (µg/L)		678	116	325	0.291 0.411
Scenario	PEC _{product} (µg/L)	PEC/RAC Ratio			
Pond	0.0286	<0.01	<0.01	<0.01	0.1 0.07
Ditch	0.717	<0.01	<0.01	<0.01	2.5 1.7
Stream	0.559	<0.01	<0.01	<0.01	1.9 1.4
10 m spray drift buffer					
Ditch	0.1247 0.443	-	-	-	0.43 1.1
Stream	0.1247 0.443	-	-	-	0.43 1.1
20 m spray drift buffer					
Ditch	0.230	-	-	-	0.56
Stream	0.230	-	-	-	0.56

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

Acceptable risk is shown for fish, daphnia and algae based on maximum predicted exposure. For *Lemna gibba*, a buffer zone of 20 m is required to show acceptable risk, which is in line with the mitigation required for thifensulfuron methyl.

zRMS comments:

The combined risk assessment provided by the Applicant and performed with consideration isoxadifen-ethyl data was not validated by the zRMS due to lack of EU agreed endpoints for the safener. Performed calculations have been retained for informative purposes, with font colour changed to grey in order to easily distinguish validated from non-validated data.

The approach to base the risk assessment on measured formulation toxicity expressed in terms of the formulated product compared with PEC for the formulation is agreed by the zRMS as being most appropriate to address the risk from the safener. Detailed discussion on most relevant - in opinion of the zRMS - approach in the mixture risk assessment is presented in point 9.5.1.1 of this document. It is noted that the risk assessment for *Lemna gibba* was based on endpoint derived from study with **adjuvant** surfactant DPX-KG691, while lower endpoint was obtained in study performed with Codacide. Recalculation of the ETR values with relevant endpoint have not changed the outcome of evaluation. Additional corrections were made in Table 9.5-35 in order to consider exposure agreed in area of Section 8.

Overall, based on the performed calculations, acceptable risk from the formulated product may be concluded when 10 m unsprayed buffer zone from surface water bodies is respected.

9.5.3 Overall conclusions

The maximum PEC_{sw} values resulted from the single application at a rate of 135 g GF-3969/ha (equivalent to a rate of 20 g rimsulfuron/ha, 12.5 g thifensulfuron methyl/ha and 15 g isoxadifen-ethyl/ha).

Based on this maximum exposure acceptable risk to all aquatic groups from isoxadifen-ethyl and its metabolites is shown at FOCUS Steps 1 and 2.

For rimsulfuron acceptable acute and chronic risk to fish, aquatic invertebrates and algae is shown at FOCUS Step 1.

For *Lemna gibba*, mitigation at FOCUS Step 4 is required to show acceptable risk for each of the uses. For the maximum application of 20 g rimsulfuron/ha, a 10-m buffer with 10 m vegetative filter strip is required to show acceptable risk in scenarios R1, R3 and R4. For remaining scenarios acceptable risk with no need for risk mitigation measures may be concluded.

An acceptable aquatic risk is concluded from the exposure to rimsulfuron metabolites at FOCUS Step 1 and 2.

For thifensulfuron methyl acceptable acute and chronic risk to fish, aquatic invertebrates, algae and sediment organisms is shown at FOCUS Step 1 and 2.

For aquatic plants a potential risk was triggered and so a refinement based on the agreed RMS geomean endpoint (from the review of confirmatory data) of 0.53 $\mu\text{g a.s./L}$ was applied to the risk assessment. Acceptable risk could be concluded provided that following risk mitigation measures are respected, depending on the use pattern:

1. Single application at 1x135 g GF-3969/ha:

- scenarios D3, D4, D5, D6: 10 m unsprayed buffer zone to surface water bodies,
- scenario R2: 10 m VFS to surface water bodies,
- scenarios R1, R3 and R4: 20 m VFS (when based on indications of FOCUS L&M) or 10 m VFS (when VFSmod is used).

2. Split application at 2x67.5 g GF-3969/ha with 7 days interval:

- scenarios D3, D4, D5, D6: 10 m unsprayed buffer zone to surface water bodies,
- scenarios R1, R2, R3: 10 m VFS to surface water bodies,
- scenario R4: 20 m VFS (when based on indications of FOCUS L&M) or 10 m VFS (when

VFSmod is used).

3. Split application at 85+50 g GF-3969/ha with 7 days interval:

- scenarios D3, D4, D5, D6: 10 m unsprayed buffer zone to surface water bodies,
- scenarios R1, R2, R4: 10 m VFS to surface water bodies,
- scenario R4: 20 m VFS (when based on indications of FOCUS L&M) or 10 m VFS (when VFSmod is used).

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

~~For the maximum single application rate of 1×12.5 g a.s./ha (equivalent to 1×135 g prod/ha) acceptable risk was shown with a 20 m buffer zone or a 10 m buffer with VFSmod. The same mitigation is required for the split application of 2×6.25 g a.s./ha (equivalent to 2×67.5 g prod/ha) to show acceptable risk. For the split application of $7.87 + 4.63$ g a.s./ha (equivalent to $85 + 50$ g prod/ha) acceptable risk is shown for all scenarios with a 10 m no-spray buffer zone with vegetated filter strip.~~

An acceptable aquatic risk is concluded from the exposure to thifensulfuron methyl metabolites at FOCUS Step 1 and 2.

~~Combined toxicity assessment for the active substances indicated the measured toxicity is comparable to predicted toxicity. For algae, potential antagonism (toxicity of the formulation is lower than expected) was identified however this can be explained by the fact that the algae endpoint for isoxadifen-ethyl is a 'greater than' value.~~

~~The assessment of endpoints for fish, daphnia and algae met the requirement for calculated mixture toxicity to be used in the risk assessment of the product. *Lemna gibba* met the criteria for the measured product endpoints to be used in the risk assessment. Based on calculated endpoints, acceptable risk to fish, *Daphnia* and algae was concluded.~~

The combined toxicity assessment demonstrated that measured and estimated toxicity endpoints for *Lemna gibba* are comparable. For fish and *Daphnia magna* the formulated product was more toxic than predicted based on data for individual active substances and for this reason measured formulation endpoints were concluded to be relevant for the risk assessment purposes in case of these two groups of species.

For algae the estimated toxicity of the mixture was clearly lower than measured. Nevertheless, in case of algae the TU analysis demonstrated that thifensulfuron-methyl contributes at >90% to the toxicity of the mixture and hence no additional calculations were deemed necessary and risk assessment for this species based on active substance data was sufficient.

Bases on measured endpoints and calculated product PEC_{sw} values, an acceptable risk was concluded following the use of GF-3969 in maize at 135 g prod/ha with the inclusion of a 10 20 m buffer zone.

~~This mitigation is in line with the required mitigation for thifensulfuron methyl.~~

zRMS comments:

The following text is added due to agreements during the Central Zone harmonisation meetings. It should be noted that this text has no impact on the outcome of zonal evaluation of formulation GF-3969, 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 honey bees have been carried out with rimsulfuron, thifensulfuron methyl and isoxadifen-ethyl. 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 honey bees and bumble bees of GF-3969 were not evaluated as part of the EU assessment of rimsulfuron and thifensulfuron methyl. 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 9.6-1: Endpoints and effect values relevant for the risk assessment for bees – rimsulfuron

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i>	Rimsulfuron	Acute Oral	LD ₅₀ >100 µg a.s./bee	EFSA 2005 Hoxter, K.A., Jaber, M., 1989 (HLO 267-89)
<i>Apis mellifera</i>	Rimsulfuron plus adjuvant surfactant DPX-KG691	Acute Oral	LD₅₀ = 41.1 µg a.s./bee	EFSA 2005 Wainwright, M., 2001 (DuPont-5654)
<i>Apis mellifera</i>	Rimsulfuron plus adjuvant surfactant DPX-KG691	Acute Contact	LD₅₀ = 27.9 µg a.s./bee	EFSA 2005 Wainwright, M., 2001 (DuPont-5654)
<i>Apis mellifera</i>	Rimsulfuron	Larvae (bee brood), 8 d test	LC ₅₀ >4.44 µg a.s./larva NOED 0.055 µg a.s./larva	EFSA, 2018
<i>Apis mellifera</i>	Rimsulfuron	Larvae (bee brood) 22-day repeated exposure	LD ₅₀ >32 µg a.s./larva NOED 32 µg a.s./larva	Cornement, M., 2018 (20170301) ^a
<i>Apis mellifera</i>	Rimsulfuron	Chronic adult, 10 d	LDD ₅₀ >18.15 µg/bee/day NOED = 18.15 µg/bee/day	EFSA, 2018
Higher-tier studies (tunnel test, field studies)				
Rimsulfuron had no impact on honeybee mortality, flight intensity, behaviour, colony condition or brood development following application to flowering <i>Phacelia tanacetifolia</i> in a cage test (80 g Rimsulfuron 25WG or Rimsulfuron 25WG + IN-KG691 adjuvant surfactant)				

Bold values are used in the risk assessment

a Summarized in Appendix 2.

Chronic studies on the toxicity of rimsulfuron to adult bees and larvae have been conducted. The studies and endpoints are presented in the EFSA conclusion 2018, the endpoints have not yet been concluded at EU level since the evaluation is currently ongoing.

However, as the chronic toxicity to bees and larvae is a data requirement, these new studies are used in the risk assessment.

A new study has also been conducted with the updated guidance for bee larvae to include the assessment of emergence over 22 days. The study has not previously been submitted for EU evaluation and so a summary is provided in Appendix 2.

The NOED from the 22-day larval study is higher (32 µg a.s./larva) than the reported NOED from the 8-day study (0.05 µg a.s./larva). To provide a conservative assessment, the endpoint from the 8-day study is therefore applied to the risk assessment.

zRMS comments:

Endpoints presented in Table 9.6-1 are EU agreed endpoints reported in EFSA Scientific Report (2005) 45, 1-61 and EFSA Journal 2018;16(5):5258 with exception of larvae endpoint derived from study by Cornement (2018). The study was, however, not validated by the zRMS since in case of GF-3969, containing 2 active compounds, respective larvae and chronic toxicity studies should be performed with the formulated product, while active substance endpoints should be generated at the EU level.

Table 9.6-2: Endpoints and effect values relevant for the risk assessment for bees – thifensulfuron methyl

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i>	Thifensulfuron methyl	Acute Oral	LD₅₀ >7.1 µg a.s./bee	EFSA 2015
<i>Apis mellifera</i>	Thifensulfuron methyl	Acute Contact	LD₅₀ >100 µg a.s./bee	EFSA 2015
Higher-tier studies (tunnel test, field studies)				
NA				

Bold values are used in the risk assessment.

zRMS comments:

Endpoints presented in Table 9.6-2 are EU agreed endpoints reported in EFSA Journal 2015;13(7):4201.

Table 9.6-3: Endpoints and effect values relevant for the risk assessment for bees – isoxadifen-ethyl

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i>	Isxadifen-ethyl (safener)	Acute Oral	LD₅₀ >151.96 µg a.s./bee	Zonal evaluation by zRMS Greece (2016)
<i>Apis mellifera</i>	Isxadifen-ethyl (safener)	Acute Contact	LD₅₀ >100.7 µg a.s./bee	Zonal evaluation by zRMS Greece (2016)

Bold values are used in the risk assessment

zRMS comments:

No EU agreed data exist for the safener, isoxadifen-ethyl, and for this reason validation of information provided in Table 9.2-3 against EU agreed endpoints was not possible. Nevertheless, data provided by the Applicant for isoxadifen-ethyl have been retained for informative purposes, with font colour changed to grey in order to easily distinguish validated from non-validated data.

Table 9.6-4: Endpoints and effect values relevant for the risk assessment for bees – GF-3969

Species	Substance	Exposure System	Results	Reference ^a
<i>Apis mellifera</i>	GF-3969 plus DPX-KG691 adjuvant surfactant	Acute Oral	LD₅₀ >100 µg product/bee	Tome, H.V.V., Porch J.R., 2018 (DuPont-48950)
		Acute Contact	LD₅₀ >100 µg product/bee	
<i>Apis mellifera</i>	GF-3969 plus adjuvant surfactant Codacide	Acute Oral	LD ₅₀ >100 µg product/bee	Tome, H.V.V., 2018 (DuPont-48892)
		Acute Contact	LD ₅₀ >100 µg product/bee	
<i>Apis mellifera</i>	GF-3969 plus DPX-KG691 adjuvant	Chronic, 10 d	LDD₅₀ = 2.98 µg product/bee/day (nominal)	KCP 10.3.1.2/01 Porch, Riles (2021a) (200439)
<i>Apis mellifera</i>	GF-3969 plus DPX-KG691 adjuvant	Larvae, 22 d	NOED = 11.0 µg product/larvae (mean measured)	KCP 10.3.1.3/02 Porch, Riles (2021b) (200438)

Bold values are used in the risk assessment

^a Summarised in Appendix 2.

zRMS comments:

Studies on acute toxicity of GF-3969 used with two **adjuvants** ~~surfactants~~ to bees were agreed by the zRMS and the endpoints reported in Table 9.6-4 above are confirmed. For summaries of the studies and details of the evaluation, please refer to Appendix 2.

Neither of **adjuvants** ~~surfactants~~ increased toxicity of GF-3969 to bees and the risk assessment will be based on LD₅₀ of >100 µg product/bee for both, oral and contact toxicity.

It is noted that no study on chronic toxicity of GF-3969 to adult bees and bee larvae were provided by the Applicant. Since GF-3969 contains two active compounds, testing of chronic and larvae toxicity is mandatory, in line with data requirements set by the Commission Regulation (EU) No 284/2013 and a data gap in this area has been identified. Nevertheless, as the results of the chronic and larvae toxicity studies are not considered in the risk assessment based on indications of the current guidance document (SANCO 10329/2002 rev 2 final), the studies must be submitted not later than the date of entry into force of EFSA bee guidance (2013). Please note that the larvae study must be performed in line with OECD TG 239.

During the commenting period the Applicant provided two studies required to fulfil the data requirements: chronic toxicity study with adult bees (Porch & Riles, 2021a, KCP 10.3.1.2/01) and repeated-exposure study with bee larvae (Porch & Riles, 2021b, KCP 10.3.1.3/02). Both studies were evaluated and agreed by the zRMS and summaries of studies together with zRMS comments may be found in Appendix 2.

It is noted that chronic adult and larvae studies were performed only with adjuvant DPX-KG691 and no study was performed with second adjuvant indicated in GAP table (Codacide). However, acute studies were performed with both proposed adjuvants and no increased toxicity from mixture of GF-3969 with Codacide was observed. Taking this into account, studies performed with addition of DPX-KG691 are considered sufficient.

9.6.1.1 Justification for new endpoints

Chronic adult and larval bee studies for rimsulfuron have been conducted according to the data requirements under Regulation No. 1107/2009.

The rimsulfuron endpoints have been confirmed in the EFSA conclusion 2018. However in addition a new study has also been conducted with the updated guidance for bee larvae to include the assessment of emergence over 22 days. The study has not previously been submitted for EU evaluation and so a summary is provided in Appendix 2.

The NOED from the 22-day larval study is higher (32 µg a.s./larva) than the reported NOED from the 8-day study (0.05 µg a.s./larva). To provide a conservative assessment the endpoint from the 8-day study is therefore applied to the risk assessment.

As herbicides, each of the active substances show a low acute and chronic toxicity to bees and are considered to have a low risk to bees. Studies conducted in other areas of the risk assessment have shown the formulation GF-3969 to have a lower toxicity than estimated from the toxicity of the active substances and so no enhanced toxicity of the formulation of the actives is shown. The available acute studies with the formulation show a low toxicity (LD₅₀ values >100 µg/bee). It is therefore expected the chronic risk to bees and larvae from the formulation will be low, especially as exposure to the formulation to larvae will be unlikely. However, to address the potential chronic adult and larval toxicity, studies with GF-3969 have been placed and conducted in 2020. The reports will be made available as soon as possible to support this risk assessment.

zRMS comments:

The new study on toxicity of rimsulfuron to bee larvae was not evaluated by the zRMS since in order to fulfil the data requirements set by the Commission Regulation (EU) No 284/2013 studies on chronic and larvae toxicity performed with GF-3969 should have been submitted, while studies addressing data requirements set by Commission Regulation (EU) No 283/2013 should be evaluated at the EU level.

During the commenting period the Applicant provided two studies required to fulfil the data requirements: chronic toxicity study with adult bees (Porch & Riles, 2021a, KCP 10.3.1.2/01) and repeated-exposure study with bee larvae (Porch & Riles, 2021b, KCP 10.3.1.3/02). See point 9.6.1 above for derived endpoints.

9.6.2 Risk assessment

The Applicant recognizes the need to review the bee pollinator risk assessment based on scientific progress. However, 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 2010 scheme^{10,11} 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.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group 1 x 135 g GF-3969/ha in maize also covers the risk for bees from all other intended uses (see Section 0).

9.6.2.1 Hazard quotients for bees

The acute risk to honey bees from use of GF-3969 was assessed using the maximum single application rate of active substances and the product and the relevant LD₅₀ values to calculate hazard quotients (EPPO/OEPP, 2003, Environmental risk assessment scheme for plant protection products, Chapter 10: Honeybees (PP 3/10(2)). Bulletin OEPP/EPPO Bulletin 33: 141-145) as follows:

$$\text{Hazard Quotient} = \frac{\text{Maximum application rate (g formulation/ha)}}{\text{Acute LD}_{50} (\mu\text{g formulation/bee})}$$

¹⁰ EPPO (2010a). Side-effects on honey bees. Bulletin OEPP/EPPO Bulletin 40: 313-319.

¹¹ EPPO (2010b). Environmental risk assessment scheme for plant protection products. Bulletin OEPP/EPPO Bulletin 40: 323-331.

Table 9.6-5: First-tier assessment of the risk for bees due to the use of GF-3969 in maize

Intended use	Maize		
Active substance	Rimsulfuron		
Application rate (g a.s./ha)	1 × 20		
Test design	LD₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q_{HO}, Q_{HC} criterion: Q_H ≤ 50
Oral toxicity	41.1 ^a	20	0.5
Contact toxicity	27.9 ^a		0.7
Intended use	Maize		
Active substance	Thifensulfuron methyl		
Application rate (g a.s./ha)	1 × 12.5		
Test design	LD₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q_{HO}, Q_{HC} criterion: Q_H ≤ 50
Oral toxicity	>7.1	12.5	1.7
Contact toxicity	>100		0.125
Product	Isoxadifen-ethyl		
Application rate (g a.s./ha)	1 × 15		
Test design	LD₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q_{HO}, Q_{HC} criterion: Q_H ≤ 50
Oral toxicity	>151.96	15	0.09
Contact toxicity	>100.7		0.15
Product	GF-3969 plus adjuvant surfactant (DPX-KG691 or Codacide)		
Application rate (g product/ha)	1 × 135		
Test design	LD₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q_{HO}, Q_{HC} criterion: Q_H ≤ 50
Oral toxicity	100	135	1.35
Contact toxicity	100		1.35

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

a Bees exposed as rimsulfuron plus adjuvant surfactant DPX-KG691.

Combination assessment: acute toxicity

Concentration addition (CA)

The following equation can be used for deriving a surrogate LD₅₀ value for a mixture of active substances with known toxicity assuming dose additivity:

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

Where:

X(a.s._i) = fraction of active substance [i] in the mixture (please note that the sum of X(a.s._i) must be 1)

LD_x(a.s._i) = toxicity value for active substance [i] (for the same endpoint).

Considering the adult oral LD₅₀ values determined for rimsulfuron, thifensulfuron methyl and isoxadifen ethyl of >100, >7.1 and >151.96 µg a.s./bee respectively, and their nominal concentrations in GF 3969 (14.82, 9.26 and 11.11% w/w respectively), the resulting LD₅₀(mix) value is >23.067 µg/bee. This value is lower than the measured LD₅₀ of >100 µg/bee, however, this is an artefact since all active substance endpoints and the product endpoint are higher than values. The laboratory data is supportive of the predicted low toxicity of GF 3969 and the risk assessment based on laboratory data is concluded acceptable.

Considering the adult contact LD₅₀ values determined for rimsulfuron, thifensulfuron methyl and isoxadifen ethyl of 27.9, >100 and 100.7 µg a.s./bee respectively, and their nominal concentrations in

GF 3969 (14.82, 9.26 and 11.11% w/w respectively), the resulting LD₅₀ (mix) value is >47.94 µg/bee. This value is slightly lower than the measured LD₅₀ of >100 µg/bee, however, this is an artefact since majority of the active substance endpoints and the product endpoint are higher than values. The laboratory data is supportive of the predicted low toxicity of GF 3969 and the risk assessment based on laboratory data is concluded acceptable.

Chronic honey bee risk assessment

This risk assessment is based upon the EPPO 2010^{10,11} risk assessment for systemic substances which is cited in the regulation as a current risk assessment scheme. The maximum application rate of GF 3969 is 135 g f.p./ha with a maximum 1 application per season. The proposed crops on the label is maize. Maize is not a melliferous crop thus it is not attractive to foraging bees (SANTE/11956/2016 rev. 9), therefore, the exposure to applications in the treated crop will be negligible.

Risk assessment for honey bee larvae

Worst case data from Rortais *et al.*, 2005¹² as proposed in the EPPO 2010^{10,11} have been used to estimate the consumption by bee larvae.

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

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

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

$$\begin{aligned} \text{Nectar dose over 5 days of consumption by larvae} &= (\text{A.R.} \times \text{RUD}) \times 198 \times 10^{-6} \text{ kg nectar/larvae} \\ \text{Pollen dose over 5 days of consumption by larvae} &= (\text{A.R.} \times \text{RUD}) \times 24.4 \text{ mg/kg pollen} \times 2 \times 10^{-6} \text{ kg pollen/larvae} \\ \text{Total dose over 5 days} &= \text{Nectar dose} + \text{Pollen dose} \end{aligned}$$

Where: A.R. = application rate in kg a.s./ha
 RUD = residue per unit dose from the EFSA bee guidance. Mean RUD_{nectar} = 2.9 mg a.s./kg (foliar sprays), Mean RUD_{pollen} = 6.1 mg a.s./kg (foliar spray).

The toxicity to exposure ratio (TER) is calculated by determined by comparing the no-observed-effect dose (NOED µg a.s./larva) to the total dose over 5 days (µg a.s./larva). For rimsulfuron the NOED from the 22 day larval study is higher (32 µg a.s./larva) then the reported NOED from the 8 day study (0.05 µg a.s./larva). To provide a conservative assessment the endpoint from the 8 day study is therefore applied to the risk assessment.

Table 9.6-6: Chronic risk for honey bee larvae due to the use of GF-3969 rimsulfuron

Test design	Endpoint	Single application rate (kg/ha)	Nectar dose ^a µg a.s./larvae	Pollen dose ^a µg a.s./larvae	Total dose µg a.s./larvae	TER
Rimsulfuron Larvae acute, lab (8-d study, single exposure)	NOED = 0.05 µg a.s./larva	0.020	0.0115	0.0002	0.00117	4

^a Assuming a foliar spray RUD of 2.9 for nectar and a RUD of 6.1 for pollen.

¹² 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

The EPPO 2010^{10,11}-scheme proposes a trigger of 1 for assessment of the risk to honeybees. With TER values of 4 for rimsulfuron, there is a clear safety margin, indicating that the proposed uses pose an acceptable risk to bee larval development.

Risk assessment for adult honey bees

The risk assessment uses NOEDD values for the endpoint so avoids the issues associated with the generation of LDD₅₀ values for substances of low toxicity, and calculates exposure in a similar way to EFSA 2013. The approach is also in line with other chronic risk assessments (e.g. birds and mammals). EPPO 2010 recommended the calculation of a TER using the following equation:

$$TER = NOEDD/DD$$

Where daily dose (DD) is based on the worst case sugar requirement for a bee at 128 mg/bee/day (Rortais *et al.* 2005) feeding exclusively from nectar containing 30% sugar:

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

Where: A.R. = application rate in kg a.s./ha
RUD = residue per unit dose from the EFSA bee guidance. Mean RUD_{nectar} = 2.9 mg a.s./kg (foliar sprays), Mean RUD_{nectar} = 0.0458 mg a.s./kg (seed treatment).

Table 9.6-7: Chronic risk for adult honey bees due to the use of GF-3969 – rimsulfuron

Test design	Endpoint	Single application rate (kg/ha)	Nectar consumption (g)	RUD – Foliar spray (mg/kg)	Daily Dose (µg a.s./bee)	TER
Rimsulfuron Lab Adult chronic oral (10 d feeding – OECD)	NOED = 18.15 µg a.s./bee/day	0.020	0.427	2.9	0.0248	733

The EPPO 2010^{10,11}-scheme proposes a trigger of 1 for assessment of the risk to larvae. With the TER value of 733 for rimsulfuron, there is a clear safety margin, indicating that the proposed uses pose an acceptable chronic risk to bees.

Combination toxicity assessment

Combination toxicity assessment is not possible, since no chronic toxicity data is not available for thifensulfuron-methyl and the safener isoxadifen-ethyl. However, chronic toxicity studies are being conducted with the GF-3969 formulation in 2020 to cover the risk assessment of the product and will be provided as soon as possible to support this submission.

zRMS comments:

The acute risk assessment for bees performed by the Applicant for rimsulfuron, thifensulfuron-methyl and formulation GF-3969 in Table 9.6-5 above is agreed by the zRMS. Evaluation was performed considering single application of both compound, covering also split applications.

It is noted that for rimsulfuron endpoints derived from studies performed with addition of **adjuvant** were considered and not for the pure active compound. Nevertheless, approach of the Applicant is accepted by the zRMS since studies performed with **adjuvant** were resulted with considerably lower endpoints.

In absence of the EU agreed **bee** avian toxicity data for isoxadifen-ethyl, validation of calculations presented in Table 9.6-5 was not possible. Nevertheless, performed calculations have been retained for informative purposes, with font colour changed to grey in order to easily distinguish validated from non-validated data. In case the endpoints were confirmed at the EU level, acceptable risk from exposure to isoxadifen-ethyl would be concluded. It should be, however, noted that the risk assessment based on formulation endpoint covers also the risk resulting from exposure to the safener, since studies performed with the formulation address effects from all formulation components.

Overall, based on the calculations performed in Table 9.6-5, acceptable risk to bees from the intended uses of

GF-3969 may be concluded.

The combined risk assessment based on the dose additivity was not validated by the zRMS since respective toxicity data for the formulated product are available and in case of bees validation of the measured against estimated endpoints is foreseen neither by the current guidance document (SANCO/10329/2002 rev 2) nor EFSA (2013). In line with both guidance documents, the risk assessment for bees is performed using either formulation or formulation+active substance toxicity data.

The chronic and larvae risk assessment was not evaluated by the zRMS as being not required according to the current guidance document (SANCO/10329/2002 rev 2 final). Furthermore, the assessment was performed in line with the revised EPPO scheme of 2010, while in opinion of the zRMS in case the chronic and larvae risk assessment is performed, it should be conducted in line with guidance considered at the EU level (i.e. EFSA, 2013). Nevertheless, as according to conclusions of the Central Zone Steering Committee (CZSC), recommendations of EFSA (2013) should not be considered for the zonal evaluations until the guidance is noted at the EU level, the risk assessment based on indications of EFSA (2013) must be performed at the national level by cMS that do require such evaluation.

During the commenting period the Applicant submitted studies on chronic toxicity of GF-3969 in mixture with adjuvant DPX-KG691 to adult bees and larvae in order to fulfil the data requirements. Studies were evaluated and agreed by the zRMS (see point 9.6.1 for details). Due to requests of some cMS, the screening step and Tier 1 risk assessment in line with EFSA (2013) has been performed by the zRMS below, using endpoints from newly submitted studies. Calculations were performed using EFSA Bee-Tool v. 3.

Screening step risk assessment (maize, BBCH 11-18, 1x0.135 kg product/ha)

Contact route of exposure				
	"calculation factor" (linked with dust)	HQ	Trigger	Risk indicator
HB	1	1.4	42	OK
Oral route of exposure (pollen and nectar)				
	"calculation factor" (Ef x SV)	ETR	Trigger	Risk indicator
HB - acute	7.6	0.01	0.2	OK
HB - chronic	7.6	0.344	0.03	!
HB - larvae	4.4	0.05	0.2	OK

Tier 1 chronic risk assessment

Crop	Category	Scenario	Ef	SV HB	TWA HB	ETR HB	Trigger	Risk indicator
Maize	chronic	treated crop	1	0.92	0.72	0.030	0.03	!
BBCH 11-18	chronic	weeds	1	2.9	0.72	0.095	0.03	!
	chronic	field margin	0.0092	2.9	0.72	0.001	0.03	OK
	chronic	adjacent crop	0.0033	5.8	0.72	0.001	0.03	OK
	chronic	next crop	1	0.54	0.72	0.018	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-3969 already at the screening step. The chronic risk to bees is acceptable in field margin, adjacent crop and next crop scenarios. However, chronic risk to bees in the treated crop and weeds scenarios is potentially unacceptable. However, the product is intended to be applied at maize BBCH 11-18, i.e. long before flowering, so the risk from the treated crop seems to be overestimated in evaluation based on EFSA (2013) indications. This issue should be further resolved at the product authorisation in Member States considering indications of the not yet noted EFSA guidance in their national assessments. Risk assessment based on EFSA (2013) is provided above for informative purposes only and is not the basis for derivation of conclusion regarding the risk to bees at the zonal level.

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

Not relevant.

9.6.3 Effects on bumble bees

Studies on the effects of the product GF-3969 and **adjuvant** ~~surfactant~~ Codacide and DPX-KG691 on bumble bees are available. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Table 9.6-8: Endpoints and effect values relevant for the risk assessment for bees – GF-3969

Species	Substance	Exposure System	Results	Reference ^a
<i>Bombus terrestris</i> L.	GF-3969 plus adjuvant surfactant DPX-KG691	Acute Oral	LD ₅₀ >225.6 µg product/bee LD₅₀ >293 µg product/bee	Vergé, E., 2018 (DuPont-48899, Revision No. 1)
		Acute Contact	LD ₅₀ >650 µg product/bee	
<i>Bombus terrestris</i> L.	GF-3969 plus adjuvant surfactant Codacide	Acute Oral	LD ₅₀ >470 µg product/bee	Vergé, E., 2018 (DuPont-48951)
		Acute Contact	LD ₅₀ >500 µg product/bee	

a Summarised in Appendix 2.

The toxicity of the formulation combined with two types of **adjuvants** ~~surfactants~~ DPX-KG691 and crop oil Codacide did not result in toxicity at the highest dose tested, suggesting low toxicity to bumble bees. The data and risk assessment available on the honey bee is considered protective of the bumble bee.

zRMS comments:

In addition to bee studies, also acute studies on effects of GF-3969 to bumblebees were performed and demonstrated that bumblebees are not more sensitive comparing to bees. The studies were evaluated and agreed by the zRMS, however their endpoints will be not used in the risk assessment since currently there is not data requirement in this area. For summaries of the studies and details of evaluation, please refer to Appendix 2.

9.6.4 Effects on solitary bees

Not relevant.

9.6.5 Overall conclusions

Regulatory testing to assess the acute toxicity to bees has been conducted with rimsulfuron, thifensulfuron methyl, isoxadifen-ethyl and GF-3969 in accordance with EU requirements. HQ values for each of the active substances and product were calculated to be less than the trigger of 50, indicating acceptable risk to bees from acute oral and contact routes of exposure based on a single maximum application rate of 135 g GF-3969/ha to maize.

Since respective chronic and larvae toxicity studies performed with the formulation GF-3969 were provided by the Applicant during the commenting period, the risk assessment based on EFSA (2013) has been also performed. Acceptable acute oral and contact risk to adult bees as well as chronic risk to larvae from the intended uses of GF-3969 could be concluded already at the screening step. The chronic risk to adult bees was unacceptable at the screening step and Tier 1 evaluation was performed which resulted with acceptable chronic risk in field margin, adjacent crop and next crop scenarios. However, ETR values calculated for the treated crop and weeds scenarios were above the respective triggers indicating potentially unacceptable risk. This issue will have to be dealt with at the product authorisation by the cMS that consider indications of EFSA (2013) at the national level, since at the

zonal level the risk assessment performed in line with EFSA (2013) is indicative only until the guidance is noted at the EU level.

~~Studies have been conducted with rimsulfuron to assess the chronic toxicity to adult bees and to assess acute and chronic toxicity to bee larva. The data from the chronic adult testing and larva testing were used in the risk assessment, the TERs were above the trigger of 1 indicating acceptable risk to adult bees and larvae based on a single maximum application rate of 135 g GF-3969/ha to maize.~~

~~Regulatory testing is being conducted with the product to assess the chronic toxicity to honey bee larvae and adults and the studies will be provided as soon as possible.~~

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

Effects on non-target arthropods of GF-3969 were not evaluated as part of the EU assessment of rimsulfuron, thifensulfuron methyl and isoxadifen-ethyl. 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. Justifications are provided below.

Studies to assess the toxicity of GF-3969 to *Typhlodromus pyri* and *Aphidius rhopalosiphi* have been conducted with both DPX-KG691 and Codacide **adjuvants** ~~surfactants~~. Each of the studies showed a low toxicity to non-target arthropods. The lowest endpoints resulted from the studies with DPX-KG691 and so these endpoints are applied to the risk assessment.

Table 9.7-1: Endpoints and effect values relevant for the risk assessment for non-target arthropods – GF-3969

Species	Substance	Exposure System	Results	Reference ^a
Tier-1				
<i>Typhlodromus pyri</i> (protonymphs)	GF-3969 plus adjuvant surfactant DPX-KG691	Laboratory test glass plates (2D)	LR₅₀ >135 g product/ha	Moll, M., 2018 (DuPont-49935)
<i>Aphidius rhopalosiphi</i> (adults)	GF-3969 plus adjuvant surfactant DPX-KG691	Laboratory test glass plates (2D)	LR₅₀ >135 g product/ha	Moll, M., 2018 (DuPont-49934)
<i>Typhlodromus pyri</i> (protonymphs)	GF-3969 plus adjuvant surfactant Codacide	Laboratory test glass plates (2D)	LR ₅₀ >135 g product/ha	Moll, M., 2018 (DuPont-49973)
<i>Aphidius rhopalosiphi</i> (adults)	GF-3969 plus adjuvant surfactant Codacide	Laboratory test glass plates (2D)	LR ₅₀ >135 g product/ha	Moll, M., 2018 (DuPont-49972)

Bold values are used in the risk assessment.

a Summarized in Appendix 2.

zRMS comments:

Studies on effects of GF-3969 used with two **adjuvants** ~~surfactants~~ on non-target arthropods were **evaluated and** agreed by the zRMS and the endpoints reported in Table 9.7-1 above are confirmed. For summaries of the studies and details of the evaluation, please refer to Appendix 2.

Neither of **adjuvants** ~~surfactants~~ increased toxicity of GF-3969 to NTAs and the risk assessment will be based on LR₅₀ of >135 g product/ha both indicator species.

9.7.1.1 Justification for new endpoints

Not relevant.

9.7.2 Risk assessment

The in-field exposure (predicted environmental rate (PER)) is calculated according to ESCORT 2 using the following equation:

$$PER_{in-field} = \text{Application rate (g/ha)} * \text{MAF}$$

The potential risk of GF-3969 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 (LR_{50}) values according to the following equation:

$$HQ_{in-field} = \frac{PER_{in-field} \left(\frac{g}{ha}\right)}{LR_{50} \left(\frac{g}{ha}\right)}$$

Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of GF-3969 in maize

Intended use	Maize		
Active substance/product	GF-3969 plus adjuvant surfactant DPX-KG691 or Codacide		
Application rate (g/ha)	1 × 135 g product/ha		
MAF	1		
Test species	LR₅₀ (lab.) (g/ha)	PER_{in-field} (g/ha)	HQ_{in-field} criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	>135	135	<1
<i>Aphidius rhopalosiph</i>	>135		<1

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient

zRMS comments:

The in-field risk assessment presented in Table 9.7-2 is agreed by the zRMS. Evaluation was performed considering single application of both compound, covering also split applications.

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-3969 may be concluded.

9.7.2.1 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-3969 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)¹³ as shown in the following equation:

$$PER_{off-field} = \frac{\text{Maximum } PER_{in-field} \times \left(\% \frac{\text{Drift}}{100}\right)}{\text{Vegetation distribution Factor}}$$

The potential risk of GF-3969 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 (LR_{50}) values according multiplied by a correction factor according to the following equation:

¹³ 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.

$$HQ_{\text{off-field}} = \frac{PER_{\text{off-field}} \left(\frac{g}{ha} \right)}{LR_{50} \left(\frac{g}{ha} \right)} \times \text{Correction factor}$$

Table 9.7-3: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of GF-3969 in maize

Intended use	Maize				
Active substance/product	GF-3969 plus adjuvant surfactant DPX-KG691				
Application rate (g/ha)	1 × 135 g product/ha				
MAF	1				
Vdf	5 (Tier 1) ^a				
Test species	LR₅₀ (lab.) (g/ha)	Drift rate	PER_{off-field} (g/ha)	CF	HQ_{off-field} criterion: HQ ≤ 2
Tier I					
<i>Typhlodromus pyri</i>	>135	2.77%	(135 × 0.0277 / 5) = 0.75	10	<0.06
<i>Aphidius rhopalosiphi</i>	>135				<0.06

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient.

a The vegetation distribution factor of 5 is used instead of 10 according to EFSA Supporting Publication 2019:EN-1673.

zRMS comments:

The off-field risk assessment presented in Table 9.7-3 is agreed by the zRMS. Evaluation was performed considering single application of both compound, covering also split applications.

As a worst case the 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-3969 may be concluded with no need for risk mitigation measures.

9.7.2.2 Additional higher-tier risk assessment

Not relevant.

9.7.2.3 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

Regulatory testing has been conducted with the product. The Tier I laboratory studies showed acceptable in-field and off-field effects for *T. pyri* and *A. rhopalosiphi* from applications of GF-3969 according to the maximum exposure without the 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 rimsulfuron, thifensulfuron methyl, isoxadifen-ethyl and their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of GF-3969 were not evaluated as part of the EU assessment of rimsulfuron or thifensulfuron methyl. 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) – rimsulfuron

Species	Substance	Exposure System	Results	Reference
Acute toxicity to earthworms				
<i>Eisenia fetida</i>	Rimsulfuron	14 d, acute	LC ₅₀ > 1000 mg a.s./kg dw	EFSA 2005 Edwards, P.J. <i>et al.</i> , 1990 (E9636/ECO 1)
Chronic toxicity earthworms				
<i>Eisenia fetida</i>	Rimsulfuron	8 week, chronic reproduction and growth, mixed into soil with 10% peat	NOEC = 100 mg a.s./kg dw	EFSA 2018
<i>Eisenia fetida</i>	IN-70941	8 week, chronic reproduction and growth, mixed into soil with 5% peat	NOEC = 0.18 mg met./kg dw	EFSA 2005 Luhrs, U., 2001a (DuPont-4155)
<i>Eisenia fetida</i>	IN-70942	8 week, chronic reproduction and growth, mixed into soil with 5% peat	NOEC = 0.18 mg met./kg dw	EFSA 2005 Luhrs, U., 2001b (DuPont-4156)
<i>Eisenia fetida</i>	IN-E9260	8 week, chronic reproduction and growth, mixed into soil with 5% peat	NOEC = 0.18 mg met./kg dw	EFSA 2005 Luhrs, U., 2001c (DuPont-4157)
Toxicity to other non-target macro-organisms				
<i>Folsomia candida</i>	Rimsulfuron	28 day, chronic, mixed into soil with 5% peat	NOEC = 500 mg a.s./kg dw	EFSA 2018
<i>Folsomia candida</i>	IN-70941	14 day, chronic, mixed into soil with 5% peat	NOEC ≥ 0.183 mg met./kg dw	EFSA 2005
<i>Folsomia candida</i>	IN-70942	14 day, chronic, mixed into soil with 5% peat	NOEC ≥ 0.183 mg met./kg dw	EFSA 2005
<i>Folsomia candida</i>	IN-E9260	14 day, chronic, mixed into soil with 5% peat	NOEC ≥ 0.183 mg met./kg dw	EFSA 2005
<i>Hypoaspis aculeifer</i>	Rimsulfuron	14 day, chronic, mixed into soil with 5% peat	NOEC = 500 mg a.s./kg dw	EFSA 2018

Bold values are used in the risk assessment.

Chronic studies on the toxicity of rimsulfuron to earthworms, *Folsomia candida* and *Hypoaspis aculeifer* have been conducted. The studies and endpoints are presented in the EFSA conclusion 2018, the endpoints have not yet been concluded at EU level since the evaluation is currently ongoing. However to complete the risk assessment for the active substance, these endpoints have been included.

zRMS comments:

Endpoints presented in Table 9.8-1 are EU agreed endpoints reported in EFSA Scientific Report (2005) 45, 1-61 and EFSA Journal 2018;16(5):5258.

Information on acute toxicity has been struck through as being no longer a data requirement.

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

Species	Substance	Exposure System	Results	Reference
Acute toxicity to earthworms				
<i>Eisenia fetida</i>	Thifensulfuron methyl	14 d, acute	LC ₅₀ > 2000 mg a.s./kg dw	EFSA 2015
<i>Eisenia fetida</i>	IN-A4098	14 d, acute	LC ₅₀ > 1000 mg met./kg dw	EFSA 2015
<i>Eisenia fetida</i>	IN-A5546	14 d, acute	LC ₅₀ > 1000 mg met./kg dw	EFSA 2015
<i>Eisenia fetida</i>	IN-JZ789	14 d, acute	LC ₅₀ > 100 mg met./kg dw	EFSA 2015
<i>Eisenia fetida</i>	IN-L9223	14 d, acute	LC ₅₀ > 1000 mg met./kg dw	EFSA 2015
<i>Eisenia fetida</i>	IN-L9223	14 d, acute	LC ₅₀ > 100 mg met./kg dw	EFSA 2015
<i>Eisenia fetida</i>	IN-L9225	14 d, acute	LC ₅₀ > 1000 mg met./kg dw	EFSA 2015
<i>Eisenia fetida</i>	IN-L9226	14 d, acute	LC ₅₀ > 891 mg met./kg dw	EFSA 2015
<i>Eisenia fetida</i>	IN-L9226	14 d, acute	LC ₅₀ > 1.0 mg met./kg dw	EFSA 2015
<i>Eisenia fetida</i>	IN-V7160	14 d, acute	LC ₅₀ > 1000 mg met./kg dw	EFSA 2015
<i>Eisenia fetida</i>	IN-W8268	14 d, acute	LC ₅₀ > 1000 mg met./kg dw	EFSA 2015
<i>Eisenia fetida</i>	2-acid-3-triuret (IN-U5F72)	14 d, acute	LC ₅₀ > 100 mg/kg dw	EFSA 2015
Chronic toxicity to earthworms				
<i>Eisenia fetida</i>	Thifensulfuron methyl 50SG plus adjuvant surfactant	56 d, chronic	NOEC = 34.3 mg a.s./kg dw (68.5 mg product/kg soil)	EFSA 2015
<i>Eisenia fetida</i>	IN-A4098	56 d, chronic	NOEC = 0.2 mg met./kg dw	EFSA 2015
<i>Eisenia fetida</i>	IN-A4098	56 d, chronic	NOEC = 0.202 mg met./kg dw	EFSA 2015
<i>Eisenia fetida</i>	IN-A4098	56 d, chronic	NOEC = 8.0 mg met./kg dw	EFSA 2015
<i>Eisenia fetida</i>	IN-L9223	56 d, chronic	NOEC = 10 mg met./kg dw	EFSA 2015
<i>Eisenia fetida</i>	IN-L9225	56 d, chronic	NOEC = 0.4 mg met./kg dw	EFSA 2015
<i>Eisenia fetida</i>	IN-L9225	56 d, chronic	NOEC = 8.0 mg met./kg dw	EFSA 2015
<i>Eisenia fetida</i>	IN-W8268	56 d, chronic	NOEC = 80 mg met./kg dw	EFSA 2015
<i>Eisenia fetida</i>	IN-W8268	56 d, chronic	NOEC = 8.0 mg met./kg dw	EFSA 2015
Toxicity to other soil macro-organisms				
<i>Folsomia candida</i>	IN-A4098	28 d, chronic	NOEC = 0.045 mg met./kg dw NOEC = 31.7 mg met./kg dw	EFSA 2015
<i>Hypoaspis aculeifer</i>	IN-A4098	14 d, chronic	NOEC = 100 mg met./kg dw	EFSA 2015
<i>Folsomia candida</i>	IN-L9223	28 d, chronic	NOEC = 100 mg met./kg dw	EFSA 2015
<i>Folsomia candida</i>	IN-L9225	28 d, chronic	NOEC = 10 mg met./kg dw NOEC = 100 mg met./kg dw	EFSA 2015
<i>Hypoaspis aculeifer</i>	IN-L9225	14 d, chronic	NOEC = 100 mg met./kg dw	EFSA 2015
<i>Folsomia candida</i>	IN-W8268	28 d, chronic	NOEC = 100 mg met./kg dw	EFSA 2015
<i>Hypoaspis aculeifer</i>	IN-W8268	14 d, chronic	NOEC = 50 mg met./kg dw	EFSA 2015
<i>Folsomia candida</i>	2-acid-3-triuret (IN-U5F72)	28 d, chronic	NOEC = 100 mg met/kg dw	Confirmatory data submitted by FMC Lührs, U., 2015b (DuPont-42481)**

Species	Substance	Exposure System	Results	Reference
<i>Folsomia candida</i>	IN-JZ789	28 d, chronic	NOEC = 90.58 mg met/kg dw	Confirmatory data submitted by FMC Lührs, U., 2015a (DuPont-42165)**
Field studies				
Not required.				
Litter bag test				
Not required.				

Bold values are used in the risk assessment.

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

** Summarised in 0.

zRMS comments:

Endpoints presented in Table 9.8-2 are EU agreed endpoints reported in EFSA Journal 2015;13(7):4201.

Information on acute toxicity has been struck through as being no longer a data requirement.

Long-term endpoint for *Folsomia candida* (metabolites IN-U5F72 and IN-JZ789) were agreed by the RMS (UK) in the course of the evaluation of the confirmatory data (for details, please refer to EFSA Supporting publication 2020:EN-1627).

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

Species	Substance	Exposure System	Results	Reference
Acute toxicity to earthworms				
<i>Eisenia fetida</i>	Isoxadifen-ethyl (safener)	Mixed into sub-strate 14 d, acute 10% peat content	LC₅₀ >1000 mg/kg dw LC_{50,corr} >500 mg/kg dw*	Zonal evaluation by zRMS Greece (2016)
<i>Eisenia fetida</i>	AE F129431	Mixed into sub-strate 14 d, acute 10% peat content	LC₅₀ >947 mg/kg dw	Zonal evaluation by zRMS Greece (2016)
<i>Eisenia fetida</i>	AE C637375	Mixed into sub-strate 14 d, acute 10% peat content	LC₅₀ >1000 mg/kg dw	Zonal evaluation by zRMS Greece (2016)

Bold values are used in the risk assessment.

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

zRMS comments:

No EU agreed data exist for the safener, isoxadifen-ethyl, and for this reason validation of information provided in Table 9.8-3 against EU agreed endpoints was not possible. Nevertheless, data provided by the Applicant for isoxadifen-ethyl have been retained for informative purposes, with font colour changed to grey in order to easily distinguish validated from non-validated data.

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-3969

Species	Substance	Exposure System	Results	Reference ^a
Chronic toxicity to earthworms				
<i>Eisenia andrei</i>	GF-3969 plus adjuvant surfactant DPX-KG691	Mixed into substrate 56 d, chronic 10% peat content	NOEC = 123 mg product/kg dw (reproduction)	Pavić, B., 2018 (DuPont-49950)
<i>Eisenia andrei</i>	GF-3969 plus adjuvant surfactant Codacide	Mixed into substrate 56 d, chronic 10% peat content	NOEC = 180 mg product/kg dw (mortality, growth, reproduction)	Pavić, B., 2018 (DuPont-49980)
Toxicity to other non-target macro-organisms				
<i>Folsomia candida</i>	GF-3969 plus adjuvant surfactant DPX-KG691	Mixed into substrate 28 d, chronic 5% peat content	NOEC (reproduction) = 125 mg product/kg dw	Pavić, B., 2018 (DuPont-49954)
<i>Folsomia candida</i>	GF-3969 plus adjuvant surfactant Codacide	Mixed into substrate 28 d, chronic 5% peat content	NOEC (reproduction) = 250 mg product/kg dw	Pavić, B., 2018 (DuPont-49981)
<i>Hypoaspis aculeifer</i>	GF-3969 plus adjuvant surfactant DPX-KG691	Mixed into substrate 28 d, chronic 5% peat content	NOEC = 1000 mg product/kg dry soil	Pavić, B., 2018 (DuPont-49955)
<i>Hypoaspis aculeifer</i>	GF-3969 plus adjuvant surfactant Codacide	Mixed into substrate 28 d, chronic 5% peat content	NOEC = 1000 mg product/kg dry soil	Pavić, B., 2018 (DuPont-49982)
Field studies				
Not required				
Litter bag test				
Not required				

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

a Summarized in Appendix 2.

zRMS comments:

Studies on toxicity of GF-3969 used with two **adjuvants** ~~surfactants~~ to soil macro- and meso-fauna were agreed by the zRMS and the endpoints reported in Table 9.8-4 above are confirmed. For summaries of the studies and details of the evaluation, please refer to Appendix 2.

In case of earthworms and *Folsomia candida* lower endpoints obtained in studies performed with **adjuvants** ~~surfactants~~ DPX-KG691 or Codacide will be used. Neither of **adjuvants** ~~surfactants~~ increased toxicity of GF-3969 to *Hypoaspis aculeifer*.

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} values for risk assessments covering the proposed use pattern are taken from the Core, Part B, Section 8.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group 1× application to maize at 135 g product/ha covers the risk to non-target soil organisms from all other intended uses. This is equivalent to a maximum rate of 20 g a.s./ha of rimsulfuron, 12.5 g a.s./ha of thifensulfuron methyl and 15 g a.s./ha of isoxadifen-ethyl.

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-3969 in maize - rimsulfuron

Intended use	Maize		
Acute effects on earthworms			
Active substance	LC ₅₀ (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _a (criterion TER ≥10)
Rimsulfuron	>1000	0.020	>50000
Chronic effects on earthworms			
Active substance/metabolite	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥5)
Rimsulfuron	100	0.020	5000
IN-70941	0.18	0.0276 0.0275	6.5 6.6
IN-70942	0.18	0.0052	34
IN-E9260	0.18	0.0091	20
Chronic effects on <i>Folsomia candida</i>			
Active substance/metabolite	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥5)
Rimsulfuron	500	0.020	25000
IN-70941	0.183	0.0276 0.0275	6.6 6.7
IN-70942	0.183	0.0052	35
IN-E9260	0.183	0.0091	20
Chronic effects on <i>Hypoaspis aculeifer</i>			
Active substance/metabolite	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥5)
Rimsulfuron	500	0.020	2500

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-3969 in maize – thifensulfuron methyl

Intended use	Maize		
Acute effects on earthworms			
Active substance	LC ₅₀ (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _a (criterion TER ≥10)
Thifensulfuron methyl	>2000	0.013	153846
IN-A4098	>1000	0.0067	149254
IN-A5546	>1000	0.0016	625000
IN-JZ789	>100	0.0049*	20408
IN-L9223	>100	0.0021	47619
IN-L9225	>1000	0.014	71429
IN-L9226	>1.0	0.0018	556
IN-V7160	>1000	0.0009	1111111
IN-W8268	>1000	0.0018	555556

2-acid-3-triuret (IN-U5F72)	>100	0.0024	41667
Chronic effects on earthworms			
Active substance/metabolite	NOEC (mg/kg dw)	PEC_{soil} (mg/kg dw)	TER_{it} (criterion TER ≥5)
Thifensulfuron methyl (Thifensulfuron methyl 50SG with adjuvant surfactant)	34.3	0.013	2638
IN-A4098	0.2	0.0067	30
IN-L9223	10	0.0021	4762
IN-L9225	0.4	0.014	29
IN-W8268	8	0.0018	4444
Chronic effects on <i>Folsomia candida</i>			
Active substance/metabolite	NOEC (mg/kg dw)	PEC_{soil} (mg/kg dw)	TER_{it} (criterion TER ≥5)
IN-A4098	0.045	0.0067	7
IN-L9223	100	0.0021	47619
IN-L9225	10	0.014	714
IN-W8268	100	0.0018	55556
2-acid-3-triuret (IN-U5F72)	100	0.0025 0.0024	40000 41667
IN-JZ789	90.58	0.0049	18486
Chronic effects on <i>Hypoaspis aculeifer</i>			
Active substance/metabolite	NOEC (mg/kg dw)	PEC_{soil} (mg/kg dw)	TER_{it} (criterion TER ≥5)
IN-A4098	100	0.0067	14925
IN-L9225	100	0.014	7143
IN-W8268	50	0.0018	27778

* PEC_{accumulation} used since DT₉₀ >365 days.

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-3969 in maize-isoxadifen-ethyl

Intended use	Maize		
Acute effects on earthworms			
Active substance/metabolite	LC₅₀ (mg/kg dw)	PEC_{soil} (mg/kg dw)	TER_a (criterion TER ≥10)
Isxadifen-ethyl (safener)	>500 [±]	0.015	>6250
AE-F129431	>947	0.013	>14134
AE-C637375	>1000	0.015 ^a	>12500

* Corrected value derived by dividing the endpoint by a factor of 2

^a As no PEC_{soil} value for the minor metabolite AE-C637375 is calculated, the PEC_{soil} value of the parent compound isoxadifen-ethyl is taken as worst case value.

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-3969 in maize

Intended use		Maize		
Chronic effects on earthworms				
Product	NOEC _{CORR} (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{tt} (criterion TER ≥5)	
GF-3969 plus adjuvant surfactant DPX-KG691	61.5 ¹²³	0.135	456 ⁹¹¹	
Chronic effects on <i>Folsomia candida</i>				
Product	NOEC _{CORR} (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{tt} (criterion TER ≥5)	
GF-3969 plus adjuvant surfactant DPX-KG691	62.5 ¹²⁵	0.135	463 ⁹²⁶	
Chronic effects on <i>Hypoaspis aculeifer</i>				
Product	NOEC _{CORR} (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{tt} (criterion TER ≥5)	
GF-3969 plus adjuvant surfactant DPX-KG691	500 ¹⁰⁰⁰	0.135	3704 ⁷⁴⁰⁷	

zRMS comments:

The risk assessment for soil macro- and meso-fauna performed above for rimsulfuron, thifensulfuron and their relevant metabolites is agreed by the zRMS with some minor corrections, having, however, no impact on the outcome of the performed calculations. Not corrected endpoints were used in the evaluation since log Pow values of both active compounds and their metabolites are <2.

It is noted that no EU agreed endpoints were available for rimsulfuron soil metabolite IN-J0290 and no risk assessment could be performed. Nevertheless, given the very low soil exposure to this compound (0.001 mg/kg dws) the risk would be acceptable even if 10 times toxicity of the parent was assumed.

In case of thifensulfuron-methyl, no EU agreed endpoints (or for not all species) were available for soil metabolites IN-USF72, IN-JZ789, IN-A5546, IN-V7160 and IN-L9226 and no risk assessment could be performed. In case of earthworms, no unacceptable risk would be expected in case 10 times toxicity of the parent (3.43 mg/kg dws) and simplified PEC_{SOIL} of 0.017 mg/kg dws calculated in area of Section 8 to cover exposure from all metabolites are assumed. The resulting TER would be 202, i.e. far above the trigger of 5. Such calculation would not be possible for *Folsomia candida* and *Hypoaspis aculeifer*, since no EU agreed endpoints for these species are available from the EU review. In such case the thifensulfuron-methyl contained in GF-3969 could be taken into account resulting with 10 times toxicity endpoints of 1.16 and 9.26 mg/kg dws for *F. candida* and *H. aculeifer*, respectively (calculation based on NOEC for the formulated product and nominal concentration of thifensulfuron-methyl in GF-3969 of 9.26%). With these endpoints and simplified PEC_{SOIL} of 0.017 mg/kg dws the TER values would be 68.2 and 544.7, respectively, indicating acceptable risk from the metabolites mentioned.

In absence of the EU agreed toxicity data for isoxadifen-ethyl, validation of calculations presented in Table 9.8-7 was not possible. However, the risk assessment has been based on acute endpoints and is thus not relevant in line with current data requirements. Nevertheless, the risk assessment based on formulation endpoint covers also the risk resulting from exposure to the safener, since studies performed with the formulation address effects from all formulation components.

With regard to the GF-3969 risk assessment, the zRMS is of the opinion that corrected endpoints should have been used due to log Pow of isoxadifen-ethyl expected to be >2. Although no EU agreed data for this compound exist, correction has been made in Table 9.8-8 for precautionary reasons. Acceptable risk could be concluded also with corrected endpoints.

Acute risk assessment has been struck through in tables above as being no longer a data requirement.

Overall, acceptable risk to soil macro- and meso-fauna from particular active compounds, their metabolites and formulation GF-3969 may be concluded. Evaluation was performed considering single application of both compound, covering also split applications.

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

The risk to earthworms and other soil organisms was assessed using the toxicity exposure ratios (TER_s) between the toxicity endpoints for GF-3969, rimsulfuron, thifensulfuron methyl, ~~isoxadifen-ethyl~~ and relevant metabolites, and the maximum PEC_{soil} or PEC_{accumulation} resulting from the single application rate of 1 × 135 g product/ha.

For each of the active substances and metabolites the ~~acute and~~ chronic TER values were greater than the trigger of 5 ~~and 10~~, indicating acceptable risk to non-target soil macro-organisms following use of GF-3969 according to the proposed use pattern.

A low toxicity of the product to soil organisms was shown and acceptable risk concluded based on maximum predicted exposure.

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 rimsulfuron, thifensulfuron methyl, isoxadifen-ethyl 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 soil microorganisms of GF-3969 were not evaluated as part of the EU assessment of rimsulfuron or thifensulfuron methyl. 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. Justifications are provided below.

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

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Rimsulfuron 25WG	28 d, aerobic	<25% effect at 28 days at 0.150 kg 0.2 mg a.s./ha corresponding to 0.2 mg a.s./kg dws	EFSA 2005 Wachter, S., 2001a (DuPont-4115)
C-mineralisation	Rimsulfuron 25WG	28 d, aerobic	<25% effect at 28 days at 0.2 mg a.s./ha	EFSA 2005 Wachter, S., 2001a (DuPont-4115)
N-mineralisation	IN-70941	28 d, aerobic	<25% effect at 28 days at 0.2 mg metab./kg dw	EFSA 2005 Wachter, S., 2001b (DuPont-4116)
C-mineralisation	IN-70941	28 d, aerobic	<25% effect at 28 days at 0.2 mg metab./kg dw	EFSA 2005 Wachter, S., 2001b (DuPont-4116)
N-mineralisation	IN-E9260	28 d, aerobic	<25% effect at 28 days at 0.2 mg metab./kg dw	EFSA 2005 Reis, K-H., 2001 (DuPont-6345)
C-mineralisation	IN-E9260	28 d, aerobic	<25% effect at 28 days at 0.2 mg metab./kg dw	EFSA 2005 Reis, K-H., 2001 (DuPont-6345)

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

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Thifensulfuron methyl	28 d, aerobic soil type	<25% effect at 28 days at 400 g a.s./ha (0.533 mg a.s./kg)	EFSA 2015
C-mineralisation	Thifensulfuron methyl	28 d, aerobic soil type	<25% effect at 28 days at 400 g a.s./ha (0.533 mg a.s./kg)	EFSA 2015
N-mineralisation	IN-A4098	28 d, aerobic soil type	<25% effect at 28 days at 0.125 mg met./kg soil dw	EFSA 2015
C-mineralisation	IN-A4098	28 d, aerobic soil type	<25% effect at 28 days at 0.125 mg met./kg soil dw	EFSA 2015
N-mineralisation	IN-A4098	42 d	<25% effect at 28 days at 0.204 mg met./kg soil dw	EFSA 2015
C-mineralisation	IN-A4098	28 d	<25% effect at 28 days at 0.204 mg met./kg soil dw	EFSA 2015

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	IN-A5546	28 d	<25% effect at 28 days at 0.827 mg met./kg soil dw	EFSA 2015
C-mineralisation	IN-A5546	28 d	<25% effect at 28 days at 0.827 mg met./kg soil dw	EFSA 2015
N-mineralisation	IN-JZ789	28 d (study not extended despite effects >25%)	Nitrate formation rate 0.1 mg met./kg soil dw ±51.6% 1.0 mg met./kg soil dw ±48.8%	EFSA 2015
C-mineralisation	IN-JZ789	28 d	<25% effect at 28 days at 1.0 mg met./kg soil dw	EFSA 2015
N-mineralisation	IN-L9223	28 d	<25% effect at 28 days at 1.0 mg met./kg soil dw	EFSA 2015
C-mineralisation	IN-L9223	28 d	<25% effect at 28 days at 1.0 mg met./kg soil dw	EFSA 2015
N-mineralisation	IN-L9223	42 d	<25% effect at 28 days at 0.849 mg met./kg soil dw	EFSA 2015
C-mineralisation	IN-L9223	28 d	<25% effect at 28 days at 0.849 mg met./kg soil dw	EFSA 2015
N-mineralisation	IN-L9225	28 d	<25% effect at 28 days at 0.42 mg met./kg soil dw	EFSA 2015
C-mineralisation	IN-L9225	28 d	<25% effect at 28 days at 0.42 mg met./kg soil dw	EFSA 2015
N-mineralisation	IN-L9225	42 d	<25% effect at 28 days at 0.413 mg met./kg soil dw	EFSA 2015
C-mineralisation	IN-L9225	28 d	<25% effect at 28 days at 0.413 mg met./kg soil dw	EFSA 2015
N-mineralisation	IN-L9226	28 d	<25% effect at 28 days at 0.39 mg met./kg soil dw	EFSA 2015
C-mineralisation	IN-L9226	28 d	<25% effect at 28 days at 0.39 mg met./kg soil dw	EFSA 2015
N-mineralisation	IN-L9226	28 d	<25% effect at 28 days at 0.827 mg met./kg soil dw	EFSA 2015
C-mineralisation	IN-L9226	28 d	<25% effect at 28 days at 0.827 mg met./kg soil dw	EFSA 2015
N-mineralisation	IN-V7160	28 d	<25% effect at 28 days at 0.843 mg met./kg soil dw	EFSA 2015
C-mineralisation	IN-V7160	28 d	<25% effect at 28 days at 0.843 mg met./kg soil dw	EFSA 2015
N-mineralisation	IN-W8268	28 d	<25% effect at 28 days at 0.20 mg met./kg soil dw	EFSA 2015
C-mineralisation	IN-W8268	28 d	<25% effect at 28 days at 0.20 mg met./kg soil dw	EFSA 2015
N-mineralisation	IN-W8268	56 d	<25% effect at 28 days at 0.8 mg met./kg soil dw	EFSA 2015
C-mineralisation	IN-W8268	56 d	<25% effect at 28 days at 0.8 mg met./kg soil dw	EFSA 2015
N-mineralisation	2-acid-3-triuret	28 d	<25% effect at 28 days at 1.0 mg/kg soil dw	EFSA 2015
C-mineralisation	2-acid-3-triuret	28 d	<25% effect at 28 days at 1.0 mg/kg soil dw	EFSA 2015

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

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Isoxadifen-ethyl (safener)	28 d, aerobic, loamy sand and clay silt	<25% effect at 28 days at 0.8 mg a.s./kg soil dw	Zonal evaluation by zRMS Greece (2016)

Table 9.9-4: Endpoints and effect values relevant for the risk assessment for soil microorganisms – GF-3969

Endpoint	Substance	Exposure System	Results	Reference ^a
N-mineralisation	GF-3969 plus adjuvant surfactant DPX-KG691	43 d, aerobic Loamy sand soil	<25% effect at 28 days at 10.4 mg product/kg soil dw	Hammesfahr, U., 2018 (DuPont-49938)
C-mineralisation	GF-3969 plus surfactant DPX-KG691	28 d, aerobic Loamy sand soil	<25% effect at 28 days at 10.4 mg product/kg soil dw	Hammesfahr, U., 2018 (DuPont-49938)
N-mineralisation	GF-3969 plus adjuvant surfactant Codacide	43 d, aerobic Loamy sand soil	<25% effect at 28 days at 10.4 mg product/kg soil dw	Hammesfahr, U., 2018 (DuPont-49976)
C-mineralisation	GF-3969 plus surfactant Codacide	28 d, aerobic Loamy sand soil	<25% effect at 28 days at 10.4 mg product/kg soil dw	Hammesfahr, U., 2018 (DuPont-49976)

a Summarized in Appendix 2.

zRMS comments:

Endpoints presented in Table 9.9-1 are EU agreed endpoints reported in EFSA Scientific Report (2005) 45, 1-61 and EFSA Journal 2018;16(5):5258. Endpoints presented in Table 9.9-2 are EU agreed endpoints reported in EFSA Journal 2015;13(7):4201.

No EU agreed data exist for the safener, isoxadifen-ethyl, and for this reason validation of information provided in Table 9.9-3 against EU agreed endpoints was not possible. Nevertheless, data provided by the Applicant for isoxadifen-ethyl have been retained for informative purposes, with font colour changed to grey in order to easily distinguish validated from non-validated data.

Studies on effects of GF-3969 used with two **adjuvants** surfactants on soil nitrogen transformation were agreed by the zRMS and the endpoints reported in Table 9.9-4 above are confirmed. For summaries of the studies and details of the evaluation, please refer to Appendix 2. Neither of **adjuvants** surfactants increased toxicity of GF-3969 to soil microflora and the risk assessment will be based on concentration of 10.4 mg product/kg dws, at which effects were <25%.

Information on effects on soil carbon transformation has been struck through as being no longer a data requirement.

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 the Core, Part B, Section 8, and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see Section 0 in this document).

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group 1× application to maize at 135 g product/ha also covers the risk to soil micro-organisms from all other intended uses.

Table 9.9-5: Assessment of the risk for effects on soil micro-organisms due to the use of GF-3969 in maize - rimsulfuron

Intended use		Maize	
N-mineralisation			
Active substance/metabolite	Max. conc. with effects ≤25% (mg/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
Rimsulfuron	0.2	0.020	Yes
IN-70941	0.2	0.0276 0.0275	Yes
IN-E9260	0.2	0.0091	Yes
C-mineralisation			
Active substance/metabolite	Max. conc. with effects ≤25% (mg/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
Rimsulfuron	0.2	0.020	Yes
IN-70941	0.2	0.0275	Yes
IN-E9260	0.2	0.0091	Yes

Table 9.9-6: Assessment of the risk for effects on soil micro-organisms due to the use of GF-3969 in maize – thifensulfuron methyl

Intended use		Maize	
N-mineralisation			
Active substance/metabolite	Max. conc. with effects ≤25% (mg/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
Thifensulfuron methyl	0.533	0.013	Yes
IN-A4098	0.125	0.0067	Yes
IN-A5546	0.827	0.0020 0.0016	Yes
IN-JZ789	No valid endpoint available, at 0.1 mg/kg effects >25% 0.1	0.0049	No Yes
IN-JZ789	0.0533 (10 times toxicity of the parent)	0.0049	Yes
IN-L9223	0.849	0.0021	Yes
IN-L9225	0.413	0.014	Yes
IN-L9226	0.39	0.0022 0.0018	Yes
IN-V7160	0.843	0.0009	Yes
IN-W8268	0.20	0.0018	Yes
2-acid-3-triuret (IN-USF72)	1.0	0.0025 0.0024	Yes
C-mineralisation			
Active substance/metabolite	Max. conc. with effects ≤25% (mg/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
Thifensulfuron methyl	0.533	0.013	Yes
IN-A4098	0.125	0.0067	Yes
IN-A5546	0.827	0.0016	Yes
IN-JZ789	1.0	0.0049*	Yes
IN-L9223	0.849	0.0021	Yes
IN-L9225	0.413	0.014	Yes

IN-L9226	0.39	0.0018	Yes
IN-V7160	0.843	0.0009	Yes
IN-W8268	0.20	0.0018	Yes
2-acid-3-triuret	1.0	0.0024	Yes

* PEC_{accumulation} used since DT₉₀ >365 days.

Table 9.9-7: Assessment of the risk for effects on soil micro-organisms due to the use of GF-3969 in maize – isoxadifen-ethyl

Intended use	Maize		
N-mineralisation			
Safener	Max. conc. with effects ≤25% (mg/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
Isoxadifen-ethyl (safener)	0.8 (at 28 d) mg/kg soil dw	0.015	Yes

Table 9.9-8: Assessment of the risk for effects on soil micro-organisms due to the use of GF-3969 in maize

Intended use	Maize		
N-mineralisation			
Product	Max. conc. with effects ≤25% (mg/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
GF-3969 plus adjuvant surfactant DPX-KG691	10.4	0.135	Yes
GF-3969 plus adjuvant surfactant Codacide	10.4	0.135	Yes
C-mineralisation			
Product	Max. conc. with effects ≤25% (mg/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
GF-3969 plus surfactant DPX-KG691	10.4	0.135	Yes
GF-3969 plus surfactant Codacide	10.4	0.135	Yes

zRMS comments:

The risk assessment for soil **microorganisms** ~~macro and meso fauna~~ performed above for rimsulfuron, thifensulfuron, majority of their relevant metabolites and GF-3969 is in general agreed by the zRMS with some minor corrections, having, however, no impact on the outcome of the performed calculations. However, for thifensulfuron-methyl metabolite IN-JZ789 no risk assessment could be performed based on the available data since at the lowest tested concentration (0.1 mg/kg dws) effects >25% were observed and the study was not extended (data gap in EFSA conclusion). In order to finalise the risk assessment, the zRMS assumed 10 times toxicity of the parent (resulting with endpoint of 0.0533 mg/kg dws, i.e. lower than the maximum tested concentration of IN-JZ789). Respective corrections were made in Table 9.9-6 above.

It is noted that no EU agreed endpoints were available for rimsulfuron soil metabolites IN-70942 and IN-J0290 and no risk assessment could be performed. Nevertheless, given the very low soil exposure to these compounds (0.0052 and 0.001 mg/kg dws) the risk would be acceptable even if 10 times toxicity of the parent (i.e. 0.02 mg/kg dws) was assumed.

In absence of the EU agreed toxicity data for isoxadifen-ethyl, validation of calculations presented in Table 9.9-7 was not possible. However, the risk assessment has been based on acute endpoints and is thus not relevant in line with current data requirements. Nevertheless, the risk assessment based on formulation endpoint covers also the risk resulting from exposure to the safener, since studies performed with the formulation address effects from all formulation components.

Risk assessment performed for carbon mineralisation has been struck through in tables above as being no longer a data requirement.

Overall, no unacceptable effects of particular active compounds, their metabolites and formulation GF-3969 on soil microbial activity are expected when GF-3969 is use according to the intended use pattern. Evaluation was performed considering single application of both compound, covering also split applications.

9.9.3 Overall conclusions

The risk of GF-3969, the active substances and relevant metabolites to soil micro-organisms was evaluated by comparison of the reported concentrations with effects <25% derived from laboratory tests, with maximum initial PEC_{soil} or PEC_{accumulation} based on the highest single application rate of 135 g product/ha.

No significant effects of >25% effect were reported at soil concentrations where exceeded the relevant PEC_{soil} values, indicating that the risk to soil micro-organisms is acceptable following the use of GF-3969 according to the proposed use pattern.

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 GF-3969. Effects on non-target terrestrial plants of GF-3969 were not evaluated as part of the EU assessment of rimsulfuron or thifensulfuron methyl. New data submitted with this application are listed in Appendix 1 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. Justifications are provided below.

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

Species	Test item	Exposure System	Results	Reference ^a
<i>Brassica napus</i> (Oilseed Rape) _d	GF-3969 + DPX-KG691 (adjuvant surfactant)	21 d Seedling emergence	ER ₅₀ = 9.74 g product/ha shoot fresh weight	Spatz, B., 2018, (DuPont-49939)
<i>Glycine max</i> (Soybean) _d			ER ₅₀ >135 g product/ha shoot fresh weight	
<i>Pisum sativum</i> (Pea) _d			ER ₅₀ = 129 g product/ha shoot fresh weight	
<i>Cucumis sativus</i> (Cucumber) _d			ER ₅₀ = 48.1 g product/ha shoot fresh weight	
<i>Beta vulgaris</i> (Sugarbeet) _d			ER ₅₀ = 9.26 g product/ha shoot dry weight	
<i>Solanum lycopersicum</i> (Tomato) _d			ER ₅₀ >45 g product/ha shoot dry weight	
<i>Sorghum bicolor</i> (Sorghum) _m			ER ₅₀ >135 g product/ha shoot dry weight	
<i>Allium cepa</i> (Onion) _m			ER₅₀ = 5.07 g product/ha shoot dry weight	
<i>Avena sativa</i> (Oat) _m			ER ₅₀ >135 g product/ha shoot dry weight	
<i>Lolium perenne</i> (Ryegrass) _m			ER ₅₀ = 22.1 g product/ha shoot dry weight	
<i>Allium cepa</i> (Onion) _m	GF-3969 + DPX-KG691 (adjuvant)	21 d Vegetative vigour	ER ₅₀ = 5.80 g product/ha shoot dry weight	Arnie, J.R., McKelvey, R.A., Aufderheide, J.A., Lockard, L.A., Zhang, L., 2020 (DuPont-49942)
<i>Avena sativa</i> (Oat) _m			ER ₅₀ = 15.9 g product/ha shoot dry weight	
<i>Sorghum bicolor</i> (Sorghum) _m			ER ₅₀ = 2.98 g product/ha shoot dry weight	
<i>Zea mays</i> (Corn) _m			ER ₅₀ >136 g product/ha visual injury/shoot dry weight	
<i>Beta vulgaris</i> (Sugarbeet) _d			ER ₅₀ = 1.61 g product/ha shoot dry weight	
<i>Brassica napus</i> (Oilseed Rape) _d			ER ₅₀ = 3.82 g product/ha shoot dry weight	
<i>Cucumis sativus</i> (Cucumber) _d			ER ₅₀ = 31.4 g product/ha shoot height	
<i>Glycine max</i> (Soybean) _d			ER ₅₀ = 11.1 g product/ha shoot dry weight	
<i>Solanum lycopersicum</i> (Tomato) _d			ER ₅₀ = >136 g product/ha shoot dry weight, visual injury, shoot height	
<i>Pisum sativum</i> (Pea) _d			ER ₅₀ = 10.6 g product/ha shoot dry weight	
Species sensitivity distribution		21 d Vegetative vigour	HR ₅ = 1.44 g/ha (95% CI 0.37—2.92)	Calculated by Applicant

m: monocotyledonous; d: dicotyledonous; CI: confidence intervals

Bold values were used in the risk assessment.

a Summarised in Appendix 2.

Based on the ER₅₀ values the most sensitive crops are onion (ER₅₀ = 5.07 g product/ha) for seedling emergence test and sugar beet (ER₅₀ = 1.61 g product/ha) for vegetative vigour test.

zRMS comments:

Studies on effects of GF-3969 used with DPX-KG691 as a **adjuvant** ~~surfactant~~ on non-target terrestrial plants were evaluated by the zRMS. The seedling emergence study (Spatz, 2018, DuPont-49939) was agreed, however the vegetative vigour test (Arnie et al., 2020, Du-Pont-49942) was **initially** considered not valid due to phytotoxic effects observed in controls (especially on day 14), while lack of phytotoxic effects is one of the validity criteria of the OECD 227. It is noted that at test termination on day 21 the control plants recovered, but OECD 227 does not indicate that this validity criterion is relevant only for test termination and for this reason no phytotoxic effects should be observed during the entire study. In the study report no explanation of the phytotoxicity observed in controls is given and it is thus not known if it was due to nutrient deficiency, overcrowding, unfavourable conditions or accidental exposure to the test item. Nevertheless, growing conditions do not seem to be the reason for these effects, since all plants were kept at the same conditions while chlorosis, necrosis or wilting were observed only in some replicates.

In opinion of the zRMS phytotoxic effects observed on day 14 could have impact on growth of the control plants and it cannot be excluded that shoot height and dry weight could be lower comparing to not affected control replicates, even if recovery from phytotoxic effects was seen on day 21. Analysis of the shoot dry weight data for oilseed rape indicates that this possible, since the lowest shoot dry weight on day 21 was observed in replicate in which most pronounced phytotoxic effects were observed. This could have impact on endpoints calculated for the test item, since reduced shoot weight of control plants could lead to lower deviation of this parameter from control in test item groups.

For summaries of the studies and details of the evaluation, please refer to Appendix 2.

~~Overall, no reliable endpoint is available for the vegetative vigour, being more sensitive than the seedling emergence.~~

During the commenting period the Applicant should provide sufficient information to address effects of GF-3969 on vegetative vigour. In addition to that, endpoints based on phytotoxic effects from the seedling emergence study should be provided, in line with the Central Zone requirements.

In addition to that it is noted that the studies on effects of GF-3969 on NTTPs were performed only with DPX-KG691 used as a **adjuvant** ~~surfactant~~. However, based on results of studies performed with *Lemna gibba* it seems that addition of Codacide leads to more pronounced toxic effects. Taking into account that GF-3969 may be used also with Codacide, studies on effects of GF-3969 with this **adjuvant** ~~surfactant~~ on non-target terrestrial plants or other sufficient information demonstrating phytotoxic effects of GF-3969+Codacide should be also provided.

During the commenting period the Applicant referred to the concerns of the zRMS regarding symptoms of phytotoxicity observed in some control plants of sorghum and oilseed rape. The full position paper by Ellis (2022, KCP 10.6.2/03) including response of the laboratory on potential cross-contamination is presented Appendix 2. In summary, the Applicant proposed to exclude the oilseed rape and sorghum control replicates with visible phytotoxic symptoms and merge the remaining replicates of water and adjuvant control in order to assure sufficient number of control plants for statistical analysis. After this procedure, the endpoints for the 2 plants were recalculated and resulted with only slightly lower ER₅₀ values, showing that the phytotoxic effects observed in some control replicates of sorghum and oilseed rape had marginal impact on the mean growth of control plants and in consequence – the study results. Of all tested plants, sugar beet remained the species with the lowest endpoint. The approach of the Applicant was agreed by the zRMS, since after exclusion of the control replicates with phytotoxic symptoms, only healthy control plants were included for comparison with the treatment groups. The results of the study were restored in Table 9.10-1 above with endpoints for oilseed rape and sorghum corrected accordingly. The HR₅ calculated by the Applicant remained unacceptable since lower HR₅ was obtained by the zRMS (see point 9.10.2.3 for details).

The endpoints for phytotoxic effects observed in the seedling emergence study (Spatz, 2018, DuPont-49939) were calculated by the Applicant (see Appendix 2 for details) and were higher than the endpoints determined for fresh weight, which are thus more relevant for the risk assessment.

Issue of potentially higher toxicity of GF-3969 applied in mixture with Codacide adjuvant was also addressed by the Applicant in the position paper by Ellis (2022, KCP 10.6.2/03) by comparison of the efficacy data obtained in trials performed with the mixture of GF-3969 with both adjuvants (DPX-KG691 and Codacide). The trials included efficacy against various monocotyledonous (100 datapoints) and dicotyledonous (204 datapoints)

weeds. Obtained results indicate that effects of GF-3969 on target weeds were similar, regardless of the adjuvant used. Some fluctuations were observed, but these were negligible (differences up to ~5%). Furthermore, in majority studies mixture of GF-3969 with DPX-KG691 (Vivolt) had more pronounced effects on investigated weeds, which is also reflected in the overall mean efficacy calculated separately for monocot and dicot weeds. Based on the obtained results it is not expected that addition of adjuvant Codacide would result with more pronounced effects in non-target terrestrial plants studies and endpoints derived from studies performed with addition of DPX-KG691 (Vivolt) cover also effects from the mixture with Codacide. Detailed comparison of the efficacy trials may be found in Appendix 2.

9.10.1.1 Justification for new endpoints

New studies have been conducted to assess the effect of formulation GF-3969 on seedling emergence and vegetative vigour. These studies are applied to the risk assessment.

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Not relevant. Since the active substances of GF-3969 have herbicidal activity, the risk to terrestrial non-target plants has been evaluated below based on dose-response data.

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.

The $PER_{\text{off-field}}$ was calculated following the below equation:

$$PER_{\text{off-field}} = \text{Application rate} \left(\frac{\text{g}}{\text{ha}} \right) * \text{MAF} * \text{drift rate}\%$$

Table 9.10-2: Assessment of the risk for non-target plants due to the use of GF-3969 in maize

Intended use		Maize		
Active substance/product		GF-3969		
Application rate (g/ha)		1 × 135 g product/ha		
MAF		1		
Test species	ER ₅₀ (g/ha)	Drift rate	PER _{off-field} (g/ha)	TER criterion: TER ≥ 5
<i>Allium cepa</i> (Onion) Seedling emergence	5.07 g product/ha	2.77% (1m)	3.73	1.4
<i>Beta vulgaris</i> (Sugar beet) Vegetative vigour	1.61 g product/ha	2.77% (1m)	3.73	0.43

MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Based on the standard drift rate of 2.77%, a potential risk from both seedling emergence and vegetative vigour effects is shown. Further refinement and mitigation is considered in the following section.

zRMS comments:

The risk assessment for the seedling emergence provided in Table 9.10-2 above is agreed by the zRMS. The calculated TER is below the trigger of 5, hence further assessment is deemed necessary and is presented in point 9.10.2.4 below.

The study on vegetative vigour by Arnie et al. (2020, DuPont-49942) has been restored after new endpoints were calculated by the Applicant after exclusion of the control replicates showing phytotoxic symptoms. For this reason the risk assessment in Table 9.10-2 could be also restored.

Based on performed calculations, no acceptable risk for vegetative vigour could be concluded and further evaluation was performed in points 9.10.2.3 and 9.10.2.4 below.

The risk assessment for the vegetative vigour is struck through in Table 9.10-2 above, since the endpoints originate from the study by Arnie et al. (2020, Du Pont 49942) which was not accepted by the zRMS due to phytotoxic effects observed in control replicates and their potential impact on growth parameters of control plants at the test termination and in consequence on the endpoints calculated for the test item groups.

As no other data exist, the risk assessment for non target plants could not be finalised.

9.10.2.3 Higher-tier risk assessment

Vegetative vigour

The lowest reported endpoints result from vegetative vigour and so higher tier refinement based on probabilistic risk assessment is conducted with vegetative vigour endpoints as these are protective of seedling emergence.

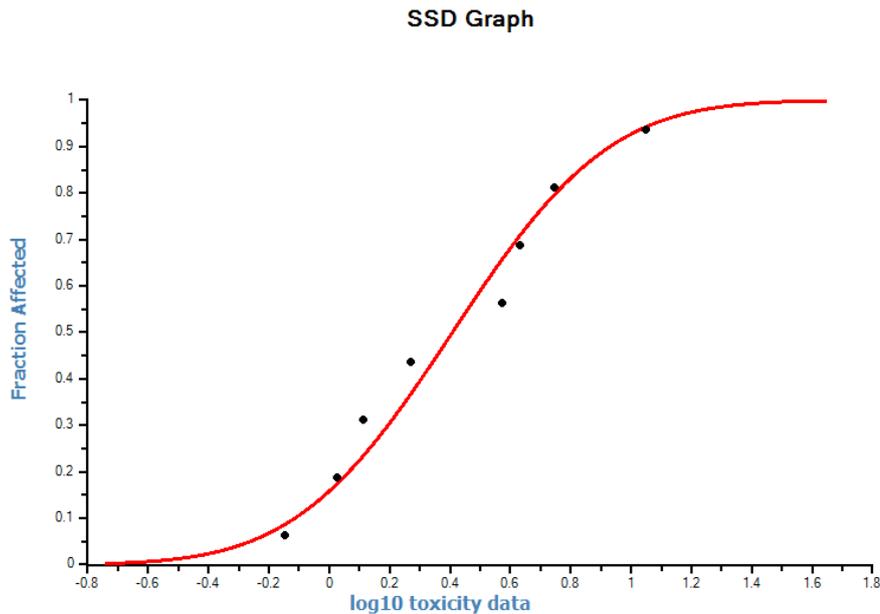
There is a sufficient number of endpoints (*i.e.* 8) available from the vegetative vigour study with GF-3969 to use a probabilistic risk assessment approach for these datasets (Guidance Document on Terrestrial Ecotoxicology, SANCO/10329/2002). Probabilistic methods that make use of species sensitivity distributions (SSD) may be used when at least 6-10 species have been tested and the SSD toxicity data fit a log normal distribution.

The SSD for vegetative vigour data was built using ETX v. 2.2 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 HR₅ in the distribution was determined. HR₅ values were derived based on the percent visual injury from the vegetative vigour study and are summarized in the following table.

Table 9.10-3: Results of HR₅ determinations for non-target terrestrial plants exposed post-emergence to GF-3969

Substance	Study type	Confidence Interval	HR ₅ value (g product/ha)		
			Lower	Median	Upper
GF-3969	Vegetative vigour	90%	0.37	1.44	2.92

Figure 9.10-1: Species Sensitivity Distribution for the lowest ER₅₀ from the vegetative vigour study for GF-3969



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 HR₅ above and accounting for different risk mitigation options in the following section.

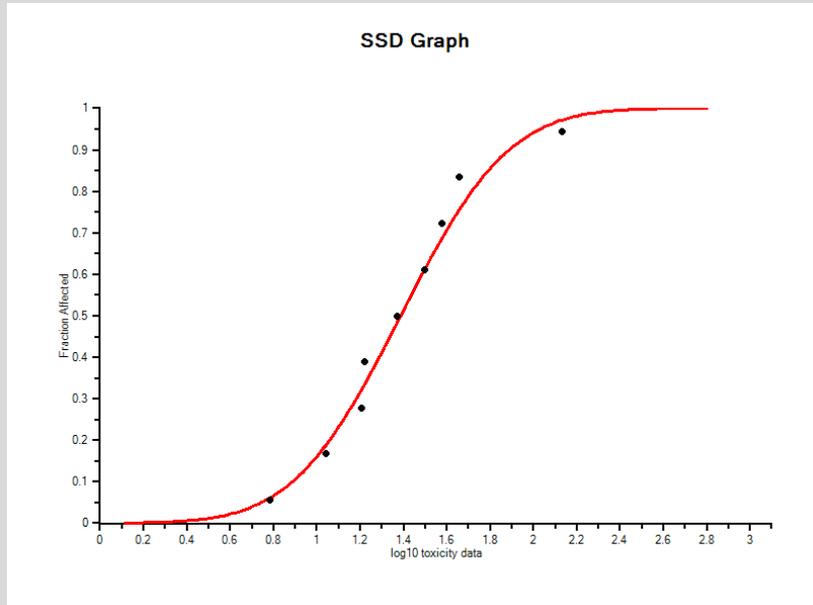
zRMS comments:

Since the vegetative vigour study by Arnie et al. (2020, DuPont-49942) was restored by the zRMS after exclusion of the control replicates showing phytotoxic symptoms, the species sensitivity distribution was evaluated taking into account corrected endpoints.

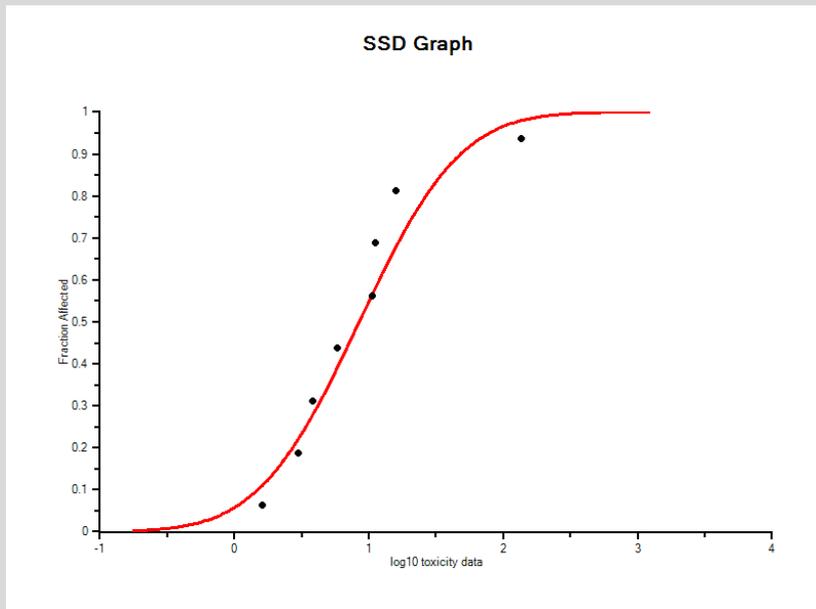
It is noted that the Applicant calculated HR₅ on the basis of the percentage visual injury observed in the vegetative vigour study, however it is not clear how these data were included in construction of the SSD and if the phytotoxicity endpoints were considered. It is also not explained if other parameters (shoot height, shoot dry weight and survival) were taken into account to calculate HR₅ in order to select the worst case value for purposes of the probabilistic risk assessment. It is further noted that the title of the Figure 9.10-1 above suggest that the lowest endpoints were considered, regardless for which parameter they were calculated. Nevertheless, in SSD approach endpoints derived for different parameters should not be mixed. Taking this into account, Applicants' calculations above remained struck through and HR₅ values for particular parameters investigated in the vegetative vigour study were calculated by the zRMS using the endpoints for oilseed rape and sorghum calculated after exclusion of the control replicates exhibiting phytotoxic effects. In case single unbound value was determined for the considered parameter, this value was included as the exact values. In case more than one unbound values were available, only one of them was used. Summary of obtained results is presented in table below. All data passed the tests for normality (Anderson-Darling, Kolmogorov-Smirnov and Cramer von Mises) build in the ETX 2.3 tool which was used in zRMS calculations.

Substance	Study type	Parameter	HR _s value (g product/ha)		
			Lower	Median	Upper
GF-3969	Vegetative vigour	Shoot height	1.598	5.249	10.02
		Shoot dry weight	0.1131	0.8272	2.319
		Survival	1.309	6.088	12.86
		Phytotoxicity	0.3497	1.979	5.081

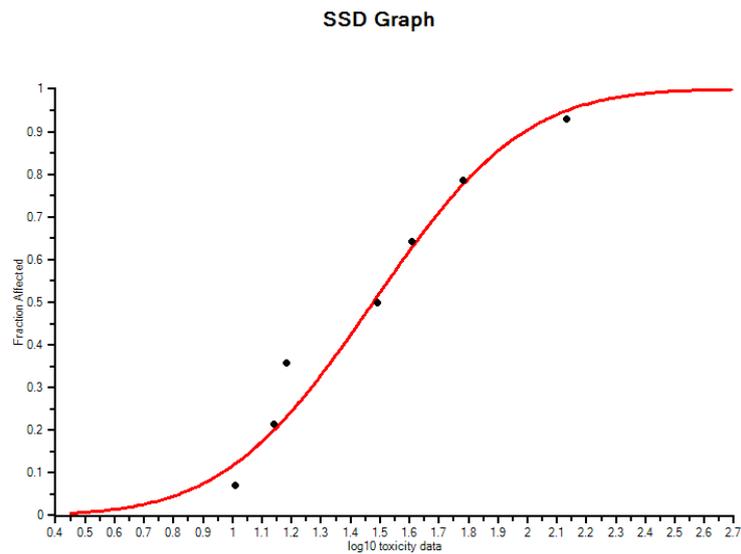
SSD graphs are presented below.



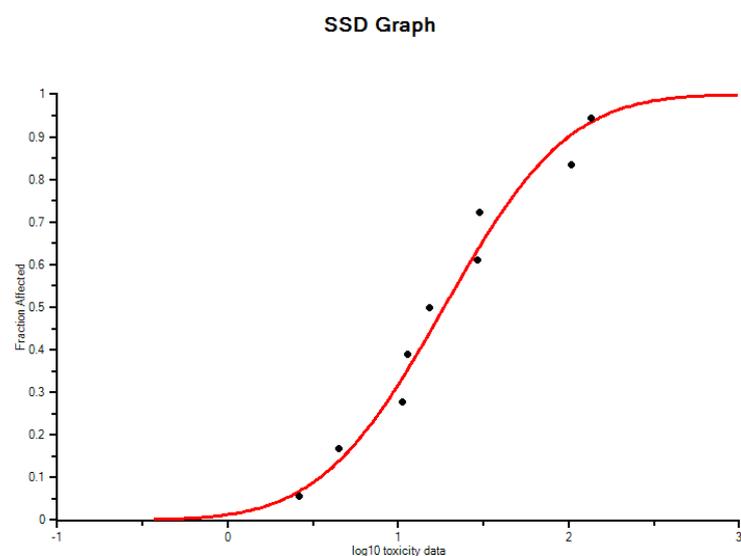
Species Sensitivity Distribution for the ER₅₀ for shoot height from the vegetative vigour study



Species Sensitivity Distribution for the ER₅₀ for shoot dry weight from the vegetative vigour study



Species Sensitivity Distribution for the ER₅₀ for survival from the vegetative vigour study



Species Sensitivity Distribution for the ER₅₀ for phytotoxicity from the vegetative vigour study

Based on the above calculations, the lowest median HR₅ of 0.8272 g/ha calculated by the zRMS for shoot dry weight in vegetative vigour test is considered relevant for purposes of the probabilistic risk assessment.

Endpoints calculated in seedling emergence study were higher, but the HR₅ was calculated by the zRMS in order to confirm that vegetative vigour study is protective also for seedling emergence. The HR₅ of 3.367 g/ha based on shoot fresh weight from seedling emergence study (the most sensitive parameter) is clearly higher than HR₅ of 0.8272 g/ha, derived from vegetative vigour test.

The calculation of the HC₅ provided above is struck through since endpoints considered in this calculation originate from vegetative vigour study by Arnie et al. (2020, Du Pont 49942) which was not accepted by the zRMS due to phytotoxic effects observed in control replicates and their potential impact on growth parameters of control plants at the test termination and in consequence on the endpoints calculated for the test item groups.

9.10.2.4 Risk mitigation measures

Deterministic risk assessment

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: Risk assessment for non-target terrestrial plants based on seedling emergence effects due to the use of GF-3969 in maize considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Maize			
Active substance/product		GF-3969			
Application rate (g/ha)		1 × 135 g product/ha			
MAF		1			
Buffer strip (m)	Drift rate (%)	PER_{off-field} (g/ha)	PER_{off-field} 50% drift red. (g/ha)	PER_{off-field} 75% drift red. (g/ha)	PER_{off-field} 90% drift red. (g/ha)
1	2.77%	3.74	1.87	0.93	0.37
5	0.57%	0.77	0.38	0.19	0.08
10	0.29%	0.39	0.20	0.10	0.04
Toxicity value		TER			
ER ₅₀ = 5.07 g/ha		criterion: TER ≥ 5			
1	2.77%	1.4	2.7	5.4	14
5	0.57%	6.6	13	26	66
10	0.29%	13.0	26	52	130

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

Table 9.10-5: Risk assessment for non-target terrestrial plants based on vegetative vigour effects due to the use of GF-3969 in maize considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Maize			
Active substance/product		GF-3969			
Application rate (g/ha)		1 × 135 g product/ha			
MAF		1			
Buffer strip (m)	Drift rate (%)	PER_{off-field} (g/ha)	PER_{off-field} 50% drift red. (g/ha)	PER_{off-field} 75% drift red. (g/ha)	PER_{off-field} 90% drift red. (g/ha)
1	2.77%	3.74	1.87	0.93	0.37
5	0.57%	0.77	0.38	0.19	0.08
10	0.29%	0.39	0.20	0.10	0.04
Toxicity value		TER			
ER ₅₀ = 1.61 g/ha		criterion: TER ≥ 5			
1	2.77%	0.43	0.86	1.7	4.4
5	0.57%	2.1	4.2	8.4	20
10	0.29%	4.1	8.1	16	40

MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

For applications to maize, the TER value calculated using the ER₅₀ value determined based on the seedling emergence data is greater than the relevant trigger of 5 with the following mitigations:

- 1 m buffer with 75% drift reducing technology,

- 5 m buffer with no drift reducing technology

The TER value calculated using the ER₅₀ value determined based on the vegetative vigour data is greater than the relevant trigger of 5 with the following mitigation:

- 5 m buffer with 75% drift reducing technology
- 10 m buffer with 50% drift reducing technology.

Probabilistic risk assessment

In the following table, the TER values are calculated, comparing the 5th percentile ER₅₀ value determined based on the vegetative vigour data for GF 3969 respectively to the predicted exposure rate (PER) in off field areas. The TER criterion for the probabilistic risk assessment is TER ≥ 1.

Table 9.10-6: Probabilistic risk assessment for non-target plants exposed to GF 3969 after application in maize

Intended use		Maize			
Active substance/product		GF 3969			
Application rate (g/ha)		1 × 135 g product/ha			
MAF		1			
Buffer strip (m)	Drift rate (%)	PER _{off-field} (g/ha)	PER _{off-field} 50% drift red. (g/ha)	PER _{off-field} 75% drift red. (g/ha)	PER _{off-field} 90% drift red. (g/ha)
1	2.77%	3.74	1.87	0.93	0.37
5	0.57%	0.77	0.38	0.19	0.08
10	0.29%	0.39	0.20	0.10	0.04
Toxicity value		TER			
HR ₅ = 1.44 g product/ha		criterion: TER ≥ 1			
1	2.77%	0.39	0.77	4.55	3.9
5	0.57%	1.9	3.8	7.6	18
10	0.29%	3.7	7.2	14	36

MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

For applications to maize, using the probabilistic risk assessment approach the TER value calculated using the HR₅ value determined based on the vegetative vigour data is greater than or equal to the relevant trigger of 1 with the following mitigation:

- 1 m buffer with 75% drift reducing technology,
- 5 m buffer with no drift reducing technology

zRMS comments:

The deterministic risk assessment for the seedling emergence and vegetative vigour performed in Table 9.10-4 with consideration of risk mitigation measures is agreed by the zRMS. Performed calculations indicate acceptable risk for this parameter provided that an unsprayed buffer zone of 5 meters to non-agricultural land is respected or the spray drift is reduced by 75% using appropriate nozzles.

The probabilistic risk assessment was recalculated by the zRMS with consideration of the agreed HR₅ of 0.8272 g/ha (for details of calculation, please refer to zRMS commenting box in point 9.10.2.3 above. Results are presented in table below.

Intended use		Maize			
Active substance/product		GF-3969			
Application rate (g/ha)		1 × 135 g product/ha			
MAF		1			
Buffer strip (m)	Drift rate (%)	PER _{off-field} (g/ha)	PER _{off-field} 50% drift red. (g/ha)	PER _{off-field} 75% drift red. (g/ha)	PER _{off-field} 90% drift red. (g/ha)
1	2.77%	0.39	0.77	4.55	3.9
5	0.57%	1.9	3.8	7.6	18
10	0.29%	3.7	7.2	14	36

1	2.77%	3.74	1.87	0.93	0.37
5	0.57%	0.77	0.38	0.19	0.08
10	0.29%	0.39	0.20	0.10	0.04
Toxicity value HR ₅₀ = 0.8272 g/ha		TER criterion: TER ≥ 1			
1	2.77%	0.22	0.44	0.9	2.2
5	0.57%	1.1	2.1	4.3	10.7
10	0.29%	2.1	4.2	8.5	21.1

Overall, based on the above calculations acceptable risk to non-target terrestrial plants may be concluded from the intended Central Zone uses of GF-3969, provided that following risk mitigation measures are respected:

1. Deterministic risk assessment:

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

2. Probabilistic risk assessment:

- 5 m unsprayed buffer zone to non-agricultural land, or
- 90% drift reduction.

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

The deterministic and probabilistic risk assessment for the vegetative vigour performed in Tables 9.10-5 and 9.10-6 above with consideration of risk mitigation measures is struck through, since the endpoints originate from the study by Arnie et al. (2020, Du Pont 49942) which was not accepted by the zRMS due to phytotoxic effects observed in control replicates and their potential impact on growth parameters of control plants at the test termination and in consequence on the endpoints calculated for the test item groups.

Most probably the risk assessment performed on the basis of endpoints derived from the correctly performed vegetative vigour study would result with acceptable risk with consideration of 10-15 m unsprayed buffer zone, but the reliable endpoints are required to determine the exact width.

As no other data exist, the risk assessment for non-target plants could not be finalised.

9.10.3 Overall conclusions

Regulatory testing has been conducted with the product, GF-3969 to assess effects on vegetative vigour and seedling emergence. The seedling emergence study was accepted by the zRMS with no concerns, but the vegetative vigour study was agreed after exclusion of control replicates of oilseed rape and sorghum which exhibited phytotoxic effects and recalculation of endpoints for these two species. The risk assessment was performed using deterministic and probabilistic approach. Overall, acceptable risk to non-target terrestrial plants could be concluded from the intended uses of GF-3969, provided that following risk mitigation measures are respected:

1. Deterministic 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
- 90% drift reduction.

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

invalidated due to phytotoxic effects observed in control replicates and their potential impact on growth parameters of control plants at the test termination and in consequence on the endpoints calculated for the test item groups.

Since no other data exist, the risk assessment for non-target plants could not be finalised and no final conclusion may be taken.

Based on the probabilistic risk assessment for vegetative vigour effects, taking into account the 5th percentile ER_{50} derived from the SSD for effects on vegetative vigour, an acceptable risk to terrestrial non-target plants can be concluded following uses of GF 3969 with

- 1 m buffer with 75% drift reducing technology,
- 5 m buffer with no drift reducing technology

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-3969 in maize.

9.11.1 Thifensulfuron methyl metabolites

Pesticidal activity information on thifensulfuron methyl metabolites – IN-JZ789 and IN-U5F72 was not available during Thifensulfuron methyl active substance renewal. Summary of the study is provided in Appendix 2.

The results demonstrate that the biological activities of metabolites IN-JZ789 and IN-U5F72 are neither comparable to nor greater than that of thifensulfuron methyl. The results of these screens compare well with the ecotoxicological data collected for aquatic plant species, which show a similar pattern of much more reduced herbicidal activity of these metabolites compared to the parent compound.

Table 9.11-1: Visual plant response ratings for thifensulfuron methyl when applied post (foliar spray) to crop and weed species. The ratings were made on a percentage scale (0 to 100, where 0 = no injury or control, and 100 = death of the plant) compared to untreated control treatment

Plant species	Thifensulfuron methyl technical @ 100 g/ha	IN-JZ789 @ 100 g/ha	IN-U5F72 @ 100 g/ha
Crop species			
Corn	95	0	0
Oilseed Rape	90	0	0
Soybean	85	0	0
Wheat	40	0	0
Weed species			
Pigweed	98	0	0
Morning glory	100	0	20
Velvetleaf	100	0	0
Ragweed	85	0	20
Lambsquarters	100	0	0
Waterhemp	95	0	0
Galium	95	0	0
Kochia	98	0	0
Chickweed	100	0	0
Foxtail	80	0	0
Crabgrass	85	0	0
Barnyardgrass	95	0	0
Nutsedge	40	0	0
Wild Oat	0	0	0
Ryegrass	85	0	0
Blackgrass	80	0	0

zRMS comments:

The study was evaluated in area of Efficacy section. Based on the results of the study it may be concluded that both tested thifensulfuron-methyl metabolites (IN-JZ789 and IN-U5F72) do not exhibit herbicidal activity.

9.12 Monitoring data (KCP 10.8)

Not relevant.

9.13 Classification and Labelling

Regulation (EC) No 1272/2008 (CLP Regulation)

Acute and chronic toxicity of GF-3969 to *Lemna gibba* showed that this product should be classified as Acute Aquatic toxicity Category 1 H400, Chronic Aquatic toxicity Category 1 H410. Hence, The GHS symbol GHS09 and signal word Warning should be added to the label together with the hazard statement H410 and precautionary statements P391 and P501.

zRMS comments:

CLP classification of GF-3969 provided by the Applicant above is agreed by the zRMS. Following classification and labelling are considered relevant:

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

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 GLP or GEP Status Published or not	Vertebrate study Y/N	Owner
KCP, 10.2.1/01	xxxxxxxxxxxxxxx	2019	DPX-V4B07 24 WG (rimsulfuron 25 SG + thifensulfuron 50 SG + isoxadifen 50 WG) A blend of paste extruded granules plus isodecylalcohol ethoxylated (DPX-KG691) surfactant: Acute toxicity to the rainbow trout, <i>Oncorhynchus mykiss</i> , determined under static-renewal test conditions DuPont-49948, Revision No. 1 Eurofins EAG Agrosience, LLC GLP: Yes Published: No	Y	DuPont
KCP, 10.2.1/02	Goudie, O.J.	2019	DPX-V4B07 24 WG (Rimsulfuron 25 SG + Thifensulfuron 50 SG + Isoxadifen 50 WG) A blend of paste extruded granules plus isodecylalcohol ethoxylated (DPX-KG691) surfactant: 48-Hour static renewal, acute toxicity test with the cladoceran, <i>Daphnia magna</i> DuPont-49949, Revision No. 1 Eurofins EAG Agrosience, LLC GLP: Yes Published: No	N	DuPont
KCP, 10.2.1/03	Hoover, E.	2019	DPX-V4B07 24 WG (Rimsulfuron 25 SG + Thifensulfuron 50 SG + Isoxadifen 50 WG) a blend of paste extruded granules plus isodecylalcohol ethoxylated (DPX-KG691) surfactant: growth inhibition test with the unicellular green alga, <i>Pseudokirchneriella subcapitata</i> DuPont-49943 Eurofins EAG Agrosience, LLC GLP: Yes Published: No	N	DuPont
KCP, 10.2.1/04	Bergfield, A.	2019	DPX-V4B07 24 WG (Rimsulfuron 25 SG + Thifensulfuron 50 SG + Isoxadifen 50 WG) A blend of paste extruded granules plus isodecylalcohol ethoxylated (DPX-KG691) surfactant: 7-Day growth inhibition test with the freshwater aquatic plant, duckweed, <i>Lemna gibba</i> DuPont-49944 Eurofins EAG Agrosience, LLC GLP: Yes Published: No	N	DuPont

Data point	Author(s)	Year	Title Company Report No. Source GLP or GEP Status Published or not	Vertebrate study Y/N	Owner
KCP, 10.2.1/05	Goudie, O.J.	2019	DPX-V4B07 24 WG (Rimsulfuron 25 SG + thifensulfuron 50 SG + isoxadifen 50 WG) A blend of paste extruded granules plus crop oil (Codacide): 7-Day growth inhibition test with the freshwater aquatic plant, duckweed, <i>Lemna gibba</i> DuPont-49978 Eurofins EAG Agrosience, LLC GLP: Yes Published: No	N	DuPont
KCP, 10.3.1.1.1/01 and KCP, 10.3.1.1.2/01	Tome, H.V.V.	2018	Isoxadifen ethyl 50WG / Rimsulfuron 25SG / Thifensulfuron methyl 50SG (DPX-V4B07), a blend of paste extruded granules (11.11% + 14.82% + 9.26 active) plus codacide oil surfactant: An acute oral and contact toxicity study with the honey bee DuPont-48892 EAG Laboratories GLP: Yes Published: No	N	DuPont
KCP, 10.3.1.1.1/02 and KCP, 10.3.1.1.2/02	Tome, H.V.V., Porch J.R.	2018	Isoxadifen ethyl 50WG / Rimsulfuron 25SG / Thifensulfuron methyl 50SG/ (DPX-V4B07), a blend of paste extruded granules (11.11% + 14.82% + 9.26% active) plus Trend 90 surfactant: An acute oral and contact toxicity study with the honey bee DuPont-48950 EAG Laboratories GLP: Yes Published: No	N	DuPont
KCP, 10.3.1.2/01	Porch, J.R., Riles, B.	2021a	GF-3969 (DPX-V4B07) + DPX-KG691 (VIVOLT): A Chronic Dietary Toxicity test with the Honey Bee (<i>Apis mellifera</i>) Rep. No. 112H-131A DAS Study No. 200439 Eurofins EAG Agrosience, LLC, USA GLP: Yes Published: No	N	Corteva
KCP, 10.3.1.3/02	Porch, J.R., Riles, B.	2021b	GF-3969 (DPX-V4B07) + DPX-KG691 (VIVOLT): A Chronic Larval Toxicity Study with the Honey Bee (<i>Apis mellifera</i>) Rep. No. 112H-130 DAS Study No. 200438 Eurofins EAG Agrosience, LLC, USA GLP: Yes Published: No	N	Corteva

Data point	Author(s)	Year	Title Company Report No. Source GLP or GEP Status Published or not	Vertebrate study Y/N	Owner
KCP, 10.3.2.1/01	Moll, M.	2018	Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) plus codacide: A laboratory rate-response test to evaluate the effects on the parasitoid <i>Aphidius rhopalosiphi</i> (Hymenoptera, Braconidae) DuPont-49972 IBACON GLP: Yes Published: No	N	DuPont
KCP, 10.3.2.1/02	Moll, M.	2018	Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) plus codacide: A laboratory rate-response test to evaluate the effects on the predatory mite, <i>Typhlodromus pyri</i> (Acari, Phytoseiidae) DuPont-49973 IBACON GLP: Yes Published: No	N	DuPont
KCP, 10.3.2.1/03	Moll, M.	2018	Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) plus DPX-KG691 Surfactant: A laboratory rate-response test to evaluate the effects on the parasitoid <i>Aphidius rhopalosiphi</i> (Hymenoptera, Braconidae) DuPont-49934 IBACON GLP: Yes Published: No	N	DuPont
KCP, 10.3.2.1/04	Moll, M.	2018	Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) plus DPX-KG691 surfactant: A laboratory rate-response test to evaluate the effects on the predatory mite <i>Typhlodromus pyri</i> (Acari, Phytoseiidae) DuPont-49935 IBACON GLP: Yes Published: No	N	DuPont
KCP, 10.4.1.1/01	Pavic, B.	2018	Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) plus DPX-KG691 surfactant: Effects on reproduction and growth of the earthworm, <i>Eisenia andrei</i> , in artificial soil DuPont-49950 IBACON GLP: Yes Published: No	N	DuPont

Data point	Author(s)	Year	Title Company Report No. Source GLP or GEP Status Published or not	Vertebrate study Y/N	Owner
KCP, 10.4.1.1/02	Pavic, B.	2018	Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) plus codacide: Effects on reproduction and growth of the earthworm, <i>Eisenia andrei</i> , in artificial soil DuPont-49980 IBACON GLP: Yes Published: No	N	DuPont
KCP, 10.4.2.1/01	Pavic, B.	2018	Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) plus DPX-KG691 surfactant: Effects on the reproduction of the predatory mite <i>Hypoaspis aculeifer</i> in artificial soil DuPont-49955 IBACON GLP: Yes Published: No	N	DuPont
KCP, 10.4.2.1/02	Pavic, B.	2018	Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) plus DPX-KG691 surfactant: Effects on the collembola <i>Folsomia candida</i> in artificial soil DuPont-49954 IBACON GLP: Yes Published: No	N	DuPont
KCP, 10.4.2.1/03	Pavic, B.	2018	Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) plus codacide: Effects on reproduction of the predatory mite <i>Hypoaspis aculeifer</i> in artificial soil DuPont-49982 IBACON GLP: Yes Published: No	N	DuPont
KCP, 10.4.2.1/04	Pavic, B.	2018	Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) plus codacide: Effects on the collembola <i>Folsomia candida</i> in artificial soil with 5% peat DuPont-49981 IBACON GLP: Yes Published: No	N	DuPont

Data point	Author(s)	Year	Title Company Report No. Source GLP or GEP Status Published or not	Vertebrate study Y/N	Owner
KCP, 10.5/01	Hammesfahr, U.	2018	Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isoxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) plus KG691 surfactant: Assessment of the effects on soil microflora DuPont-49938 IBACON GLP: Yes Published: No	N	DuPont
KCP, 10.5/02	Hammesfahr, U.	2018	Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isoxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) plus codacide: Assessment of the effects on soil microflora DuPont-49976 IBACON GLP: Yes Published: No	N	DuPont
KCP, 10.6.2/01	Spatz, B.	2018	Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isoxadifen ethyl 50WG (DPX-V4B07) A blend of paste extruded granules (14.82% + 9.26% active) plus DPX-KG691 surfactant: Effects on terrestrial (non-target) plants: Seedling emergence and seedling growth test DuPont-49939 IBACON GLP: Yes Published: No	N	DuPont
KCP, 10.6.2/02	Arnie, J.R., McKelvey, R.A., Aufderheide, J.A., Lockard, L.A., Zhang, L.	2020	Isoxadifen ethyl 50WG/Rimsulfuron 25SG/Thifensulfuron methyl 50SG (DPX-V4B07), A Blend of Paste Extruded Granules Plus Isodecylalcohol Ethoxylated (DPX-KG691) Surfactant: A Greenhouse Study to Investigate the Effects on Vegetative Vigor of Ten Terrestrial Plants Following Foliar Exposure DuPont-49942 Eurofins EAG Agrosience, LLC GLP: Yes Published: No	N	DuPont
KCP, 10.6.2/03	Ellis, S.	2022	Position paper to address zRMS comments on the risk to non-target plants from GF-3969 GLP: Not relevant, position paper Published: No	N	Corteva

List of data relied on but not submitted– all documents

The following studies are relied upon and have not been evaluated at the EU level, but are not submitted in this dossier. FMC-provided studies are in 0.

zRMS comments:
 Please note that below studies were agreed by the RMS (UK) in the course of the evaluation of the confirmatory data (for details, please refer to EFSA Supporting publication 2020:EN-1627).

Annex No., OECD Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Owner	Relied upon Y/N
KCP, 10.2.1	xxxxxxxxxxxxxx	2010	Thifensulfuron Methyl (DPX-M6316) Technical: Early Life-Stage Toxicity Test with the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Under Flow-Through Conditions ABC Laboratories, Inc. (USA) GLP: Yes DuPont-28722 Published: No	Y	FMC*, Rotam	Y
KCP, 10.2.1	Brouger, D.S., Lockard, L., Gallagher, S.P.	2017	Thifensulfuron methyl (DPX-M6316) technical: A 48-hour static acute toxicity test with the cladoceran (<i>Daphnia magna</i>) Wildlife International Ltd (USA) DuPont-46007, Revision No. 1 GLP: Yes Published: No	N	FMC*, Rotam	Y
KCP, 10.2.1	Hutton, D.G.	1989	Chronic toxicity of IN-M6316-25 to <i>Daphnia magna</i> DuPont Haskell Laboratory HLR 70-89 GLP: Yes Published: No	N	FMC*	Y
KCP, 10.2.1	Arnie, J.R., Lockard, L., Martin, K.H., Porch, J.R.	2017	Thifensulfuron methyl (DPX-M6316) technical: A 72-hour toxicity test with the freshwater alga (<i>Pseudokirchneriella subcapitata</i>) Wildlife International Ltd (USA) DuPont-46004, Revision No. 1 GLP: Yes Published: No	N	FMC*, Rotam	Y

Annex No., OECD Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Owner	Relied upon Y/N
KCP, 10.2.1	Arnie, J.R., Zhang, L., Porch, J.R., Martin, K.H.	2016	IN-D8858: A 72-hour toxicity test with the freshwater alga (<i>Pseudokirchneriella subcapitata</i>) Wildlife International Ltd. (USA) DuPont-42163, Revision No. 1 GLP: Yes Published: No	N	FMC*, Rotam	Y
KCP, 10.2.2	Arnie, J.R., Zhang, L., Porch, J.R., Martin, K.H.	2016	IN-D8858: A 7-day static-renewal toxicity test with duckweed (<i>Lemna gibba</i> G3) Wildlife International Ltd. (USA) DuPont-42164, Revision No. 1 GLP: Yes Published: No	N	FMC*, Rotam	Y
KCP, 10.4.2.1	Lührs, U.	2015a	IN-JZ789: Effects on the Collembola <i>Folsomia candida</i> in artificial soil with 5% peat IBACON DuPont-42165 GLP: Yes Published: No	N	FMC*, Rotam	Y
KCP, 10.4.2.1	Lührs, U.	2015b	IN-U5F72: Effects on the Collembola <i>Folsomia candida</i> in artificial soil with 5% peat IBACON DuPont-42481 GLP: Yes Published: No	N	FMC*, Rotam	Y
KCP, 10.7.1/01	Pur, A. Ochoa-Acuna, H.	2015	Herbicide non-relevance screen results for Thifensulfuron methyl metabolites (IN-JZ789 and IN-U5F72) DuPont-43667 E.I. du Pont de Nemours and Company GLP: No Published: No	N	FMC*	Y

* DuPont has Letter of Access (LoA) from FMC

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

zRMS comments:

As all of endpoints for particular active compounds and their relevant metabolites were taken from the EU review, for the list of respective studies please refer to Volume 2 of the RAR for rimsulfuron and thifensulfuron-methyl.

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, 10.3.1.1.1/03 and KCP, 10.3.1.1.2/03	Verge, E.	2018	Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isoxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) + codacide oil: Acute oral and contact toxicity to the bumble bee, <i>Bombus terrestris</i> L. under laboratory conditions DuPont-48951 Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH GLP: Yes Published: No	N	DuPont	Not a data requirement
KCP, 10.3.1.1.1/04 and KCP, 10.3.1.1.2/04	Verge, E.	2019	Rimsulfuron 25SG/thifensulfuron methyl 50SG/isoxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) + surfactant DPX-KG691: Acute oral and contact toxicity to the bumble bee, <i>Bombus terrestris</i> L. under laboratory conditions DuPont-48899, Revision No. 1 Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH GLP: Yes Published: No	N	DuPont	Not a data requirement
KCP, 10.3.1.3/01	Cornement, M.	2018	Rimsulfuron-toxicity to Honey bees (<i>Apis mellifera</i> L.) larvae after repeated exposure under <i>In Vitro</i> laboratory conditions 20170301 Innovative Environmental Services (IES) LtdKC GLP: Yes Published: No	N	DuPont	Active substance study, not relevant for zonal 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	Reason for rejection
KCP-10.6.2/02	Arnie, J.R., McKelvey, R.A., Aufderheide, J.A., Lockard, L.A., Zhang, L.	2020	Isxadifen ethyl 50WG/Rimsulfuron 25SG/Thifensulfuron methyl 50SG (DPX-V4B07), A Blend of Paste Extruded Granules Plus Isodecylalcohol Ethoxylated (DPX-KG691) Surfactant: A Greenhouse Study to Investigate the Effects on Vegetative Vigor of Ten Terrestrial Plants Following Foliar Exposure DuPont 49942 Eurofins EAG Agroscience, LLC GLP: Yes Published: No	N	DuPont	Study not valid

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

Data point	Author(s)	Year	Title Company Report No. Source GLP or GEP Status Published or not	Vertebrate study Y/N	Owner
There were no studies 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

No new or additional studies have been submitted.

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

A 2.2 KCP 10.2 Effects on aquatic organisms

A 2.2.1 KCP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

A 2.2.1.1 Study 1, DuPont-49948, Revision No. 1

Comments of zRMS:	<p>The study was conducted in line with OECD 203 (1992) guideline with a minor deviation.</p> <p>It was noted that the current guideline OECD 203 (2019) recommends culture temperature in the range of 10-14°C for the rainbow trout while the test was conducted in line with OECD 203 (1992) when the recommended culture temperature for the rainbow trout was in the range of 13-17°C. This deviation is considered to not invalidate the present study and to have no impact on its outcome as all the validity criteria were met:</p> <ul style="list-style-type: none"> - Mortality in the control sample was below 10 % at the end of the exposure (actually 0 %), - Dissolved oxygen concentration was ≥ 60 % of air saturation throughout the exposure (actually 61-108 %), - Measured concentrations of the active substances were within 80-120% of nominal concentrations throughout the exposure (actually 92-112% of nominal for rimsulfuron and 93-111% of nominal for thifensulfuron methyl; mean calculated concentration of the total product was 98-105% of nominal). <p>Even though the concentrations of both active substances were maintained within 80-120% of nominal throughout the study, the endpoint was reported in the study based on the mean calculated concentrations from the mean measured concentrations for both active substances.</p> <p>Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>96 h LC₅₀ = 6.78 mg product/L (based on the mean calculated concentrations from the active substances)</p>
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Reference:	KCP 10.2.1/01
Report:	xxxxxxxxxxxxxxxxxxxxx (2019); DPX-V4B07 24 WG (rimsulfuron 25 SG + thifensulfuron 50 SG + isoxadifen 50 WG) A blend of paste extruded granules plus isodecylalcohol ethoxylated (DPX-KG691) surfactant: Acute toxicity to the rainbow trout, <i>Oncorhynchus mykiss</i> , determined under static-renewal test conditions
DuPont Report No.:	DuPont-49948, Revision No. 1
Testing Facility Report No.:	86361
Guidelines	OECD 203 (1992)
Deviations:	Minor (see the commenting box above) <i>None</i>
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

EXECUTIVE SUMMARY

In a 96-hr acute toxicity study, rainbow trout were exposed to GF-3969 under static-renewal conditions in accordance with OECD Guideline 203. A definitive test was performed at nominal concentrations of 0 (blank control), 0.75, 1.5, 3.0, 6.0, and 12 mg GF-3969 total product (TP)/L.

Based on the combined analysis of rimsulfuron (DPX-E9636) and thifensulfuron methyl (DPX-M6316) total active substances, the mean calculated concentrations in the test substance treatment solutions during the 96-hour exposure were 0.573, 1.18, 2.45, 4.85, and 9.47 mg GF-3969 total product (TP)/L or 98 to 105% of the nominal concentrations calculated from the active substances. No residues of GF-3969, based on the combined rimsulfuron (DPX-E9636) and thifensulfuron methyl (DPX-M6316) total active substances were detected in the control. The biological response results were reported based upon the mean calculated GF-3969 concentrations.

Water quality parameters of temperature, dissolved oxygen concentration and pH remained within acceptable limits throughout the definitive test. The control and 0.573 mg GF-3969 total product (TP)/L test substance solutions were clear and colourless with no visible particulates, surface film, undissolved test substance, or precipitate throughout the test. The 1.18 mg GF-3969 total product (TP)/L fresh solutions were clear and colourless with no visible particulates, surface film, undissolved test substance, or precipitate at initiation and had a very slight surface foam at 24, 48, and 72 hours. The 1.18 mg GF-3969 total product (TP)/L spent solutions were clear and colourless with no visible particulates, surface film, undissolved test substance, or precipitate throughout the test. The 2.45 and 4.85 mg GF-3969 total product (TP)/L fresh solutions had scattered foam on the surface at initiation and a slight surface foam at 24, 48, and 72 hours. The 2.45 and 4.85 mg GF-3969 total product (TP)/L spent solutions were clear and colourless with no visible particulates, surface film, undissolved test substance, or precipitate throughout the test. The 9.47 mg GF-3969 total product (TP)/L fresh solution had surface foam at initiation. The 9.47 mg GF-3969 total product (TP)/L spent solution was clear and colourless with no visible particulates, surface film, undissolved test substance, or precipitate at 24 hours. The 9.47 mg GF-3969 total product (TP)/L test substance solutions were not observed after 24 hours due to 100% mortality.

After 96 hours, mortality was 0, 0, 0, 0, 0, and 100% in the 0 (control), 0.573, 1.18, 2.45, 4.85, and 9.47 mg GF-3969 total product (TP)/L treatments, respectively. No sublethal effects were observed in the control, 0.573, 1.18, and 2.45 mg GF-3969 total product (TP)/L test treatments throughout the test. Sublethal effects were observed in the 4.85 mg GF-3969 total product (TP)/L test treatment at 24, 48, 72, and 96 hours. Observed effects included fish on the bottom of the test chamber, loss of equilibrium, and discoloration.

The 24-, 48-, 72-, and 96-hour LC₅₀ value, based on mortality and mean calculated GF-3969 concentrations, was 6.78 mg GF-3969 total product (TP)/L, with estimated 95% confidence limits of 4.85 and 9.47 mg GF-3969 total product (TP)/L.

The lowest mean calculated GF-3969 concentration that caused 100% mortality at test end was 9.47 mg GF-3969 total product (TP)/L. The highest mean calculated GF-3969 concentration causing 0% mortality at test end was 4.85 mg GF-3969 total product (TP)/L.

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):

GF-3969 (names used in the study: DPX-V4B07-002 or DPX-V4B07 24 WG)

Purity:

DPX-V4B07-002 is a formulation containing nominal concentrations (w/w):

25.1% DPX-E9636

49.8% DPX-M6316

50.4% DPX-X4145

Composition:

DPX-V4B07-002 is a blend of paste extruded granules containing nominal concentrations (w/w):

42.1 % DPX-E9636 (rimsulfuron)

26.3 % DPX-M6316 (thifensulfuron methyl)

31.6 % DPX-X4145 (isoxadifen-ethyl)

DPX-KG691 (ethoxylated isodecylalcohol as adjuvant surfactant.)

Purity of granules of particular
paste components:

DPX-E9636 – 25.1% of rimsulfuron
DPX-M6316 – 49.8% of thifensulfuron methyl
DPX-X4145 – 50.4% of isoxadifen-ethyl

Description (physical state):

DPX-V4B07 24 WG is prepared by combining 22.1%
Isoxadifen ethyl 50WG, 59.2% Rimsulfuron 25SG and
18.7% Thifensulfuron methyl 50SG ~~Not provided~~

Lot/batch no.:

DPX-E9636-227 (Lot number: MAR15EL004)
DPX-M6316-323 (Lot number: APR15EL002)
DPX-X4145-021 (Lot number: DEC15EL001)

Test System

Organism (*Species*):

Rainbow trout (*Oncorhynchus mykiss*)

Study type:

Acute

Study design:

Static-renewal

Test concentrations:

Nominal: 0 (blank control), 0.75, 1.5, 3.0, 6.0, and 12
mg GF-3969 total product (TP)/L
Mean calculated: <LOD (control), 0.573, 1.18, 2.45,
4.85, and 9.47 mg GF-3969 total product (TP)/L

Parameters measured:

Mortality

Observation intervals:

24, 48, 72, and 96 hours

Age, weight and length of fish at test
initiation:

Age: >12 days

Mean blotted wet weight: 1.1252 ± 0.2272 g (0.7822 to
1.3963 g)

Mean total length: 49 ± 3.2 mm (45 to 54 mm)

Analytical confirmation of test
concentrations:

On hours: 0 (fresh), 24 (spent), 72 (fresh), and
96 (spent)

No. of holding days before dosing:

7

Number of fish per dose group:

7

Number of fish per control group:

7

Feeding regime:

Fish were fed salmon starter daily during holding, none
during exposure

Environmental conditions:

Loading rate: instantaneous biomass loading rate was
0.44 g/L

Temperature: 15.1 to 16.0 °C

Photoperiod: 16-hr light:8-hr dark

Dissolved oxygen concentration:

5.9 to 10.5 mg/L (61 to 108% saturation)

pH: 7.9 to 8.5

Total hardness: 150 mg CaCO₃/L

Salinity: not applicable

Reference substance:

DPX-M6316-258 (Lot number: OCT05MA040)

DPX-E9636-022 (Lot number: E58246-070D)

Methodology

The static-renewal definitive test was performed from 15 to 19 May 2018 at nominal concentrations of 0 (blank control), 0.75, 1.5, 3.0, 6.0, and 12 mg GF-3969 total product (TP)/L. Seven fish were added to the blank control and each test substance treatment. Observations for mortality and sublethal responses were made every 24 hours (± 1 hour) from the time of test initiation for the duration of the test. In an effort to maintain maximal exposure to the test substance, the control and treated test solutions were freshly prepared and renewed at 24, 48, and 72 hours. Temperature, dissolved oxygen concentration, and pH were measured daily in all replicates with live animals. An electronic data logger and thermistor probe was used to make a continuous recording of the temperature from a centrally located test chamber during the test. Total hardness and alkalinity of the dilution water were

measured using titrimetric methods adapted from Standard Methods. The light intensity at definitive test initiation was 876 lux.

RESULTS AND DISCUSSION

The concentration of the GF-3969 was calculated, based on the combined rimsulfuron (DPX-E9636) and thifensulfuron methyl (DPX-M6316) total active substances, in fresh solutions at 0 (test initiation) and 72 hours, and in spent solutions at 24 and 96 hours (termination). Mean calculated concentrations of GF-3969 during the 96-hour exposure were 0.573, 1.18, 2.45, 4.85, and 9.47 mg GF-3969 total product (TP)/L or 98 to 105% of the nominal concentrations calculated from the active substances. The biological response results were reported based on the mean calculated GF-3969 concentrations. After 96 hours, mortality was 0, 0, 0, 0, and 100% in the 0 (control), 0.573, 1.18, 2.45, 4.85, and 9.47 mg GF-3969 total product (TP)/L treatments. No sublethal effects were observed in the control, 0.573, 1.18, and 2.45 mg GF-3969 total product (TP)/L test treatments throughout the test. Sublethal effects were observed in the 4.85 mg GF-3969 total product (TP)/L test treatment at 24, 48, 72, and 96 hours. Observed effects included fish on the bottom of the test chamber, loss of equilibrium, and discoloration.

Table A 1: Effect of GF-3969 on mortality of rainbow trout

Treatment (mg GF-3969 total product (TP)/L)		No. of fish	Cumulative mortality (%)			
Nominal	Mean calculated		24-hr	48-hr	72-hr	96-hr
Negative control	Negative control	7	0 (0)	0 (0)	0 (0)	0 (0)
0.75	0.573	7	0 (0)	0 (0)	0 (0)	0 (0)
1.5	1.18	7	0 (0)	0 (0)	0 (0)	0 (0)
3.0	2.45	7	0 (0)	0 (0)	0 (0)	0 (0)
6.0	4.85	7	0 (0)	0 (0)	0 (0)	0 (0)
12	9.47	7	7 (100)	7 (100)	7 (100)	7 (100)
LC ₅₀		6.78 mg GF-3969 total product (TP)/L				
95% C.I.		4.85 and 9.47 mg GF-3969 total product (TP)/L				
NOEC		NA				

Table A 2: Sub-lethal effects of GF-3969 in rainbow trout

Treatment (mg GF-3969 total product (TP)/L)		Observation period							
Nominal	Mean calculated	On bottom of test chamber (% affected)				Loss of equilibrium (% affected)			
		24-hr	48-hr	72-hr	96-hr	24-hr	48-hr	72-hr	96-hr
Negative control	Negative control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.75	0.573	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1.5	1.18	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
3.0	2.45	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
6.0	4.85	2 (29)	1 (14)	2 (29)	2 (29)	0 (0)	2 (29)	0 (0)	0 (0)
12	9.47	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Table A 2: Sub-lethal effects of GF-3969 in rainbow trout (continued)

Treatment (mg GF-3969 total product (TP)/L)		Observation period			
Nominal	Mean calculated	Discoloration (% affected)			
		24-hr	48-hr	72-hr	96-hr
Negative control	Negative control	0 (0)	0 (0)	0 (0)	0 (0)
0.75	0.573	0 (0)	0 (0)	0 (0)	0 (0)
1.5	1.18	0 (0)	0 (0)	0 (0)	0 (0)
3.0	2.45	0 (0)	0 (0)	0 (0)	0 (0)
6.0	4.85	0 (0)	0 (0)	2 (29)	0 (0)
12	9.47	0 (0)	0 (0)	0 (0)	0 (0)

CONCLUSION

The 24-, 48-, 72-, and 96-hour LC₅₀ value, based on mortality and mean calculated GF-3969 concentrations, was 6.78 mg GF-3969 total product (TP)/L, with estimated 95% confidence limits of 4.85 and 9.47 mg GF-3969 total product (TP)/L. The lowest mean calculated GF-3969 concentration that caused 100% mortality at test end was 9.47 mg GF-3969 total product (TP)/L. The highest mean calculated GF-3969 concentration causing 0% mortality at test end was 4.85 mg GF-3969 total product (TP)/L.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Rainbow trout	<i>Oncorhynchus mykiss</i>	GF-3969	96-hr	LC ₅₀	6.78	mg GF-3969 total product (TP)/L

A 2.2.1.2 Study 2, DuPont-49949, Revision No. 1

Comments of zRMS:	<p>The study was conducted in line with OECD 202 with no major deviations.</p> <p>It was noted that the temperature was not constant within $\pm 1^\circ\text{C}$. However, this slight deviation is not considered to have an impact on the study outcome as all the validity criteria were met:</p> <ul style="list-style-type: none"> - In the control no more than 10% of the daphnids should be immobilised or show other signs of stress or disease (actually 0% immobilised and none with signs of stress or disease), - Dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in the control and treatment groups (actually 8.2-8.3 mg/L). <p>Even though the concentrations of both active substances were maintained within 80-120% of nominal throughout the study, the endpoint was reported in the study based on the mean calculated concentrations from the mean measured concentrations for both active substances.</p> <p>Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>48h EC₅₀ = 11.6 mg product/L (based on the mean calculated concentration from the active substances)</p>
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Reference:	KCP 10.2.1/02
Report:	Goudie, O.J., (2019); DPX-V4B07 24 WG (Rimsulfuron 25 SG + Thifensulfuron 50 SG + Isoxadifen 50 WG) A blend of paste extruded granules plus isodecylalcohol ethoxylated (DPX-KG691) surfactant: 48-Hour static renewal, acute toxicity test with the cladoceran, <i>Daphnia magna</i>
DuPont Report No.:	DuPont-49949, Revision No. 1
Testing Facility Report No.:	86363
Guidelines	OECD 202 (2004)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable

EXECUTIVE SUMMARY

The acute toxicity of GF-3969 to the cladoceran, *Daphnia magna*, was determined in a 48-hour static-renewal test. The test was conducted in accordance with the OECD Guidelines for the Testing of Chemicals: Guideline No. 202. The study was conducted with nominal concentrations of 7.5, 15, 30, 60, and 120 mg GF-3969 total product (TP)/L and a dilution water control at a temperature range of 19.9 to 21.2°C. Four replicates with five daphnids per replicate were used for each of the test

substance concentrations and control. Exposure of *Daphnia magna* to the dilution water control and mean calculated concentrations of 5.35, 10.8, 21.5, 45.0, and 94.8 mg GF-3969 total product (TP)/L resulted in 0, 0, 35, 100, 100, and 100% immobility at the end of 48 hours. Mean calculated concentrations (0 to 48 hours) of GF-3969 were <LOD (control), 5.35, 10.8, 21.5, 45.0, and 94.8 mg GF-3969 total product (TP)/L, and ranged from 92 to 101% of the nominal concentrations of GF-3969 calculated from the active substances. No sublethal effects were observed in any treatment in the study. The 48-hour EC₅₀ value, determined from immobility data, was 11.6 mg GF-3969 total product (TP)/L, with 95% confidence limits of 9.40 and 14.0 mg GF-3969 total product (TP)/L. The lowest mean calculated concentration that caused 100% immobility at test end was 21.5 mg GF-3969 total product (TP)/L. The highest mean calculated concentration causing 0% immobility at test end was 5.35 mg GF-3969 total product (TP)/L.

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-3969
Composition Purity:	DPX-V4B07-002 is a formulation containing nominal concentrations (w/w): 31.6% Isoxadifen-ethyl safener 42.1% Rimsulfuron active ingredient 26.3% Thifensulfuron methyl active ingredient DPX-KG691 (ethoxylated isodecylalcohol as adjuvant surfactant)
Synonym:	DPX-V4B07-002, DPX-V4B07 24 WG GF-3969
Lot/Batch #:	None for formulation MAR15EL004 for rimsulfuron (DPX-E9636) APR15EL002 for thifensulfuron methyl (DPX-M6316) DEC15EL001 for isoxadifen-ethyl (DPX-X4145)
Purity of granules of particular paste components:	None for formulation 25.1% for DPX-E9636 49.8% for DPX-M6316 50.4% for DPX-X4145
Description:	DPX-V4B07-002 is prepared by combining 22.1% Isoxadifen 50 WG, 59.2% Rimsulfuron 25 SG, and 18.7% Thifensulfuron 50 SG
CAS#:	None for formulation 122931-48-0 for DPX-E9636 79277-27-3 for DPX-M6316 163520-33-0 for DPX-X4145
Stability of test compound:	Determined to be stable in the test system
Control:	Dilution (blended) water
Test solvent:	None
Toxic reference:	None

Test System

Organism (<i>Species</i>):	Cladoceran (<i>Daphnia magna</i>)
Age at dosing:	Neonates (<24 hours old)
Weight at dosing:	NA
Initial population :	5 daphnids per test chamber/20 daphnids per treatment
Source:	In house culture
Acclimation period:	Continuous culture
Diet:	Test period: unfed
Test chamber:	250-mL glass container containing ~200 mL of test solution (6.5-cm test solution depth), covered with a clear plastic Petri dish

Test medium: Blended freshwater
 Environmental conditions Temperature: 19.9 to 21.2°C
 Photoperiod: 16 hr photoperiod (646 lux) and 8 hr darkness which included 30 min transitional light preceding and following the 16-hr light interval
 Dissolved oxygen: 8.1 to 8.5 mg/L (93 to 100% saturation)
 pH: 8.4 to 8.6

Methodology

1. In-life initiated/completed
 23-May-2018 to 25-May-2018
2. Experimental treatments
 The acute toxicity of GF-3969 to unfed *Daphnia magna* (<24-hour old) was determined in an unaerated, static-renewal, 48-hour test. Daphnids were from the 12th brood of at least 19 day-old parents. Treatments consisted of a dilution water control and five nominal concentrations of 7.5, 15, 30, 60, and 120 mg GF-3969 total product (TP)/L. Five daphnids were used per replicate with four replicates per test concentration and control.
3. Observations
 Immobility and sublethal (behavioural) observations were made every 24 hours.
4. Statistics
 Estimates of EC₅₀ values and their 95% confidence limits were calculated using the probit method or moving average angle method. When there was no evidence of questionable convergence, the probit method was selected for reporting. When this criterion was not achieved, moving average angle method was selected for reporting.

RESULTS AND DISCUSSION

Mean calculated concentrations, (0 to 48 hours) were <LOD (control), 5.35, 10.8, 21.5, 45.0, and 94.8 mg GF-3969 total product (TP)/L, and ranged from 92 to 101% of the nominal concentrations of GF-3969 calculated from the active substances. No sublethal effects were observed in any treatment in the study. Summaries of observed immobility and sublethal effects are presented in Table A 3 and Table A 4, respectively.

Table A 3: Observed immobility of the Cladoceran, *Daphnia magna*, exposed to GF-3969 for 48 hours in an unaerated, static-renewal, acute test

Mean Calculated GF-3969 Concentration (mg GF-3969 total product (TP)/L)	Cumulative immobility ^a (Number immobile /Number at test start ^b)								Mean % Immobile (after 48-hours)
	24 Hours				48 Hours				
	A	B	C	D	A	B	C	D	
0 (Control)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0
5.35	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0
10.8	0/5	1/5	0/5	3/5	1/5	1/5	1/5	4/5	35 *
21.5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	100 *
45.0	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	100 *
94.8	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	100 *

a Immobile. No observed movement of appendages or postabdomen within 15 seconds after gentle agitation of the test chamber or gentle disturbance of the daphnid itself. Affected numbers are cumulative.

b Replicate test chambers contained 5 daphnids each for a total of 20 per concentration at test initiation.

* Statistically significant increase (Fisher's Exact test and Cochran-Armitage trend test, $p = 0.05$) in immobility compared to the control.

Table A 4: Observed sublethal effects of the Cladoceran, *Daphnia magna*, exposed to GF-3969 for 48 hours in an unaerated, static-renewal, acute test

Mean Calculated GF-3969 Concentration (mg GF-3969 total product (TP)/L)	Sublethal Effects / Number Alive ^a							
	24 Hours				48 Hours			
	A	B	C	D	A	B	C	D
0 (Control)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
5.35	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
10.8	0/5	0/4	0/5	0/2	0/4	0/4	0/4	0/1
21.5	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
45.0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
94.8	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0

a Replicate test chambers contained 5 daphnids each for a total of 20 per concentration at test initiation.

CONCLUSION

The 48-hour EC₅₀ value, based on immobility, was 11.6 mg GF-3969 total product (TP)/L, with 95% confidence limits of 9.40 and 14.0 mg GF-3969 total product (TP)/L. The lowest mean calculated concentration that caused 100% immobility at test end was 21.5 mg GF-3969 total product (TP)/L. The highest mean calculated concentration causing 0% immobility at test end was 5.35 mg GF-3969 total product (TP)/L.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
cladoceran	<i>Daphnia magna</i>	GF-3969	48-hr	EC ₅₀	11.6	mg GF-3969 total product (TP)/L

A 2.2.1.3 Study 3, DuPont-49943

Comments of zRMS:	<p>The study was conducted in line with OECD 201 with minor deviations.</p> <p>It was noted that the AAP medium was indicated as the test diluent and control in the study protocol but in the actual study the FWAM nutrient medium equivalent to the AAP medium was used. The medium change was not reported nor a reason for it was given in the study report. The AAP medium was used as the medium for the abiotic control (single replicate with the highest nominal concentration of product to determine its stability during the exposure time). Additionally, the temperature was not maintained within ± 2°C and was slightly outside of the recommended range of 21-24°C (23.2-25.6°C). In zRMS opinion these deviations are considered to have no impact on the outcome of the study as all the validity criteria were met:</p> <ul style="list-style-type: none"> - the biomass in the control cultures should increase by a factor of at least 16 within the 72-h test period (reported factor of 93), - the mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35% (reported 21-27%, overall mean 25%), - the coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% (reported 1%). <p>The analytical measurements demonstrated that the concentrations of thifensulfuron methyl were maintained within 80-120% of nominal during the whole study while the concentrations of rimsulfuron dropped below the required 80% of nominal at test termination in some treatment groups (see table below).</p>
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Nominal concentration of formulation [mg/L]	Nominal concentration of rimsulfuron [mg/L]	Measured concentration of rimsulfuron				% of nominal over the test period (0-72 h)
		Test start		Test end (72 h)		
		[µg/L]	% of nominal	[µg/L]	% of nominal	
0.036	0.00535	0.00501	94	0.00381	71	81.7
0.11	0.0164	0.0130	80	0.0114	70	74.8
0.37	0.0550	0.0595	108	0.0475	86	96.4
1.2	0.178	0.177	99	0.148	83	90.6
3.8	0.565	0.504	89	0.419	74	81.2
12	1.78	1.59	89	1.42	80	84.4

The mean recoveries of rimsulfuron over the whole study period were >80% in majority of the test item groups with exception of the second lowest concentration (0.11 mg product/L) where the mean recovery was 74.8%. It seems, however, that this was not a result of rapid dissipation of the active compound, but some error during dosing since at test initiation the recovery of rimsulfuron of 80% in this test item group was also quite low comparing to other test item groups with recovery at 89-108%. As in all other test item groups the recovery of rimsulfuron was within 80-120% of nominal and at this test item concentration (0.11 mg product/L) no effects on algae were observed (the NOEC was determined to be 0.37 mg product/L, which is the next higher concentration with recovery >80%), the zRMS is of the opinion that the endpoints may be based on nominal concentrations, especially based on the results provided in the table above, the geometric mean measured concentration over the whole study period was determined to be 84.6% of nominal for all test item groups.

Although the concentrations of rimsulfuron dropped below 80% of nominal at test termination in some treatment groups, the geometric mean measured concentration over the whole study period was 84.6% of nominal, thus the endpoints may be expressed in terms of nominal concentrations.

Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:

72h E_bC₅₀ = 0.510 mg product/L (based on nominal concentration)
72h NOE_bC = 0.37 mg product/L (based on nominal concentration)

72h E_rC₅₀ = 3.25 mg product/L (based on nominal concentration)
72h NOE_rC = 0.37 mg product/L (based on nominal concentration)

72h E_yC₅₀ = 0.532 mg product/L (based on nominal concentration)
72h NOE_yC = 0.37 mg product/L (based on nominal concentration)

Reference:	KCP 10.2.1/03
Report:	Hoover, E., (2019); DPX-V4B07 24 WG (Rimsulfuron 25 SG + Thifensulfuron 50 SG + Isoxadifen 50 WG) a blend of paste extruded granules plus isodecylalcohol ethoxylated (DPX-KG691) surfactant: Growth inhibition test with the unicellular green alga, <i>Pseudokirchneriella subcapitata</i>
DuPont Report No.:	DuPont-49943
Testing Facility Report No.:	86355
Guidelines	OECD 201 (2006)
Deviations:	Minor (see the commenting box above) None
GLP:	Yes
Acceptability:	Acceptable

EXECUTIVE SUMMARY

The acute toxicity of GF-3969 to the unicellular green alga, *Pseudokirchneriella subcapitata*, was determined in a 72-hour growth inhibition test. The test was conducted in accordance with Organization for Economic Cooperation and Development (OECD) Guideline No. 201. Treatments consisted of an untreated control and six nominal concentrations of 0.036, 0.11, 0.37, 1.2, 3.8, and 12 mg total product (TP)/L. The 72-hour geometric mean calculated concentrations of GF-3969 were 0.0248, 0.0716, 0.288, 0.891, 2.61, and 8.39 mg TP/L. The 72-hour EC₅₀ and NOEC values for *Pseudokirchneriella subcapitata* were based on nominal calculated concentrations of GF-3969 and area under the growth curve, growth rate, and yield. The 72-hour E_bC₅₀, E_rC₅₀, and E_yC₅₀ values based on area, growth rate, and yield were 0.510, 3.25, and 0.532 mg TP/L, respectively.

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-3969
Composition Purity:	DPX-V4B07 is a formulation containing nominal concentrations (w/w): 31.6% Isoxadifen ethyl safener 42.1% Rimsulfuron active ingredient 26.3% Thifensulfuron methyl active ingredient DPX-KG691 (ethoxylated isodecylalcohol as adjuvant surfactant)
Synonym:	DPX-V4B07 24 WG, DPX-V4B07
Lot/Batch #:	None for formulation MAR15EL004 for rimsulfuron (DPX-E9636) APR15EL002 for thifensulfuron methyl (DPX-M6316) DEC15EL001 for isoxadifen-ethyl (DPX-X4145)
Purity of granules of particular paste components:	None for formulation 25.1% for DPX-E9636 48.9% for DPX-M6316 50.4% for DPX-4145
Description:	DPX-V4B07 24 WG is prepared by combining 22.1% Isoxadifen ethyl 50WG, 59.2% Rimsulfuron 25SG and 18.7% Thifensulfuron methyl 50SG
CAS#:	None for formulation 122931-48-0 for DPX-E9636 79277-27-3 for DPX-M6316 163520-33-0 for DPX-X4145
Stability of test compound:	Determined to be stable in the test system
Control:	FWAM nutrient medium
Test solvent:	None
Toxic reference:	None

Test System

Organism (<i>Species</i>):	Unicellular green alga (<i>Pseudokirchneriella subcapitata</i>)
Initial population :	5000 cells/mL
Source:	Eurofins, Columbia, Missouri in-house culture, parent culture from University of Texas
Test chamber:	250-mL Erlenmeyer flask with a foam stopper, containing 100 mL of test solution.
Growth medium:	FWAM nutrient medium
Environmental conditions	Temperature: 23.2 to 25.6°C Photoperiod: 24-hour light photoperiod (7209 to 7234 lux) pH: 7.4 to 7.5 at test initiation and 7.7 to 8.5 at test termination

Methodology

1. In-life initiated/completed
25-May-2018 to 28-May-2018
2. Experimental treatments
The effect of GF-3969 to the green alga *Pseudokirchneriella subcapitata* was determined in a static acute 72-hour test. The algae were exposed to an untreated control and six nominal concentrations of 0.036, 0.11, 0.37, 1.2, 3.8, and 12 mg total product (TP)/L in FWAM nutrient medium for 72 hours, without test medium renewal. The 72-hour geometric mean calculated concentrations of GF-3969 were 0.0248, 0.0716, 0.288, 0.891, 2.61, and 8.39 mg TP/L. An abiotic (stability) control was included in the test to determine the stability of GF-3969 in AAP nutrient medium under the same environmental conditions without the algae. The untreated control was tested as six replicates and each test concentration was tested as three replicates. The abiotic control was tested as a single test unit. The initial cell density was 5000 cells/mL. Test units were incubated in an environmental chamber for 72 hours.
3. Observations
Test concentrations for GF-3969 were measured on hour 0 (initiation) and hour 72 (termination) to verify target test concentrations and stability of the test item.
Biomass, based on cell count, was determined approximately 24, 48, and 72 hours after test initiation. Yield was determined by subtracting the initial cell count from the test end cell count.
Area under the growth curve and growth rate were determined for each day of exposure and were based on cell count.
Area, yield, and growth rate, all based on cell count, were recorded and expressed as percent inhibition relative to the control following exposure to GF-3969 for 72 hours.
4. Statistics
The LOEC and NOEC values, based on area under the growth curve, growth rate, and yield, were estimated using a one-way analysis of variance (ANOVA) procedure and a one-tailed Dunnett's test and/or Jonckheere Trend test ($p = 0.05$) where the alternate hypothesis was that the mean for the growth parameter was reduced in comparison to the control. Prior to the Dunnett's test and Jonckheere Trend test, a Shapiro-Wilk's test and a Levene's test were conducted to test for normality and homogeneity of variance, respectively, over treatments at each time point. If the results from the Shapiro-Wilk's and Levene's tests indicated normality and insignificant heterogeneity (i.e., $p > 0.01$), the analysis was performed on the non-transformed raw data. In instances of non-normality or heterogeneity (i.e., $p < 0.01$), a square root transformation was performed. If both the non-transformed raw data and the transformed data exhibited non-normality or inequality of variance, a non-parametric analysis of variance was performed on the ranks of the raw data values. Parametric analyses were performed on the 72-hour area under the curve data, growth rate and yield data.

RESULTS AND DISCUSSION

The nominal concentrations of GF-3969 were 0.036, 0.11, 0.37, 1.2, 3.8, and 12 mg TP/L. The 72-hour measured concentration of the 9.34 mg TP/L, calculated using the nominal calculated active substance (a.s.) concentrations (12 mg TP/L nominal) abiotic control was 8.27 mg TP/L representing 88% of nominal based on calculated a.s. concentrations. The untreated control solutions contained no detectable concentrations of GF-3969, based on the combined DPX-E9636 and DPX-M6316 total a.s. GF-3969 was determined to be stable over the course of the test without the presence of algae. All validation criteria were met for the study.

A summary of algal growth inhibition following exposure of *Pseudokirchneriella subcapitata* to GF-3969 for 72 hours is presented in the tables that follow.

Table A 5: Summary of algal growth inhibition following exposure of *Pseudokirchneriella subcapitata* to GF-3969 for 72 hours

Nominal GF-3969 Concentration (mg TP/L)	% Inhibition relative to the control ^a		
	Area	Growth Rate	Yield
Untreated control (0.0)	—	—	—
0.036	-2	0	1
0.11	-2	0	-2
0.37	42	12	42
1.2	69 *	25 *	68 *
3.8	93 *	53 *	92 *
12	101 *	92 *	100 *

a positive values indicate inhibition

* Significant reduction as compared to the blank control (Jonckheere-Terpstra test, p = 0.05).

CONCLUSION

Growth inhibition values based nominal GF-3969 concentrations on *Pseudokirchneriella subcapitata* were as follows:

Area: 72-hr E_bC_{50} = 0.510 mg GF-3969 total product (TP)/L ^a
72-hr NOEC = 0.37 mg GF-3969 total product (TP)/L

Growth Rate: 72-hr E_rC_{50} = 3.25 mg GF-3969 total product (TP)/L ^b
72-hr NOEC = 0.37 mg GF-3969 total product (TP)/L

Yield: 72-hr E_yC_{50} = 0.532 mg GF-3969 total product (TP)/L ^a
72-hr NOEC = 0.37 mg GF-3969 total product (TP)/L

a Schabenberger Weighted Hormetic model.

b OECD Model 2.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Unicellular green alga	<i>Pseudokirchneriella subcapitata</i>	GF-3969	72-hr	E_rC_{50}	3.25	mg GF-3969 total product (TP)/L
				E_bC_{50}	0.510	
				E_yC_{50}	0.532	

A 2.2.1.4 Study 4, DuPont-49944

Comments of zRMS:	<p>The study was performed in line with OECD 221 with no deviations.</p> <p>The analytical measurements demonstrated that the concentrations of thifensulfuron methyl were maintained within 80-120% of nominal during the whole study while the concentrations of rimsulfuron exceeded the required 120% of nominal in fresh solutions and dropped below the required 80% of nominal in spent solutions in some treatment groups. For that reason, the endpoints were reported in the study based on the mean calculated concentrations from the mean measured concentrations for both active substances.</p> <p>All the validity criteria were met:</p> <ul style="list-style-type: none"> - The doubling time of the frond number in the control must be < 2.5 days (60 h), corresponding to approximately a 7-fold increase in 7 days and an average specific growth rate of 0.275 d⁻¹ (actual doubling time was 1.5 days, corresponding to approximately a 24-fold increase in 7 days and an average specific growth rate of 0.456 d⁻¹). <p>Overall the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>E_rC_{10} (growth rate) = 0.000625 mg product/L (based on the mean calculated concentration from the active substances)</p> <p>E_rC_{20} (growth rate) = 0.00132 mg product/L (based on the mean calculated</p>
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	<p>concentration from the active substances) E_rC_{50} (growth rate) = 0.00411 mg product/L (based on the mean calculated concentration from the active substances) NOE_rC (growth rate) = 0.00296 mg product/L (based on the mean calculated concentration from the active substances)</p> <p>E_yC_{20} (yield) = 0.000734 mg product/L (based on the mean calculated concentration from the active substances) E_yC_{50} (yield) = 0.00228 mg product/L (based on the mean calculated concentration from the active substances) NOE_yC (yield) = 0.000804 mg product/L (based on the mean calculated concentration from the active substances)</p> <p>E_rC_{10} (biomass growth rate) = 0.00192 mg product/L (based on the mean calculated concentration from the active substances) E_rC_{20} (biomass growth rate) = 0.00407 mg product/L (based on the mean calculated concentration from the active substances) E_rC_{50} (biomass growth rate) > 0.00958 mg product/L (based on the mean calculated concentration from the active substances) NOE_rC (biomass growth rate) = 0.00296 mg product/L (based on the mean calculated concentration from the active substances)</p> <p>E_yC_{10} (biomass yield) = 0.000571 mg product/L (based on the mean calculated concentration from the active substances) E_yC_{20} (biomass yield) = 0.00121 mg product/L (based on the mean calculated concentration from the active substances) E_yC_{50} (biomass yield) = 0.00376 mg product/L (based on the mean calculated concentration from the active substances) NOE_yC (biomass yield) = 0.00296 mg product/L (based on the mean calculated concentration from the active substances)</p>
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Reference:	KCP 10.2.1/04
Report:	Bergfield, A., (2019); DPX-V4B07 24 WG (Rimsulfuron 25 SG + Thifensulfuron 50 SG + Isoxadifen 50 WG) A blend of paste extruded granules plus isodecylalcohol ethoxylated (DPX-KG691) surfactant: 7-Day growth inhibition test with the freshwater aquatic plant, duckweed, <i>Lemna gibba</i>
DuPont Report No.:	DuPont-49944
Testing Facility Report No.:	86356
Guidelines	OECD 221 (2006)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable

EXECUTIVE SUMMARY

The acute toxicity of GF-3969 to the freshwater aquatic plant, duckweed, *Lemna gibba*, was determined in a 7-day growth inhibition test. The test was conducted in accordance with Organization for Economic Cooperation and Development (OECD) Guidelines for Testing of Chemicals, Guideline No. 221. The exposure test was conducted with a filter-sterilized 20X AAP nutrient medium (blank) control and five concentrations of GF-3969. Three replicates were initiated per test concentration and blank control.

Calculated concentrations of GF-3969, based on the combined DPX-E9636 and DPX-M6316 total active substances, ranged from 91 to 135% of nominal concentrations in fresh test substance treatment solutions and from 53 to 92% of the nominal concentrations in spent test substance solutions. The geometric mean calculated concentrations of GF-3969 in the test concentration solutions ranged from 83 to 97% of nominal GF-3969 concentrations. Frond counts increased in the blank control by at least

a factor of approximately 24 in 7 days and the doubling time in the blank control based on frond count was 1.5 days, thereby satisfying the appropriate test acceptance criteria.
The analytical results are summarized as follows:

Nominal concentrations of GF-3969, as mg GF-3969 total product (TP)/L	Blank control, 0.00012, 0.00038, 0.0012, 0.0039, and 0.013
Geometric mean calculated concentrations of GF-3969, as mg GF-3969 total product (TP)/L	<LOD ^a , 0.0000772, 0.000267, 0.000804, 0.00296, and 0.00958

a <LOD denotes not detected. The limit of detection for GF-3969 was calculated as 0.0000300 mg GF-3969 total product (TP)/L.

Inhibition of growth of *Lemna gibba* exposed to geometric mean calculated GF-3969 concentrations as compared to the control for 7 days is reported below.

Parameter	Percent inhibition in 0.0000772, 0.000267, 0.000804, 0.00296, and 0.00958 mg GF-3969 total product (TP)/L geometric mean calculated test substance concentrations
Growth Rate, Frond Count	-2, -2, -3, 43, and 76%
Yield, Frond Count	-8, -7, -9, 78, and 95%
Growth Rate, Dry Weight	-2, -2, -2, 21, and 36%
Yield, Dry Weight	-6, -6, -6, 49, and 69%

Geometric mean calculated concentrations of GF-3969 were used for the estimation of the E_yC₅₀ and E_rC₅₀ (0-7 day) values (effect concentration producing a 50% inhibition of growth based on yield and growth rate, respectively, relative to the control), 95% confidence interval (CI), LOEC (lowest concentration that had a significant effect relative to the control, and NOEC (highest concentration that had no significant effect relative to the control) values. Geometric mean measured concentrations producing 20 and 10% inhibition were also estimated (EC₂₀ and EC₁₀).

The results are summarized as follows:

Endpoint	Effect Concentration as mg GF-3969 total product (TP)/L			
	Growth Rate, Frond Count (95% CI)	Yield, Frond Count (95% CI)	Biomass Yield, Dry Weight (95% CI)	Biomass Growth Rate, Dry Weight (95% CI)
7-day NOEC	0.00296	0.000804	0.00296	0.00296
7-day LOEC	0.00958	0.00296	0.00958	0.00958
7-day EC ₁₀ ^a	0.000625 (0.000475 – 0.000776)	Not statistically sound (Not calculated)	0.000571 (0.000350 – 0.000792)	0.00192 (0.00149 – 0.00235)
7-day EC ₂₀ ^a	0.00132 (0.00101 – 0.00164)	0.000734 (0.000354 – 0.00111)	0.00121 (0.000742 – 0.00168)	0.00407 (0.00316 – 0.00498)
7-day EC ₅₀ ^a	0.00411 (0.00312 – 0.00510)	0.00228 (0.00110 – 0.00346)	0.00376 (0.00230 – 0.00521)	>0.00958 (Not statistically sound)

a Determined using OECD Model 2.

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):

GF-3969

Composition/Purity:

DPX-V4B07-002 is a formulation containing nominal concentrations (w/w):
31.6% Isoxadifen-ethyl safener

	42.1% Rimsulfuron active ingredient 26.3% Thifensulfuron methyl active ingredient DPX-KG691 (ethoxylated isodecylalcohol as adjuvant surfactant.)
Synonym:	GF-3969
Lot/Batch #:	None for formulation MAR15EL004 for rimsulfuron (DPX-E9636) APR15EL002 for thifensulfuron methyl (DPX-M6316) DEC15EL001 for isoxadifen-ethyl (DPX-X4145)
Purity:	None for formulation 25.1% for DPX-E9636 49.8% for DPX-M6316 50.4% for DPX-X4145
Description:	DPX-V4B07-002 is prepared by combining 22.1% Isoxadifen 50 WG, 59.2% Rimsulfuron 25 SG, and 18.7% Thifensulfuron 50 SG
CAS#:	None for formulation 122931-48-0 for DPX-E9636 79277-27-3 for DPX-M6316 163520-33-0 for DPX-X4145
Stability of test compound:	Determined to be stable in the test system
Control:	20X AAP nutrient medium
Test solvent:	None
Toxic reference:	None

Test System

Organism (<i>Species</i>):	Duckweed (<i>Lemna gibba</i> G3)
Initial population:	3 plant with 4 fronds each
Source:	Eurofins, Columbia, Missouri In house culture, parent culture from USDA/ARS Beltsville Agricultural Research Center, Beltsville, Maryland
Test chamber:	500-mL Erlenmeyer flask with a foam stopper, containing 200 mL of test solution.
Growth medium:	20X AAP nutrient medium
Environmental conditions:	Temperature: 22.5 to 24.5°C Photoperiod: 24 hr photoperiod (7401 to 7830 lux) pH: 7.4 to 9.0 throughout the exposure period

Methodology

1. In-life initiated/completed
27 February to 06 March 2019
2. Experimental treatments
The effect of GF-3969 to the floating freshwater vascular plant *Lemna gibba* G3 was determined in a static-renewal, 7-day test. The plants were exposed to an untreated control and five nominal concentrations of 0.00012, 0.00038, 0.0012, 0.0039, and 0.013 mg GF-3969 total product (TP)/L in 20X AAP nutrient medium for 7 days, with daily test medium renewal. Each test concentration and the untreated control were tested as three replicates. Test units were incubated in an environmental chamber for 7 days.
3. Observations
Test solutions were measured for GF-3969 on days 0, 1, 3, 4, 6, and 7 to verify stability of the test item.
Frond counts were made on Days 0, 3, 5, and 7.
Dry weight was determined at the completion of the 7-day test.

Fronnd count yield and dry weight yield were determined by subtracting the initial frond count or dry weight from the test end values.

Growth rate was determined on Day 7 and was based on frond count and dry weight.

Biomass, yield, and growth rate based on frond count or dry weight after 7 days were expressed as percent inhibition relative to the untreated control following exposure to GF-3969 for 7 days.

4. Statistics

The LOEC and NOEC values, based on growth and yield were estimated using a one-way analysis of variance (ANOVA) procedure and a one-tailed Dunnett's test and/or Jonckheere Trend test ($p = 0.05$). Prior to the Dunnett's test and Jonckheere Trend test, a Levene's test were conducted to test for normality and homogeneity of variance, respectively, over treatments at each time point. Parametric analyses were performed for all data at all intervals.

RESULTS AND DISCUSSION

The geometric mean calculated concentrations of GF-3969 over the 7-day exposure period in the test concentrations ranged from 83 to 97% of nominal. The blank control solution contained no detectable concentrations of GF-3969. All validation criteria were met for the study.

Data on biomass, yield, and growth rate based on frond count and dry weight after 7 days following exposure of *Lemna gibba* G3 to GF-3969 for 7 days are summarized in the tables that follow.

Table A 6: Summary of growth inhibition (frond counts) following exposure of *Lemna gibba* to GF-3969 for 7 days

Geometric Mean Measured GF-3969 Concentration (mg GF-3969 total product (TP)/L)	Fronnd count	
	Mean total fronds	% Inhibition relative to control
Blank control (0.0)	293	---
0.0000772	315	-8
0.000267	312	-6
0.000804	319	-9
0.00296	73.3	75
0.00958	26.0	91

Table A 7: Summary of growth inhibition (average specific growth rate and yield) following exposure of *Lemna gibba* to GF-3969 for 7 days

Geometric Mean Measured GF-3969 Concentration (mg GF-3969 total product (TP)/L)	Average specific growth rate		Yield of fronds	
	Mean average specific growth rate (day ⁻¹)	% Inhibition relative to control	Mean yield of total fronds	% Inhibition relative to control
Blank control (0.0)	0.456	---	281	---
0.0000772	0.466	-2	303	-8
0.000267	0.465	-2	300	-7
0.000804	0.469	-3	307	9
0.00296	0.258	43	61.3**	78
0.00958	0.109*	76	14.0**	95

* Significant reduction in the 7-day average specific growth rate as compared to the blank control (Jonckheere-Terpstra test, $p = 0.05$).

** Significant reduction in the 7-day yield as compared to the blank control (Dunnett's test, $p = 0.05$).

Table A 8: Summary of growth rate inhibition following exposure of *Lemna gibba* to GF-3969 for 7 days

Geometric Mean Measured GF-3969 Concentration (mg GF-3969 total product (TP)/L)	7-day growth rate based on yield (dry weight)		7-day growth rate based on biomass (dry weight)	
	Mean 7-Day yield (dry weight)	% Inhibition relative to control	Mean 7-Day biomass (dry weight)	% Inhibition relative to control
Blank control (0.0)	0.0418	---	0.428	--
0.0000772	0.0443	-6	0.435	-2
0.000267	0.0443	-6	0.436	-2
0.000804	0.0442	-6	0.436	-2
0.00296	0.0212	49	0.336	21
0.00958	0.0129*	69	0.274**	36

* Significant reduction in yield (dry weight) as compared to the blank control (Jonckheere-Terpstra test, $p = 0.05$).

** Significant reduction in biomass growth rate (dry weight) as compared to the blank control (Jonckheere-Terpstra test, $p = 0.05$).

CONCLUSION

GF-3969 was assessed for toxicity to *L. gibba* in a 7-day growth inhibition test. Based on the average specific growth rate, the estimated E_rC_{50} values on day 7 were 0.00411 mg GF-3969 total product (TP)/L (geometric mean measured concentration), with 95% confidence limits of 0.00312 and 0.00510 mg GF-3969 total product (TP)/L. Based on mean yield, the estimated E_yC_{50} values on day 7 were 0.00228 mg GF-3969 total product (TP)/L, with 95% confidence limits of 0.00110 and 0.00346 mg GF-3969 total product (TP)/L. Based on mean biomass yield, the estimated E_yC_{50} values on day 7 were 0.00376 mg GF-3969 total product (TP)/L, with 95% confidence limits of 0.00230 and 0.00521 mg GF-3969 total product (TP)/L. Based on the biomass growth rate, the estimated E_rC_{50} values on day 7 were >0.00958 mg GF-3969 total product (TP)/L, the highest concentration tested. The NOEC and LOEC values on day 7 were 0.00296 and 0.00958 mg GF-3969 total product (TP)/L (geometric mean measured concentration), respectively, for average specific growth rate, biomass yield, and biomass growth rate. The NOEC and LOEC values on day 7 were 0.000804 and 0.00296 mg GF-3969 total product (TP)/L (geometric mean measured concentration), respectively, for yield. The biomass, yield, and growth rate, all based on frond count or dry weight, did indicate a concentration-dependent response for exposure to the test substance, GF-3969.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Duckweed	<i>Lemna gibba</i>	GF-3969	7 d	E_rC_{50} (growth rate) E_yC_{50} (yield) = mg product/L _{mm} E_rC_{50} (biomass growth rate) >0.00958 mg product/L _{mm} E_yC_{50} (biomass yield) = 0.00376 mg product/L _{mm}	0.00411 0.00228	mg GF-3969 total product (TP)/L

A 2.2.1.5 Study 5, DuPont-49978

Comments of zRMS:	The study was performed in line with OECD 221 with no deviations. The analytical measurements demonstrated that the concentrations of thifensulfuron methyl exceed the required 120% of nominal in fresh solutions in some treatment groups while the concentrations of rimsulfuron exceeded the required 120% of nominal in fresh solutions and dropped below the required 80% of nominal in spent solutions in some treatment groups. For that reason, the endpoints were reported in the study based
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on the mean calculated concentrations from the mean measured concentrations for both active substances.

All the validity criteria were met:

- The doubling time of the frond number in the control must be < 2.5 days (60 h), corresponding to approximately a 7-fold increase in 7 days and an average specific growth rate of 0.275 d^{-1} (actual doubling time was 1.4 days, corresponding to approximately a 31-fold increase in 7 days and an average specific growth rate of 0.493 d^{-1}).

Overall the study is considered acceptable with the following endpoints relevant for the risk assessment:

E_rC_{10} (growth rate) = 0.00131 mg product/L (based on the mean calculated concentration from the active substances)

E_rC_{20} (growth rate) = 0.00180 mg product/L (based on the mean calculated concentration from the active substances)

E_rC_{50} (growth rate) = 0.00291 mg product/L (based on the mean calculated concentration from the active substances)

NOE_rC (growth rate) = 0.0000706 mg product/L (based on the mean calculated concentration from the active substances)

E_yC_{10} (yield) = 0.000827 mg product/L (based on the mean calculated concentration from the active substances)

E_yC_{20} (yield) = 0.00864 mg product/L (based on the mean calculated concentration from the active substances)

E_yC_{50} (yield) = 0.000940 mg product/L (based on the mean calculated concentration from the active substances)

NOE_yC (yield) = 0.0000706 mg product/L (based on the mean calculated concentration from the active substances)

E_rC_{10} (biomass growth rate) = 0.000727 mg product/L (based on the mean calculated concentration from the active substances)

E_rC_{20} (biomass growth rate) = 0.00164 mg product/L (based on the mean calculated concentration from the active substances)

E_rC_{50} (biomass growth rate) = 0.00853 mg product/L (based on the mean calculated concentration from the active substances)

NOE_rC (biomass growth rate) = 0.0000706 mg product/L (based on the mean calculated concentration from the active substances)

E_yC_{10} (biomass yield) > 0.0101 mg product/L (based on the mean calculated concentration from the active substances)

E_yC_{20} (biomass yield) = 0.000658 mg product/L (based on the mean calculated concentration from the active substances)

E_yC_{50} (biomass yield) = 0.00204 mg product/L (based on the mean calculated concentration from the active substances)

NOE_yC (biomass yield) = 0.000254 mg product/L (based on the mean calculated concentration from the active substances)

Reference:	KCP 10.2.1/05
Report:	Goudie, O.J., (2019); DPX-V4B07 24 WG (Rimsulfuron 25 SG + thifensulfuron 50 SG + isoxadifen 50 WG) A blend of paste extruded granules plus crop oil (Codacide): 7-Day growth inhibition test with the freshwater aquatic plant, duckweed, <i>Lemna gibba</i>
DuPont Report No.:	DuPont-49978
Testing Facility Report No.:	86359
Guidelines	OECD 221 (2006)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable

EXECUTIVE SUMMARY

In a 7-day static-renewal toxicity test, the freshwater aquatic plant, *Lemna gibba*, was exposed to GF-3969 plus Crop Oil (Codacide). The in-life phase of the definitive test was conducted at nominal concentrations of 0.00012, 0.00038, 0.0012, 0.0039, and 0.013 mg GF-3969 total product (TP)/L.

Calculated GF-3969 concentrations, based on the combined rimsulfuron (DPX-E9636) and thifensulfuron methyl (DPX-M6316) total active substances, ranged from 80 to 128% of nominal concentrations in fresh test substance treatment solutions and from 40 to 96% of the nominal concentrations in spent test substance solutions. The biological response results were reported based on the geometric mean calculated GF-3969 concentrations.

Based on the average specific growth rate, the estimated E_rC_{50} value on day 7 was 0.00291 mg GF-3969 total product (TP)/L, with 95% confidence limits of 0.00274 and 0.00308 mg GF-3969 total product (TP)/L.

Based on the yield, the estimated E_yC_{50} value on day 7 was 0.000940 mg GF-3969 total product (TP)/L with 95% confidence limits of 0.000877 and 0.00101 mg GF-3969 total product (TP)/L.

Based on the biomass, yield the estimated E_yC_{50} value on day 7 was 0.00204 mg GF-3969 total product (TP)/L, the 95% confidence limits of 0.00140 and 0.00269 mg GF-3969 total product (TP)/L.

Based on the dry weight biomass growth rate, the estimated E_rC_{50} value on day 7 was 0.00853 mg GF-3969 total product (TP)/L, with 95% confidence limits of 0.00520 and 0.0118 mg GF-3969 total product (TP)/L.

The test acceptability criteria for control growth (i.e., frond doubling time <2.5 days, greater than a seven-fold increase in the number of fronds) set by OECD 221 test guideline were met for this study. The doubling time for the control fronds was 1.4 days, corresponding to a 31-fold increase in the number of fronds, and the average specific growth rate was 0.493 day⁻¹.

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):

GF-3969 plus Crop Oil (Codacide)

Composition Purity:

DPX-V4B07-002 is a formulation containing nominal concentrations (w/w):

31.6% Isoxadifen-ethyl safener

42.1% Rimsulfuron active ingredient

26.3% Thifensulfuron methyl active ingredient

Crop oil (Codacide)

Purity of granules of particular paste components:

DPX-E9636 – 25.1% of rimsulfuron

DPX-M6316 – 49.8% of thifensulfuron methyl

DPX-X4145 – 50.4% of isoxadifen-ethyl

Description (physical state):

DPX-V4B07 24 WG is prepared by combining 22.1% Isoxadifen ethyl 50WG, 59.2% Rimsulfuron 25SG and 18.7% Thifensulfuron methyl 50SG Not provided

Lot/batch no.:

Rimsulfuron: MAR15EL004

Thifensulfuron methyl: APR15EL002

Isoxadifen-ethyl safener: DEC15EL001

Test System

Organism (<i>Species</i>):	Freshwater aquatic plant, <i>Lemna gibba</i>
Study type:	Laboratory study Static-renewal
Study duration:	7 days
Parameters measured:	Test solution pH (range): 7.4 to 9.0 Test solution temperature (range): 22.0 to 24.7 °C
Environmental conditions:	Photoperiod: continuous Light intensity (range): 7244 to 7813 lux
Observation intervals:	0, 3, 5 and 7 days
Test concentrations:	Nominal: Control, 0.00012, 0.00038, 0.0012, 0.0039, and 0.013 mg GF-3969 total product (TP)/L Geometric mean calculated concentrations: <LOD, 0.0000706, 0.000254, 0.000831, 0.00310, and 0.0101 mg GF-3969 total product (TP)/L
Acclimation period/conditions:	The <i>Lemna gibba</i> cultures used to inoculate the definitive test were transferred to fresh nutrient medium seven days prior to study initiation and the number of fronds in the cultures had increased approximately 8-fold in seven days.
Growth medium:	Name: 20X freshwater algal nutrient medium pH at test initiation: 7.6 to 7.7 pH at test termination: 8.5 to 9.0
Dilution water:	Type: 20X freshwater algal nutrient medium
Method of test item added to the test medium:	A 100 mg GF-3969 total product (TP)/L primary standard solution was prepared daily from 08 to 14 March 2019 by mixing together 0.0111 g of DPX-X4145, 0.0296 g of DPX-E9636, and 0.0093 g of DPX-M6316 dry product and adding the dry product mixture to 0.5 L of test medium. Following the dry product addition, 0.154 mL of the crop oil adjuvant surfactant, Codacide, was added to the primary standard solution to maintain the ratio of adjuvant surfactant to dry mixed product at 100 mL of adjuvant surfactant per 32.5 g of total dry mixed product. Primary standard solution aliquots of 0.60, 1.9, 6.0, 19.5, and 65 mL were each diluted in 1.0 L of test medium daily from 08 to 14 March 2019 to prepare the 0.00012, 0.00038, 0.0012, 0.0039, and 0.013 mg GF-3969 total product (TP)/L test treatment solutions. The blank control consisted of test medium only.
Initial frond number:	12
No. of control replicates:	4
No. of test concentration replicates:	4
Analytical verification:	Method: The concentration of the GF-3969 was calculated, based on the combined rimsulfuron (DPX-E9636) and thifensulfuron methyl (DPX-M6316) total active substances, using a liquid chromatography system with tandem mass spectrometry (LC-MS/MS). Samples taken : fresh parent solutions at day 0 (test initiation) day 3, and day 6, and, and in a composite of spent replicate solutions at day 1, day 4, and day 7 (termination)

Limit of Detection: 0.0000300 mg GF-3969 total product (TP)/L
Limit of Quantitation: 0.000100 mg GF-3969 total product (TP)/L
Recoveries from QC fortifications: 77 to 111%
Daily
Test substance renewal days:

Methodology

The test chambers used for the test were 500-mL Erlenmeyer flasks with foam stoppers. The control and each test substance treatment were replicated three times and each replicate contained 200 mL of the appropriate test solution. Each flask was labelled with the study number, treatment, replicate, and grid position. Each test flask received three plants, for a total of 12 fronds, at test initiation. Aseptic addition of *Lemna gibba* was initiated within 30 minutes of test solution preparation. The blank control and test substance treatments were renewed daily throughout the exposure. Beginning with the blank control, plants were aseptically transferred, without harming roots or fronds, from old test solutions to new test solutions. Growth was measured by determining the change in the number of total fronds during the exposure period. Every frond that visibly projected beyond the edge of the parent frond was counted as a separate frond. Any change in plant development, frond size, appearance, necrosis or chlorosis was noted if observed. Frond observations and counts were performed on days 3, 5, and 7 for all replicates of the blank control and each test substance treatment. Biomass (dry weight) measurements of each control and test substance treatment replicate were performed on day 7 (test termination), after frond observations had been conducted, as well as on three representative samples at test initiation. The concentration of GF-3969 was calculated, based on the combined rimsulfuron (DPX-E9636) and thifensulfuron methyl (DPX-M6316) total active substances, in fresh parent solutions at day 0 (test initiation) day 3, and day 6, and, and in a composite of spent replicate solutions at day 1, day 4, and day 7 (termination). The samples were analysed on a liquid chromatography system with tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Calculated concentrations of GF-3969, based on the combined rimsulfuron (DPX-E9636) and thifensulfuron methyl (DPX-M6316) total active substances, in fresh test substance treatment solutions were 0.0000899, 0.000268, 0.000950, 0.00335, and 0.0108 mg GF-3969 total product (TP)/L on day 0; 0.0000984, 0.000314, 0.00108, 0.00368, and 0.0117 mg GF-3969 total product (TP)/L on day 3; and 0.0000748, 0.000380, 0.000940, 0.00334, and 0.0104 mg GF-3969 total product (TP)/L on day 6. Calculated concentrations of GF-3969, based on the combined rimsulfuron (DPX-E9636) and thifensulfuron methyl (DPX-M6316) total active substances, in spent test substance treatment solutions were 0.0000757, 0.000244, 0.000811, 0.00286, and 0.00932 mg GF-3969 total product (TP)/L on day 1; 0.0000984, 0.000314, 0.00108, 0.00368, and 0.0117 mg GF-3969 total product (TP)/L on day 4; and 0.0000373, 0.000148, 0.000529, 0.00263, and 0.00889 mg GF-3969 total product (TP)/L on day 7. No detectable concentrations of GF-3969, based on the combined DPX-E9636 and DPX-M6316 total active substances, above the LOD (0.0000300 mg GF-3969 total product (TP)/L). Geometric mean calculated concentrations of GF-3969 during the 7 day exposure were 0.0000706, 0.000254, 0.000831, 0.00310, and 0.0101 mg GF-3969 total product (TP)/L or 75 to 102% of the nominal concentrations calculated from the active substances. All results from biological responses were based on the geometric mean calculated GF-3969 concentrations. The percent inhibition of frond average specific growth rate as compared to the control was 2, 4, 5, 56, and 79% for the 0.0000706, 0.000254, 0.000831, 0.00310, and 0.0101 mg a.s./L geometric mean calculated test substance treatments, respectively. The percent inhibition of frond yield as compared to the control was 7, 13, 17, 88, and 97% for the 0.0000706, 0.000254, 0.000831, 0.00310, and 0.0101 mg a.s./L geometric mean calculated test substance treatments, respectively. The percent inhibition of biomass yield (dry weight), as compared to the control, was 8, 15, 24, 69, and 83% for the 0.0000706, 0.000254, 0.000831, 0.00310, and 0.0101 mg a.s./L geometric mean calculated test substance treatments, respectively. The percent inhibition of biomass (dry weight) average specific growth rate

as compared to the control was 3, 5, 8, 34, and 52% for the 0.0000706, 0.000254, 0.000831, 0.00310, and 0.0101 mg a.s./L geometric mean calculated test substance treatments, respectively.

Table A 9: Mean frond density and growth rate

Concentration (mg GF-3969 total product (TP)/L)	Mean Number of Total Fronds	% Inhibition	Mean Average Specific Growth Rate (day ⁻¹)	% Inhibition
	Day 7	Day 7	Day 0 - 7	Day 7
control	380	NA	0.493	NA
0.0000706	355	6	0.483	2
0.000254	334	12	0.474 *	4
0.000831	319	16	0.468 *	5
0.00310	54.7	86	0.216 *	56
0.0101	24.7	94	0.102 *	79

* Significant reduction as compared to the blank control (Jonckheere-Terpstra test, $p = 0.05$).

Table A 10: Mean frond yield and biomass

Concentration (mg GF-3969 total product (TP)/L)	Mean Yield of Total Fronds	% Inhibition	Treatment Mean Yield (dry weight g)	% Inhibition
	Day 7	Day 7	Day 0 - 7	Day 7
control	368	NA	0.0505	NA
0.0000706	343	7	0.0463	8
0.000254	322 *	13	0.0429	15
0.000831	307 *	17	0.0384 *	24
0.00310	42.7 *	88	0.0159 *	69
0.0101	12.7 *	97	0.0084 *	83

* Significant reduction as compared to the blank control (Jonckheere-Terpstra test, $p = 0.05$).

Table A 11: Statistical endpoints

Endpoint	Frond yield	Frond average specific growth rate	Biomass yield as dry weight	Biomass average specific growth rate as dry weight
NOEC	0.0000706	0.0000706	0.000254	0.0000706
LOEC	0.00254	0.000254	0.000831	0.000254
EC ₅₀	0.000940	0.00291	0.00204	0.00853

CONCLUSION

The test acceptability criterion for control growth was met for this study. The doubling time for the control fronds was 1.4 days. This study is classified as acceptable, and satisfies the guideline requirement for a growth inhibition test with *Lemna gibba*.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Duckweed	<i>Lemna gibba</i>	GF-3969	7 day	Frond average specific growth rate EC ₅₀	0.00291	mg GF-3969 total product (TP)/L
Duckweed	<i>Lemna gibba</i>	GF-3969	7 day	Frond yield EC ₅₀	0.000940	mg GF-3969 total product (TP)/L
Duckweed	<i>Lemna gibba</i>	GF-3969	7 day	Biomass yield as dry weight EC ₅₀	0.00204	mg GF-3969 total product (TP)/L
Duckweed	<i>Lemna gibba</i>	GF-3969	7 day	Biomass average specific growth rate as dry weight EC ₅₀	0.00853	mg GF-3969 total product (TP)/L

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

No new or additional studies have been submitted.

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

No new or additional studies have been submitted.

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

A 2.3.1.1.1.1 Study 1, DuPont-48950

Comments of zRMS:	The study was performed in line with OECD 213 and 214 with no deviations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: 48 h LD ₅₀ (oral) > 100 µg product/bee 48 h LD ₅₀ (contact) > 100 µg product/bee
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Reference:	KCP 10.3.1.1.1/02
Report:	Tome, H.V.V.,Porch J.R., (2018); Isoxadifen ethyl 50WG / Rimsulfuron 25SG / Thifensulfuron methyl 50SG/ (DPX-V4B07), a blend of paste extruded granules (11.11% + 14.82 + 9.26% active) plus Trend 90 surfactant: An acute oral and contact toxicity study with the honey bee
DuPont Report No.:	DuPont-48950
Testing Facility Report No.:	112H-116
Guidelines	OCSPP 850.3020 (2012), OECD 213 (1998), OECD 214 (1998)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable

EXECUTIVE SUMMARY

For the *contact exposure bioassay*, 1 µL droplets of the test-item solutions were applied to the dorsal surface of the thorax of anaesthetised worker bees. The test item was mixed with Trend-90 **adjuvant surfactant** at a ratio of 1 g GF-3969 : 345 µL Trend-90 **adjuvant surfactant**. An untreated solution of 50% w/v sugar in water was provided as sustenance for the bees throughout the bioassay.

The bees were exposed to five doses in a geometric series, up to 100 µg test item/bee.

In a contact-exposure laboratory test with the honeybee *Apis mellifera*, both the 24-h and 48-h LD₅₀ for GF-3969 plus Trend 90 **adjuvant surfactant** were >100 µg test item/bee.

For the *oral exposure bioassay*, worker bees were exposed to the treatment solutions (20 µL/bee) via feeding vials placed in each cage. These vials contained the diluted products mixed into a 50% w/v solution of sugar in purified water. Upon consumption of the dose, or at 6 hours after first exposure, the treated feeding vials were replaced with ones containing untreated 50% w/v sucrose solution. After six hours, the amount of unconsumed dosing solution remaining in each feeder was measured using an HPLC syringe.

The bees were exposed to five doses, up to 100 µg test item/bee.

In an oral-exposure laboratory test with the honeybee *Apis mellifera*, both the 24-h and 48-h LD₅₀ for DPX-V4B07 plus Trend 90 **adjuvant surfactant** were >100 µg test item/bee.

MATERIALS AND METHODS

Test Item(s)

Test item (Common name): Isoxadifen-ethyl 50WG / Rimsulfuron 25SG / Thifensulfuron methyl 50SG (GF-3969) plus Trend-90 **adjuvant surfactant**

Purity: 11.11% + 14.82% + 9.26%, respectively
Description (physical state): Wettable granules (WG) and soluble granules (SG)
Formulation is a blend of three formulated components
Lot/batch no.: DPX-X4145-021, DPX-E9636-227, DPX-M6316-323

Test System

Organism (*Species*): *Apis mellifera*
Study type: oral and contact acute toxicity
Study design: multiple dose test; 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 concentrations: Oral: 3.1, 6.3, 13, 25 and 100 µg total formulation/bee
Contact: 6.3, 13, 25, 50 and 100 µg total formulation/bee
Information on bee colony (health etc.): The bees used in each 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 was incomplete, however test results were based on nominal doses.
Feeding method: 50% w/v sucrose solution *ad libitum*; 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°C oral
25°C contact
Relative Humidity: 52 - 69% oral
59-70% 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 0.30 µg Dimethoate per bee (contact test)
Solvent substance (if applicable): none

Methodology

Young adult worker honey bees were exposed to five nominal test doses of GF-3969 plus Trend-90 **adjuvant surfactant**, ranging from 3.1 to 100 µg/bee, administered orally in a sucrose solution and five test doses of GF-3969 plus Trend-90 **adjuvant surfactant** ranging from 6.3 to 100 µg/bee administered topically. Negative and **adjuvant surfactant** control (deionized water containing Trend 90 **adjuvant surfactant**) groups were maintained concurrently. Three replicate test chambers were maintained in each control and treatment group, with 10 bees in each test chamber. The nominal test concentrations were established based upon known toxicity information and in consultation with the Sponsor. Additional groups of bees from the same source were dosed with dimethoate, at 0.05, 0.10, and 0.30 µg a.s./bee (orally) and 0.05, 0.10, and 0.30 µg a.s./bee (topically) as a positive control substance. The positive control test was conducted concurrently with the definitive test. Positive control doses were selected to approximate the oral LD₅₀ of dimethoate to honey bees. Observations of mortality and other signs of toxicity were made for up to approximately 48 hours after dosing. The cumulative mortality observed in the test groups was used to determine the LD₅₀ and NOEC.

RESULTS AND DISCUSSION

Incomplete dose consumption was observed in some replicates throughout the test. Since the incomplete consumption did not appear to be treatment-related, results were based on nominal doses.

There was no mortality in the negative control at the end of the test. Mortality in the **adjuvant surfactant** control, 3.1, 6.3, 13, 25 and 100 µg/bee groups was 10, 10, 10, 3, 3 and 3%, respectively. Therefore, the oral NOEC and oral LD₅₀ were determined to be 100 µg/bee and >100 µg/bee, respectively, based on nominal doses. One bee in the negative control group exhibited lethargy, one bee in the **adjuvant surfactant** control group control group was immobile, and one bee in the 13 µg/bee group exhibited loss of equilibrium during the test. However, these behaviours were considered incidental to treatment.

In the contact exposure test, mortality in the negative and **adjuvant surfactant** control at the end of the test was 7 and 3%, respectively. Mortality in the 3.1, 6.3, 13, 25, 50 and 100 µg/bee groups was 0, 3, 3, 0 and 3%, respectively. Therefore, the contact NOEC and contact LD₅₀ were determined to be 100 µg/bee and >100 µg/bee, respectively, based on nominal doses. Two lethargic bees were observed in the 100 µg/bee group at test termination, and bees in the positive control groups exhibited signs of toxicity common for exposure to dimethoate. Otherwise, all living bees appeared normal throughout the test.

Table A 12: Toxicity of GF-3969 plus Trend-90 **adjuvant surfactant to honeybees in oral and contact toxicity tests**

A		Oral	Contact
Nominal	Mean consumed dose	Mortality (%)	
		48-hr	48-hr
Negative Control (0)	-	0	7
Adjuvant surfactant Control (0)	-	10	3
3.1	3.0	10	-
6.3	6.3	10	0
13	10	3	3
25	19	3	3
50	-	-	0
100	36	3	3
Contact 48-hr LD ₅₀		>100 µg/bee (95% CI not available)	
Oral 48-hr LD ₅₀		>100 µg/bee (95% CI not available)	
Contact LD ₅₀ (24-hr) value of the reference item: 0.168 0.17 µg dimethoate/bee			
Oral LD ₅₀ (24-hr) value of the reference item: 0.228 0.23 µg dimethoate/bee			

Table A 13: Sublethal effects of GF-3969 plus Trend-90 **adjuvant surfactant to honey bees in oral and contact toxicity tests**

Treatment µg GF-3969 plus Trend-90 adjuvant surfactant /bee				
Nominal	Consumed (Oral Test)	Sublethal effects after 48 hrs (number of bees)		
		On back	Lethargic	Other
Contact:				
Negative Control (0)	-	0%	0%	0%
Adjuvant surfactant Control (0)	-	0%	0%	0%
6.3	-	0%	0%	0%
13	-	0%	0%	0%
25	-	0%	0%	0%
50	-	0%	0%	0%
100	-	0%	7%	0%
Oral:				
Negative Control (0)	-	0%	3%	0%
Adjuvant surfactant Control (0)	-	0%	0%	0%
3.1	3.0	0%	0%	0%
6.3	6.3	0%	0%	3%
13	10	0%	0%	0%
25	19	0%	0%	0%
100	36	0%	0%	0%

CONCLUSION

The 48-hour acute contact LD₅₀ for honey bees exposed to GF-3969 plus Trend-90 **adjuvant** ~~surfactant~~ was determined to be greater than the highest test dose of 100 µg/bee. The NOEC in the contact test was 100 µg/bee. In the acute oral test, the 48-hour LD₅₀ was determined to be greater than the highest test dose of 100 µg/bee and the corresponding NOEC was 100 µg/bee.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Honey bee	<i>Apis mellifera</i>	GF-3969 plus Trend-90 adjuvant surfactant	48-hr – oral	LD ₅₀	>100	µg/bee
Honey bee	<i>Apis mellifera</i>	GF-3969 plus Trend-90 adjuvant surfactant	48-hr – contact	LD ₅₀	>100	µg/bee

A 2.3.1.1.1.2 Study 2, DuPont-48892

Comments of zRMS:	<p>The study was performed in line with OECD 213 and 214 with no deviations.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>48 h LD₅₀ (oral) > 100 µg product/bee 48 h LD₅₀ (contact) > 100 µg product/bee</p>
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Reference:	KCP 10.3.1.1/01
Report:	Tome, H.V.V., (2018); Isoxadifen ethyl 50WG / Rimsulfuron 25SG / Thifensulfuron methyl 50SG (DPX-V4B07), a blend of paste extruded granules (11.11% + 14.82% + 9.26 active) plus codacide oil surfactant: An acute oral and contact toxicity study with the honey bee
DuPont Report No.:	DuPont-48892
Testing Facility Report No.:	112H-112
Guidelines	OCSPP 850.3020 (2012), OECD 213 (1998), OECD 214 (1998)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable

EXECUTIVE SUMMARY

For the *contact exposure bioassay*, 1 µL droplets of the test-item solutions were applied to the dorsal surface of the thorax of anaesthetised worker bees. The test item was mixed with Codacide Oil **adjuvant** ~~surfactant~~ at a ratio of 1 g GF-3969 : 345 µL Codacide Oil **adjuvant** ~~surfactant~~. An untreated solution of 50% w/v sugar in water was provided as sustenance for the bees throughout the bioassay.

The bees were exposed to five doses in a geometric series, up to 100 µg test item/bee

In a contact-exposure laboratory test with the honeybee *Apis mellifera*, both the 24-h and 48-h LD₅₀ for GF-3969 plus Codacide Oil **adjuvant** ~~surfactant~~ were >100 µg test item/bee.

For the *oral exposure bioassay*, worker bees were exposed to the treatment solutions (20 µL/bee) via feeding vials placed in each cage. These vials contained the diluted products mixed into a 50% w/v solution of sugar in purified water. Upon consumption of the dose, or at 6 hours after first exposure, the treated feeding vials were replaced with ones containing untreated 50% w/v sucrose solution. After six hours, the amount of unconsumed dosing solution remaining in each feeder was measured using an HPLC syringe.

The bees were exposed to five doses, up to 100 µg test item/bee.

In an oral-exposure laboratory test with the honeybee *Apis mellifera*, both the 24-h and 48-h LD₅₀ for GF-3969 plus Codacide Oil **adjuvant** ~~surfactant~~ were >100 µg test item/bee.

MATERIALS AND METHODS

Test Item(s)

Test item (Common name): Isoxadifen Ethyl 50WG / Rimsulfuron 25SG / Thifensulfuron Methyl 50SG (GF-3969) plus Codacide Oil **adjuvant** ~~surfactant~~

Purity: 11.11% + 14.82% + 9.26%, respectively

Description (physical state): Wettable granules (WG) and soluble granules (SG)
Formulation is a blend of three formulated components

Lot/batch no.: DPX-X4145-021, DPX-E9636-227, DPX-M6316-323

Test System

Organism (*Species*): *Apis mellifera*

Study type: oral and contact acute toxicity

Study design: multiple dose test; 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 concentrations: Oral: 3.1, 6.3, 13, 25 and 100 µg total formulation/bee
Contact: 6.3, 13, 25, 50 and 100 µg total formulation/bee

Information on bee colony (health etc.): The bees used in each 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 was incomplete, however test results were based on nominal doses.

Feeding method: 50% w/v sucrose solution *ad libitum*; 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°C oral
25°C contact
Relative Humidity: 51-70% oral
66-70% 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 0.30 µg Dimethoate per bee (contact test)

Solvent substance (if applicable): none

Methodology

Young adult worker honey bees were exposed to five nominal test doses of GF-3969 plus Codacide Oil **adjuvant** ~~surfactant~~, ranging from 3.1 to 100 µg/bee, administered orally in a sucrose solution and five test doses of GF-3969 plus Codacide Oil **adjuvant** ~~surfactant~~ ranging from 6.3 to 100 µg/bee administered topically. Negative and **adjuvant** ~~surfactant~~ control (deionized water containing Codacide Oil **adjuvant** ~~surfactant~~) groups were maintained concurrently. Three replicate test chambers were maintained in each control and treatment group, with 10 bees in each test chamber. The nominal test concentrations were established based upon known toxicity information and in consultation with the Sponsor. Additional groups of bees from the same source were dosed with dimethoate, at 0.05, 0.10, and 0.30 µg a.s./bee (orally) and 0.05, 0.10, and 0.30 µg a.s./bee (topically) as a positive control substance. The positive control test was conducted concurrently with the definitive test. Positive control doses were selected to approximate the oral LD₅₀ of dimethoate to honey bees. Observations

of mortality and other signs of toxicity were made for up to approximately 48 hours after dosing. The cumulative mortality observed in the test groups was used to determine the LD₅₀ and NOEC.

RESULTS AND DISCUSSION

Incomplete dose consumption was observed in some replicates throughout the oral exposure test. Since the incomplete consumption did not appear to be treatment-related, results were based on nominal doses. There was no mortality in the negative or **adjuvant surfactant** control at the end of the oral exposure test. Mortality in the 3.1, 6.3, 13, 25 and 100 µg/bee groups was 0, 3, 0, 7 and 0%, respectively. The incidental mortality observed in the 6.3 and 25 µg/bee groups was determined not to be dose responsive nor attributed to treatment. Therefore, the oral NOEC and oral LD₅₀ were determined to be 100 µg/bee and >100 µg/bee, respectively, based on nominal doses. One bee in the 100 µg/bee group exhibited loss of equilibrium during the test. However, this behaviour was considered incidental to treatment.

In the contact exposure test, there was no mortality in the negative or **adjuvant surfactant** control, or any treatment group at the end of the test. Therefore, the contact NOEC and contact LD₅₀ were determined to be 100 µg/bee and >100 µg/bee, respectively, based on nominal doses. All living bees appeared normal throughout the test.

Table A 14: Toxicity of GF-3969 plus Codacide Oil **adjuvant surfactant** to honeybees in oral and contact toxicity tests

Treatment µg GF-3969 plus Codacide Oil adjuvant surfactant /bee		Oral	Contact
Nominal	Mean consumed dose	Mortality (%)	
		48-hr	48-hr
Negative Control (0)	-	0	0
Adjuvant surfactant Control (0)	-	0	0
3.1	3.1	0	-
6.3	6.1	3	0
13	12	0	0
25	25	7	0
50	-	-	0
100	89	0	0
Contact 48-hr LD ₅₀		>100 µg/bee (95% CI not available)	
Oral 48-hr LD ₅₀		>100 µg/bee (95% CI not available)	
Contact LD ₅₀ (24-hr) value of the reference item: 0.173 µg dimethoate/bee			
Oral LD ₅₀ (24-hr) value of the reference item: 0.167 0.23 µg dimethoate/bee			

Table A 15: Sublethal effects of GF-3969 plus Codacide Oil **adjuvant surfactant to honey bees in oral and contact toxicity tests**

Treatment		Sublethal effects after 48 hrs (number of bees)		
µg GF-3969 plus Codacide Oil adjuvant surfactant /bee		On back	Lethargic	Other
Nominal	Consumed (Oral Test)			
Contact:				
Negative Control (0)	-	0%	0%	0%
Adjuvant surfactant Control (0)	-	0%	0%	0%
6.3	-	0%	0%	0%
13	-	0%	0%	0%
25	-	0%	0%	0%
50	-	0%	0%	0%
100	-	0%	0%	0%
Oral:				
Negative Control (0)	-	0%	0%	0%
Adjuvant surfactant Control (0)	-	0%	0%	0%
3.1	3.0	0%	0%	0%
6.3	6.3	0%	0%	0%
13	10	0%	0%	0%
25	19	0%	0%	0%
100	36	0%	0%	3%

CONCLUSION

The 48-hour acute contact LD₅₀ for honey bees exposed to GF-3969 plus Codacide Oil **adjuvant surfactant** was determined to be greater than the highest test dose of 100 µg/bee. The NOEC in the contact test was 100 µg/bee. In the acute oral test, the 48-hour LD₅₀ was determined to be greater than the highest test dose of 100 µg/bee and the corresponding NOEC was 100 µg/bee.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Honey bee	<i>Apis mellifera</i>	GF-3969 plus Codacide Oil adjuvant surfactant	48-hr – oral	LD ₅₀	>100	µg/bee
Honey bee	<i>Apis mellifera</i>	GF-3969 plus Codacide Oil adjuvant surfactant	48-hr – contact	LD ₅₀	>100	µg/bee

A 2.3.1.1.1.3 Study 3, DuPont-48899, Revision No. 1

Comments of zRMS:	The study was performed in line with OECD 246 and 247 with no deviations.				
	The analytical measurements of the lowest and highest concentrations of the test solutions demonstrated that the concentrations of rimsulfuron were maintained within 80-120% of nominal during the study while the concentrations of thifensulfuron methyl dropped below the required 80% of nominal (see table below).				
	Nominal application rate [µg product/bumble bee]	Nominal concentration of thifensulfuron-methyl [mg/L]	Measured concentration of thifensulfuron-methyl		
			[mg/L]	% of nominal	Mean recovery
	Oral toxicity test				
	31.3	72.5	53.5	74	76
			56.5	78	
	500	1160	850	73	78
			960	83	
	Contact toxicity test				

40.6	750	585	78	79
		600	80	
650	12000	10700	89	89

Based on the results provided in the table above, the geometric mean measured concentration was determined to be 77 and 83.9% of nominal for the oral and contact toxicity tests, respectively.

Although the concentrations of thifensulfuron methyl dropped below 80% of nominal, the geometric mean measured concentration in the contact toxicity study was 83.9% of nominal, thus the endpoint may be expressed in terms of the nominal concentration.

In the case of the oral toxicity study the geometric mean measured concentration of thifensulfuron methyl was below 80% and for this reason and in line with conclusions of the Central Zone harmonisation meetings in area of ecotoxicology, the endpoint should be expressed as the mean measured concentration of the least stable active substance - thifensulfuron-methyl in the tested formulation. Therefore, the endpoint was recalculated accordingly.

All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:

48 h LD₅₀ (contact) > 650 µg product/bumblebee (based on nominal concentration)
48 h LD₅₀ (oral) > 225.6 µg product/bumblebee (based on geometric mean measured concentration)

Reference:	KCP 10.3.1.1/04
Report:	Verge, E., (2019); Rimsulfuron 25SG/thifensulfuron methyl 50SG/isoxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) + surfactant DPX-KG691: Acute oral and contact toxicity to the bumble bee, <i>Bombus terrestris</i> L. under laboratory conditions
DuPont Report No.:	DuPont-48899, Revision No. 1
Testing Facility Report No.:	S18-00130
Guidelines	OECD 247 (2017), OECD 246 (2017)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable

EXECUTIVE SUMMARY

Acute 48 hour oral and contact toxicity tests on bumble bees (*Bombus terrestris* L.) were conducted with GF-3969 mixed with DPX-KG691 (ratio: 100 mL DPX-KG691 to 32.5 g of GF-3969) in the laboratory based on OECD Guidelines No. 247 (2017) and No. 246 (2017).

The oral toxicity test treatments consisted of one toxic reference treatment, one control (50% (w/v) aqueous sucrose solution) and five target doses of 31.3, 62.5, 125, 250 and 500 µg product/bumble bee of the test item GF-3969. The doses for the oral toxicity test based on measured consumption of application solution (actual consumption) were 26.8, 43.7, 91.1, 175 and 293 µg product/bumble bee.

The contact toxicity treatments consisted of one reference treatment, two controls (2 µL and 5 µL deionized water containing 0.1% Triton X-100) and five target doses 40.6, 81.3, 162.5, 325 and 650 µg product/bumble bee.

Analytical verification of the test item concentration in the control and application solution(s) of the highest and lowest dose levels was performed by liquid chromatography and mass spectrometric detection (LC MS/MS). Samples were taken directly after preparation and before application.

In the lowest and highest oral application solutions, the actual concentrations of rimsulfuron were equivalent to mean recoveries of 86% of nominal. The actual concentrations of thifensulfuron methyl were equivalent to mean recoveries of 76 and 78% of nominal, respectively.

In the lowest and highest contact application solutions, the actual concentrations of rimsulfuron were equivalent to mean recoveries of 89% of nominal. The actual concentrations of thifensulfuron methyl were equivalent to mean recoveries of 79 and 89% of nominal, respectively.

No residues of rimsulfuron or thifensulfuron methyl above the LOD (4.44 mg rimsulfuron/L and 2.78 mg thifensulfuron methyl/L) were found in any of the control samples.

The acute oral 48 hour LD₅₀ was determined to be >293 µg product/bumble bee. The acute contact 48 hour LD₅₀ was determined to be >650 µg product/bumble bee.

The 48 hour oral NOED (No Observed Effect Dose) was determined to be 293 µg product/bumble bee. The 48 hour contact NOED was determined to be 162.5 µg product/bumble bee.

MATERIALS AND METHODS

Test Item(s)

Test material:	GF-3969 (formulation is a blend of three formulated components)
Lot/Batch #:	119695-102-6
Content of active ingredient, analysed	14.82% rimsulfuron 9.26% thifensulfuron methyl 11.11% isoxadifen ethyl safener
CAS Name (uninverted):	Not available
CAS #:	Not available
Stability of test compound:	No information available
Control:	Oral toxicity test: 50% (w/v) aqueous sucrose solution Contact toxicity test: deionized water containing 0.1% Triton X-100
Test vehicle:	Oral toxicity test: 50% (w/v) aqueous sucrose solution Contact toxicity test: deionized water containing 0.1% Triton X-100
Toxic reference:	BAS 152 11 I (dimethoate a.s.) applied at 1.5 and 13.0 µg a.s./bumblebee in the oral and contact test, respectively.

Test System

Test organism:	Bumble bees (<i>Bombus terrestris</i> L)
Source:	Biobest Belgium, Ilse Velden 18, 2260 Westerlo, Belgium (BE)
Diet:	50% (w/v) aqueous sucrose solution
Water:	See diet
Test chamber:	Housing in Nicot cages (perforated plastic cylinder; base: ~1 cm radius, height: 7 cm)
Environmental conditions (In-life phase)	oral toxicity test contact toxicity test
Temperature:	24.6 to 25.2°C 24.5 to 25.0°C
Relative humidity:	54.3 to 63.3% 55.2 to 71.1%
Photoperiod:	Continuous darkness except during the assessments. Observations were made under neon light.

Methodology

1. In life initiated/completed
26 FEB 2018 to 29 JUN 2018
Analytical Phase
26 MAR 2018 to 01 AUG 2018
2. Experimental treatments
The acute 48 hour oral and contact toxicity of GF-3969 was determined in bumble bees (*Bombus terrestris* L.). The oral toxicity test treatments consisted of one toxic reference treatment, one control (50% (w/v) aqueous sucrose solution) and five target doses of 31.3,

62.5, 125, 250 and 500 µg product/bumble bee of the test item GF-3969. Due to exclusion of bumble bees that consumed less than 80% of the mean consumption of feeding solution, 26 to 33 replicates per treatment group with one bumble bee per replicate were used.

The contact toxicity treatments consisted of one reference treatment, two controls (2 µl and 5 µL deionized water containing 0.1% Triton X-100), and five target doses of 40.6, 81.3, 162.5, 325 and 650 µg product/bumble bee of the test item GF-3969. Thirty replicates per treatment group with one bumble bee per replicate were used.

BAS 152 11 I (dimethoate a.s.) was used as toxic reference. In the oral toxicity test, bumble bees were offered the test solutions in 50% (w/v) aqueous sucrose solution. In the contact toxicity test, bumble bees were dosed with GF-3969 by topical application with a 5-µL droplet applied to the dorsal thorax of each bumble bee.

3. Observations

Assessments for mortalities and behavioural abnormalities were carried out 4, 24 and 48 hours after treatment in the oral and contact toxicity tests.

4. Statistics

For the oral and contact toxicity Cochran-Armitage test (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there was a statistically significant difference between the mortality data of the test item groups and the control group to determine the NOED.

The LD₅₀ values could not be determined, since there was no mortality above 50%.

All statistical analysis was done using the statistical program, ToxRat Professional 3.2.1 (ToxRat Solutions GmbH).

RESULTS AND DISCUSSION

In the lowest and highest oral application solutions, the actual concentrations of rimsulfuron were equivalent to mean recoveries of 86% of nominal. The actual concentrations of thifensulfuron methyl were equivalent to mean recoveries of 76 and 78% of nominal, respectively.

In the lowest and highest contact application solutions, the actual concentrations of rimsulfuron were equivalent to mean recoveries of 89% of nominal. The actual concentrations of thifensulfuron methyl were equivalent to mean recoveries of 79 and 89% of nominal, respectively.

In the control group of the oral toxicity test treated with 50% (w/v) aqueous sucrose solution 3.1% mortality occurred during the 48 hour observation period. In the toxic reference item group of the oral toxicity test the mortality was 96.9% (corrected mortality: 96.8%) at the end of the test.

In the control group of the contact toxicity test treated with deionized water containing 0.1% Triton X-100 no mortality was recorded at the end of the 48 hours observation period. In the toxic reference item group of the contact toxicity test the mortality was 90.0% at the end of the test. Consequently, validity criteria for both control and reference item mortality were met and the test was deemed valid.

In the oral toxicity test at the dose levels of 31.3, 62.5, 125, 250 and 500 µg product/bumble bee (actual doses: 26.8, 43.7, 91.1, 175 and 293 µg product/bumble bee) mortalities of 0.0, 0.0, 3.7, 3.8 and 7.4% (corrected mortality: -3.2, -3.2, 0.6, 0.7 and 4.4%) occurred during the 48 hours observation period, respectively. No behavioural abnormalities were observed during the 48 hour observation period.

In the contact toxicity test at the dose levels of 40.6, 81.3, 162.5, 325 and 650 µg product/bumble bee mortalities of 3.3, 6.7, 6.7, 20.0 and 26.7% occurred during the 48 hours observation period. Affected and moribund bumble bees were observed in all dose levels, except at 81.3 µg product/bumble bee, during the 48 hour observation period.

Actual test item uptake in the oral toxicity test and mortality results for the oral and contact toxicity tests at 24 and 48 hours are given in the tables below.

Table A 16: Mortality of the bumble bee, *Bombus terrestris* L., exposed to GF-3969 in the oral toxicity test

GF-3969 (µg product/bumble bee)		Mortality [%]		Corrected mortality [%]	
Treatment (Target Dose)	Test item consumed ^a	24 h	48 h	24 h	48 h
C (0)		3.1	3.1	-	-
31.3	26.8	0.0	0.0	-3.2	-3.2
62.5	43.7	0.0	0.0	-3.2	-3.2
125	91.1	3.7	3.7	0.6	0.6
250	175	3.8	3.8	0.7	0.7
500	293	7.4	7.4	4.4	4.4

C: control

a: based on the actual consumption of application solution, rounded values

Table A 17: Mortality of the bumble bee, *Bombus terrestris* L., exposed to GF-3969 in the contact toxicity test

GF-3969 (µg product/bumble bee)	Mortality [%]	
Treatment (Target Dose, nominal)	24 h	48 h
C (0)	0.0 3.3	0.0 3.3
40.6	0.0	3.3
81.3	6.7	6.7
162.5	3.3	6.7
325	16.7 ^a	20.0 ^a
650	23.3 ^a	26.7 ^a

C: Control

a: Statistically significantly different compared to the control (Cochran Armitage Test; one-sided greater; $\alpha = 0.05$)

CONCLUSION

The effects of GF-3969 were assessed in an acute oral and contact bumble bee toxicity test conducted in the laboratory.

The acute oral 48-hour LD₅₀ was determined to be >293 µg product/bumble bee.

The acute contact 48-hour LD₅₀ was determined to be >650 µg product/bumble bee.

The 48-hour oral NOED (No Observed Effect Dose) was determined to be 293 µg product/bumble bee.

The 48-hour contact NOED was determined to be 162.5 µg product/bumble bee.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Bumble bee	<i>Bombus terrestris</i> L	GF-3969 plus adjuvant surfactant DPX-KG691	48-hr – oral	LD ₅₀	>293	µg/bee
Bumble bee	<i>Bombus terrestris</i> L	GF-3969 plus adjuvant surfactant DPX-KG691	48-hr – contact	LD ₅₀	>650	µg/bee

A 2.3.1.1.1.4 Study 4, DuPont-48951

Comments of zRMS:	<p>The study was performed in line with OECD 246 and 247 with no deviations.</p> <p>The concentrations of both active substances were maintained within 80-120% of nominal and the endpoints can be expressed based on nominal concentrations.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>48h LD₅₀ (oral) > 470 µg product/bumblebee (based on nominal concentration) 48h LD₅₀ (contact) > 500 µg product/bumblebee (based on nominal concentration)</p>
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Reference:	KCP 10.3.1.1.1/03
Report:	Verge, E., (2018); Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) + codacide oil: Acute oral and contact toxicity to the bumble bee, <i>Bombus terrestris</i> L. under laboratory conditions
DuPont Report No.:	DuPont-48951
Testing Facility Report No.:	S18-00132
Guidelines	OECD 247 (2017), OECD 246 (2017)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable

EXECUTIVE SUMMARY

Acute 48 hour oral and contact toxicity tests on bumble bees (*Bombus terrestris* L.) were conducted with GF-3969 mixed with Codacide Oil (ratio: 100 mL Codacide Oil to 32.5 g of GF-3969) in the laboratory based on OECD Guidelines No. 247 (2017) and No. 246 (2017).

The oral and contact toxicity test treatments consisted of one toxic reference treatment, one control (50% (w/v) aqueous sucrose solution) and five target doses of 31.1, 62.5, 125, 250 and 500 µg product/bumble bee of the test item GF-3969. The doses for the oral toxicity test based on measured consumption of application solution (actual consumption) were 30.4, 60.2, 120, 237 and 470 µg product/bumble bee.

Analytical verification of the test item concentration in the solvent controls and application solution(s) of the highest and lowest dose levels was performed by liquid chromatography and mass spectrometric detection (LC MS/MS). Samples were taken directly after preparation and before application.

In the lowest and highest oral application solutions, the actual concentrations of rimsulfuron were equivalent to recoveries of 91 and 84% of nominal, respectively. The actual concentrations of thifensulfuron methyl were equivalent to recoveries of 90 and 93% of nominal, respectively.

In the lowest and highest contact application solutions, the actual concentrations of rimsulfuron were equivalent to mean recoveries of 97 and 96% of nominal. The actual concentrations of thifensulfuron methyl were equivalent to mean recoveries of 94 and 95% of nominal, respectively.

No residues of rimsulfuron or thifensulfuron methyl above the LOD (4.44 mg rimsulfuron/L and 2.78 mg thifensulfuron methyl /L) were found in any of the control samples.

The acute oral 48-hour LD₅₀ was determined to be >470 µg product/bumble bee. The acute contact 48-hour LD₅₀ was determined to be >500 µg product/bumble bee.

The 48-hour oral NOED (No Observed Effect Dose) was determined to be 470 µg product/bumble bee. The 48-hour contact NOED was determined to be 500 µg product/bumble bee.

MATERIALS AND METHODS

Test material:	GF-3969 (blend of 59.26% DPX-E9636-227 25SG, 18.52% DPX-M6316-323 50SG and 22.22% DPX-X4145-021 50WG)
Lot/Batch #:	119695-102-6
Content of substances:	14.82% rimsulfuron 9.26% thifensulfuron methyl 11.11% isoxadifen ethyl safener
CAS Name (uninverted):	Not available
CAS #:	Not available
Stability of test compound:	No information available
Control:	Oral toxicity test: 50% (w/v) aqueous sucrose solution Contact toxicity test: deionized water
Test vehicle:	Oral toxicity test: 50% (w/v) aqueous sucrose solution Contact toxicity test: deionized water
Toxic reference:	BAS 152 11 I (dimethoate a.s.) applied at 1.5 and 13.0 µg a.s./bumblebee in the oral and contact test, respectively.

Test organism: Bumble bees
Species: *Bombus terrestris* L
Source: Biobest Belgium, Ilse Velden 18, 2260 Westerlo, Belgium (BE)
Diet: 50% (w/v) aqueous sucrose solution
Water: See diet
Test chamber: Housing in Nicot cages (perforated plastic cylinder; base: ~ 1 cm radius, height: 7 cm)

Environmental conditions
(In-life phase oral and contact toxicity)

Temperature: 24.5 to 25.2°C
Relative humidity: 51.9 to 60.7%
Photoperiod: Continuous darkness except during the assessments.
Observations were made under neon light.

1. In life initiated/completed
20 MAR 2018 to 22 MAR 2018
Analytical Phase
19 MAR 2018 to 02 JUL 2018
2. Experimental treatments
The acute 48 hour oral and contact toxicity of GF-3969 mixed with Codacide oil was determined in bumble bees (*Bombus terrestris* L.). The oral toxicity test treatments consisted of one toxic reference treatment, one control (50% (w/v) aqueous sucrose solution) and five target doses of 31.1, 62.5, 125, 250 and 500 µg product/bumble bee of the test item GF-3969 missed with Codacide oil. Due to exclusion of bumble bees that consumed less than 80% of the mean consumption of feeding solution, 29 to 35 replicates per treatment group with one bumble bee per replicate were used.
The contact toxicity treatments consisted of one reference treatment, one control (deionized water), and five target doses of 31.1, 62.5, 125, 250 and 500 µg product/bumble bee of the test item GF-3969 mixed with Codacide oil. Thirty replicates per treatment group with one bumble bee per replicate were used.
BAS 152 11 I (dimethoate a.s.) was used as toxic reference. In the oral toxicity test, bumble bees were offered the test solutions in 50% (w/v) aqueous sucrose solution. In the contact toxicity test, bumble bees were dosed by topical application with a 5-µL droplet applied to the dorsal thorax of each bumble bee.
3. Observations
Assessments for mortalities and behavioural abnormalities were carried out 4, 24 and 48 hours after treatment in the oral and contact toxicity tests.
4. Statistics
For the oral and contact toxicity test Multiple Fisher's exact test with Bonferroni-Holm adjustment (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there was a statistically significant difference between the mortality data of the test item groups and the control group in order to determine the NOED (No Observed Effect Dose).
The LD₅₀ values could not be determined, since there was no mortality above 50%.
All statistical analysis was done using the statistical program, ToxRat Professional 3.2.1 (ToxRat Solutions GmbH).

RESULTS AND DISCUSSION

In the lowest and highest oral application solutions, the actual concentrations of rimsulfuron were equivalent to recoveries of 91 and 84% of nominal, respectively. The actual concentrations of thifensulfuron methyl were equivalent to recoveries of 90 and 93% of nominal, respectively.

In the lowest and highest contact application solutions, the actual concentrations of rimsulfuron were equivalent to mean recoveries of 97 and 96% of nominal. The actual concentrations of thifensulfuron methyl were equivalent to mean recoveries of 94 and 95% of nominal, respectively.

In the control groups of the oral and contact toxicity test treated with 50% (w/v) aqueous sucrose solution or deionized water no mortality occurred during the 48 hour observation period. In the toxic reference item groups of the oral and contact toxicity test the mortality at the end of the test was 82.4 and 93.3%, respectively.

Consequently, validity criteria for both control and reference item mortality were met and the test was deemed valid.

In the oral toxicity test at the dose levels of 31.3, 62.5, 125, 250 and 500 µg product/bumble bee (actual doses: 30.4, 60.2, 120, 237 and 470 µg product/bumble bee) no mortality occurred during the 48-hour observation period, respectively. No behavioural abnormalities were observed during the 48-hour observation period.

In the contact toxicity test at the dose levels of 31.3, 62.5, 125, 250 and 500 µg product/bumble bee mortalities of 0.0, 10.0, 6.7, 0.0 and 6.7% occurred during the 48-hour observation period. Few affected and moribund bumble bees were observed in all dose levels except at 250 µg product/bumble bee during the 48 hour observation period.

Actual test item uptake in the oral toxicity test and mortality results for the oral and contact toxicity tests at 24 and 48 hours are given in the tables below.

Table A 18: Mortality of the bumble bee, *Bombus terrestris* L., exposed to GF-3969 in the oral toxicity test

GF-3969 (µg product/bumble bee)		Mortality [%]	
Treatment (Target Dose)	Test item consumed ^a	24 h	48 h
C (0)		0.0	0.0
31.3	30.4	0.0	0.0
62.5	60.2	0.0	0.0
125	120	0.0	0.0
250	237	0.0	0.0
500	470	0.0	0.0

C: control

a based on the actual consumption of application solution, rounded values

Table A 19: Mortality of the bumble bee, *Bombus terrestris* L., exposed to GF-3969 in the contact toxicity test

GF-3969 (µg product/bumble bee)	Mortality [%]	
Treatment (Target Dose)	24 h	48 h
C (0)	0.0	0.0
31.3	0.0	0.0
62.5	10.0	10.0
125	3.3	6.7
250	0.0	0.0
500	3.3	6.7

C: Control

CONCLUSION

The effects of GF-3969 were assessed in an acute oral and contact bumble bee toxicity test conducted in the laboratory.

The acute oral 48-hour LD₅₀ was determined to be >470 µg product/bumble bee.

The acute contact 48-hour LD₅₀ was determined to be >500 µg product/bumble bee.

The 48-hour oral NOED (No Observed Effect Dose) was determined to be 470 µg product/bumble bee.

The 48-hour contact NOED was determined to be 500 µg product/bumble bee.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Bumble bee	<i>Bombus terrestris</i> L	GF-3969 plus adjuvant surfactant Codacide	48-hr – oral	LD ₅₀	>470	µg/bee
Bumble bee	<i>Bombus terrestris</i> L	GF-3969 plus adjuvant surfactant Codacide	48-hr – contact	LD ₅₀	>500	µg/bee

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

Reference:	KCP 10.3.1.1.2/01
Report:	Tome, H.V.V., (2018); Isoxadifen ethyl 50WG / Rimsulfuron 25SG / Thifensulfuron methyl 50SG (DPX-V4B07), a blend of paste extruded granules (11.11% + 14.82% + 9.26 active) plus codacide oil surfactant: An acute oral and contact toxicity study with the honey bee
DuPont Report No.:	DuPont-48892
Testing Facility Report No.:	112H-112
Guidelines	OCSPP 850.3020 (2012), OECD 213 (1998), OECD 214 (1998)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable

Reference:	KCP 10.3.1.1.2/02
Report:	Tome, H.V.V.,Porch J.R., (2018); Isoxadifen ethyl 50WG / Rimsulfuron 25SG / Thifensulfuron methyl 50SG/ (DPX-V4B07), a blend of paste extruded granules (11.11% + 14.82 + 9.26% active) plus Trend 90 surfactant: An acute oral and contact toxicity study with the honey bee
DuPont Report No.:	DuPont-48950
Testing Facility Report No.:	112H-116
Guidelines	OCSPP 850.3020 (2012), OECD 213 (1998), OECD 214 (1998)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable

Reference:	KCP 10.3.1.1.2/03
Report:	Verge, E., (2018); Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isoxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) + codacide oil: Acute oral and contact toxicity to the bumble bee, <i>Bombus terrestris</i> L. under laboratory conditions
DuPont Report No.:	DuPont-48951
Testing Facility Report No.:	S18-00132
Guidelines	OECD 247 (2017), OECD 246 (2017)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable

Reference:	KCP 10.3.1.1.2/04
Report:	Verge, E., (2019); Rimsulfuron 25SG/thifensulfuron methyl 50SG/isoxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) + surfactant DPX-KG691: Acute oral and contact toxicity to the bumble bee, <i>Bombus terrestris</i> L. under laboratory conditions
DuPont Report No.:	DuPont-48899, Revision No. 1
Testing Facility Report No.:	S18-00130
Guidelines	OECD 247 (2017), OECD 246 (2017)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable

Refer to A.2.3.1.1.1 above for summaries of both oral and contact studies.

A 2.3.1.2 KCP 10.3.1.2 Chronic toxicity to bees

A 2.3.1.2.1 Study 1, 200439

Comments of zRMS:	<p>The study was performed in line with OECD 245 with no major deviations.</p> <p>It is noted that the maximum relative humidity of 71.7% slightly exceeded the maximum of 70% recommended by the test guideline. Nevertheless, this deviation is considered to have no impact on the study results since all validity criteria were met.</p> <p>The measured concentrations of rimsulfuron and thifensulfuron-methyl in fresh and aged diets were within 80-120% of nominal (111-137% and 96-111%, respectively). In addition to active compounds, also measured concentrations of isoxadifen-ethyl were determined and were in range 77-92% of nominal in fresh test diets and 74-83% in aged test diets. Since isoxadifen-ethyl is not an active compound, its concentration was not considered in derivation of the endpoints.</p> <p>The daily doses of the test item were corrected for evaporation, in line with indications of OECD 245.</p> <p>Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>10 day LDD₅₀ = 2.98 µg product/bee/day 10 day NOEDD = 0.81 µg product/bee/day</p>
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Reference:	KCP 10.3.1.2/01
Report:	Porch, J.R., Riles, B. (2021a); GF-3969 (DPX-V4B07) + DPX-KG691 (VIVOLT): A Chronic Dietary Toxicity test with the Honey Bee (<i>Apis mellifera</i>)
DAS Study No.:	200439
Testing Facility Report No.:	112H-131A
Guidelines	OECD 245
Deviations:	Minor (see commenting box above)
GLP:	Yes
Acceptability:	Acceptable

EXECUTIVE SUMMARY

Newly emerged worker bees (10 bees/cage, three cages/treatment) were exposed for 10 days to untreated control, reference item or test item treatment solutions dissolved or dispersed

homogeneously in 50 % sucrose via syringes placed in the top of each cage. Every 24 hours, the treated feeding vials were replaced with ones containing freshly prepared diet. The mean amount of test item solution consumed per bee was determined by weighing the feeding vials before and after being placed in the cages and by analytical confirmation of the tested concentrations in the final diets. Mortality, behavioural effects, and diet consumption were observed on each day of the study.

The bees were exposed to nominal dietary concentrations of 12.8, 32, 80, 200 and 500 mg GF-3969/kg diet, equivalent to daily dietary dosages of 0.37, 0.81, 1.6, 3.4 and 8.0 µg GF-3969/bee/day, based on measured daily diet consumption. Analytical confirmation was conducted for thifensulfuron-methyl, rimsulfuron and isoxadifen-ethyl using LC-MS/MS with an LOQ of 11.2 mg GF-3969/kg.

The test item caused statistically significant increases in mortality and statistically significant decreases in food consumption at the three highest concentrations tested.

Based on nominal concentrations and measured food consumption, the LDD_{10,20,50} (10 day) were 0.82, 1.28 and 2.98 µg GF-3969/bee, respectively. The LC_{10, 20,50} (10 day) were 35.6, 59.9 and 162 mg GF-3969/kg diet, respectively.

The NOEDD and LOEDD (10 day) were 0.81 and 1.6 µg GF-3969/bee, respectively, and the NOEC and LOEC (10 day) were 32 and 80 mg GF-3969/kg diet, respectively.

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-3969 (Isoxadifen Ethyl 50WG / Rimsulfuron 25SG / Thifensulfuron Methyl 50SG (DPX-V4B07) plus DPX-KG691
Purity:	11.11 % + 14.82 % + 9.26 %, respectively + adjuvant surfactant
Description (physical state):	Wettable granules (WG), soluble granules (SG) and adjuvant surfactant
Lot/batch no.:	GF-3966 [TSN315416], GF-3866 [TSN316738], GF-3968 [TSN315297], TSN401051

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), 12.8, 32, 80, 200 and 500 mg GF-3969/kg diet
Information on bee colony (health etc):	The bees used in the test were from disease-free colonies which 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 °C and 63 to 64 % humidity.
Amount of treated diet consumed:	Consumption of the treated diets ranged from 15.9 to 28.8 mg of diet per bee per day. Calculated daily dosages ranged from 0.37 to 8.0 µg GF-3969/bee/day.

Feeding method: The bees were housed in cages containing pre-weighed feeders (syringes) containing approximately 2.5 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: 33 – 34 °C
Relative humidity: 57 – 72 %
Photoperiod: The environmental chamber was kept dark except when room lighting was used during observation periods.

Reference substance: Dimethoate: 0.65 mg a.i./kg diet

Solvent substance (if applicable): -

Methodology

Test diets were prepared by diluting the test substance in sucrose solution and performing proportional dilutions. A positive control stock was prepared by dissolving dimethoate in acetone.

Honey bees were collected from local hives maintained by Eurofins as capped brood. Upon receipt, the capped brood was incubated until to allow emergence of adult bees. One day prior to test initiation, bees were collected from the incubator and 10 bees were impartially placed into each test chamber for 24-hour acclimation. At the end of the acclimation period, cages with apparently healthy bees were selected for use in the study.

Test cages were impartially allocated to the treatment and control groups at test initiation. Starting at test initiation, the appropriate diet was presented to the bees through feeders inserted through the lid of the test chamber. In addition, three replicate cages with sucrose solution and containing no bees were included in order to determine evaporative loss during each feeding period. Test and control diets were provided *ad libitum* for the 10-day duration of the test. Feeders containing diet were replaced daily at 24 ± 2 hour intervals. The amount of diet consumed in each replicate was measured by weighing the feeders before and after use. The daily change in weight was adjusted to account for evaporative loss estimated by the mean of these three replicates from the same day.

The negative control replicates were handled in a manner identical to the treated and positive control bees, but were not administered any test substance, while positive control bees received diet containing dimethoate.

Observations of the bees were made daily during the test.

RESULTS AND DISCUSSION

Mean mortality in the negative control, 12.8, 32, 80, 200 and 500 mg GF-3969/kg groups was 7, 3, 7, 40, 60 and 80 %, respectively.

Mean daily food consumption in the negative control, 12.8, 32, 80, 200 and 500 mg GF-3969/kg groups was 26.3, 28.8, 25.4, 19.7, 17.0 and 15.9 mg/bee/day, respectively.

Measured food consumption and nominal concentrations resulted in dietary doses in the negative control, 12.8, 32, 80, 200 and 500 mg GF-3969/kg groups of 0, 0.37, 0.81, 1.6, 34 and 8.0 µg GF-4320/bee/day.

Based on nominal concentrations and measured food consumption, the LDD_{10,20,50} (10 day) were 0.82, 1.28 and 2.98 µg GF-3969/bee, respectively. The LC_{10, 20,50} (10 day) were 35.6, 59.9 and 162 mg GF-3969/kg diet, respectively. The NOEDD and LOEDD (10 day) were 0.81 and 1.6 µg GF-3969/bee, respectively, and the NOEC and LOEC (10 day) were 32 and 80 mg GF-3969/kg diet, respectively.

All living bees appeared normal throughout the test except for a small number of affected bees in the 200 and 500 mg GF-3969/kg and positive control groups between days 6 and 10 of the test.

Table A 20: Toxicity of GF-3969 to honey bees in the chronic oral toxicity test

Treatment Dietary Dose µg GF-3969/bee/day (mg GF-3969/kg)	Oral 10 day test									
	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	7	7	7
0.37 (12.8)	0	0	0	0	0	0	0	0	0	3
0.81 (32)	0	0	0	0	0	0	0	0	0	7
1.6 (80)	0	3	3	3	3	3	3	13	20	40
3.4 (200)	0	0	0	3	3	3	3	23	43	90
8.0 (500)	0	0	3	3	3	3	17	37	43	80
Reference Item	0	0	3	3	13	40	63	100	100	100
10 day LDD ₁₀	0.82 µg GF-3969/bee/day (95 % CI 0.072 – 1.58)									
10 day LDD ₂₀	1.28 µg GF-3969/bee/day (95 % CI 0.24 – 2.20)									
10 day LDD ₅₀	2.98 µg GF-3969/bee/day (95 % CI 1.53 – 5.31)									
10 day NOEDD	0.81 µg GF-3969/bee/day									
10 day LOEDD	1.6 µg GF-3969/bee/day									
10 day LC ₁₀	35.6 mg GF-3969/kg diet (95 % CI 2.08 – 77.6)									
10 day LC ₂₀	59.9 mg GF-3969/kg diet (95 % CI 7.73 – 114)									
10 day LC ₅₀	162 mg GF-3969/kg diet (95 % CI 72 – 314)									
10 day NOEC	32 mg GF-3969/kg diet									
10 day LOEC	80 mg GF-3969/kg diet									

Table A 21: Effect of GF-3969 on diet consumption in honey bees in the chronic oral toxicity test

Treatment Dietary Dose µg GF-3969/bee/day (mg GF-3969/kg)	Oral 10 day test									
	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)	17.03	14.00	24.53	23.27	23.77	30.65	27.53	41.07	21.05	40.14
0.37 (12.8)	14.50	26.33	31.70	15.47	22.77	33.80	30.97	36.83	30.87	44.93
0.81 (32)	7.67	31.93	18.70	26.00	20.90	39.13	14.60	37.38	20.77	36.77
1.6 (80)	11.17	26.47	18.28	12.90	19.55	18.70	23.45	17.62	20.02	29.17
3.4 (200)	6.13	17.70	15.20	16.33	14.76	17.38	19.01	22.28	14.64	26.24
8.0 (500)	7.70	20.33	12.47	12.00	19.60	19.92	13.47	24.75	13.88	15.24
Reference Item	16.63	20.50	25.47	12.93	14.12	15.66	7.93	4.23	-	-
10 day EDD ₁₀	0.60 µg GF-3969/bee/day (95 % CI 0.12 – 1.27)									
10 day EDD ₂₀	1.47 µg GF-3969/bee/day (95 % CI 0.75 – 2.50)									
10 day EDD ₅₀	> 8 µg GF-3969/bee/day									
10 day NOEDD	0.81 µg GF-3969/bee/day									
10 day LOEDD	1.6 µg GF-3969/bee/day									
10 day EC ₁₀	24.6 mg GF-3969/kg diet (95 % CI 4.11 – 58.5)									
10 day EC ₂₀	70.5 mg GF-3969/kg diet (95 % CI 32.0 – 130)									
10 day EC ₅₀	> 500 mg GF-3969/kg diet									
10 day NOEC	32 mg GF-3969/kg diet									
10 day LOEC	80 mg GF-3969/kg diet									

Table A 22: Sublethal effects of GF-3969 to honey bees in the chronic oral toxicity test

Treatment Dietary Dose µg GF-3969/bee/day (mg GF-3969/kg)	Oral 10 day test		
	Sublethal effects (Number of bees, Day observed)		
	On back	Lethargic	Other
Control (0)	0 %	0 %	0 %
0.37 (12.8)	0 %	0 %	0 %
0.81 (32)	0 %	0 %	0 %
1.6 (80)	0 %	0 %	0 %
3.4 (200)	0 %	0 %	3 % (days 7 and 8)
8.0 (500)	0 %	0 %	3 % (days 8 and 9)

CONCLUSION

Based on nominal concentrations and measured food consumption, the LDD_{10,20,50} (10 day) were 0.82, 1.28 and 2.98 µg GF-3969/bee, respectively. The LC_{10, 20,50} (10 day) were 35.6, 59.9 and 162 mg GF-3969/kg diet, respectively. The NOEDD and LOEDD (10 day) were 0.81 and 1.6 µg GF-3969/bee, respectively, and the NOEC and LOEC (10 day) were 32 and 80 mg GF-3969/kg diet, respectively.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Honey bee	<i>Apis mellifera</i>	GF-3969	10 days	LDD ₁₀	0.82	µg GF-3969/bee/day
Honey bee	<i>Apis mellifera</i>	GF-3969	10 days	LDD ₂₀	1.28	µg GF-3969/bee/day
Honey bee	<i>Apis mellifera</i>	GF-3969	10 days	LDD ₅₀	2.98	µg GF-3969/bee/day
Honey bee	<i>Apis mellifera</i>	GF-3969	10 days	NOEDD	0.81	µg GF-3969/bee/day
Honey bee	<i>Apis mellifera</i>	GF-3969	10 days	LOEDD	1.6	µg GF-3969/bee/day
Honey bee	<i>Apis mellifera</i>	GF-3969	10 days	LC ₁₀	35.6	mg GF-3969/kg diet
Honey bee	<i>Apis mellifera</i>	GF-3969	10 days	LC ₂₀	59.9	mg GF-3969/kg diet
Honey bee	<i>Apis mellifera</i>	GF-3969	10 days	LC ₅₀	162	mg GF-3969/kg diet
Honey bee	<i>Apis mellifera</i>	GF-3969	10 days	NOEC	32	mg GF-3969/kg diet
Honey bee	<i>Apis mellifera</i>	GF-3969	10 days	LOEC	80	mg GF-3969/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 1, 20170301

Comments of zRMS:	<p>The study was submitted by the Applicant in order to address the toxicity of rimsulfuron to bee larvae. However, the study was not validated since GF-3969 contains more than one active substance and for this reason in order to fulfil the data requirements respective study with the formulated product should be provided for purposes of the zonal evaluation, while active substance endpoints should be generated in the course of the EU renewal process.</p> <p>The study summary is struck through and shaded as being not evaluated at the zonal level.</p>
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Reference:	KCP 10.3.1.3/01
Report:	Cornement, M., (2018); Rimsulfuron-toxicity to Honey bees (<i>Apis mellifera</i> L.) larvae after repeated exposure under <i>In Vitro</i> laboratory conditions
DuPont Report No.:	20170301
Testing Facility Report No.:	20170301
Guidelines	OECD 239 (2016)
Deviations:	None
GLP:	Yes
Acceptability:	Not evaluated, not relevant for the zonal assessment of GF-3969

EXECUTIVE SUMMARY

The purpose of this study was to determine the chronic toxicity of rimsulfuron after repeated exposure to honey bee larvae (*A. mellifera* L.) followed by observations of mortality and adult emergence for a period of 22 days. Mortality of the larvae, developmental effects and emergence of adults were used as the toxic endpoints.

Nominal dietary concentrations (mg a.s./kg diet) and nominal larval dosages (μg a.s./larva) were 0.0043, 0.013, 0.039, 0.012 and 0.35 mg a.s./kg diet and 0.61, 1.8, 5.5, 16 and 49 μg a.s./larva. Mean measured dietary concentrations and larval dosages were 0.0019, 0.0071, 0.017, 0.056 and 0.23 mg a.s./kg diet and 0.26, 1.0, 2.4, 7.8 and 32 μg a.s./larva. Analytical determination of samples taken on the days of application from stock solutions ranged from 98–127% of nominal and treated diets resulted in recoveries of 39 to 67% of the nominal value.

The LD₅₀ (8 day) was >32 μg a.s./larva, corresponding to 0.23 mg a.s./kg diet.

The NOED (22 day) was 32 μg a.s./larva, corresponding to 0.23 mg a.s./kg diet.

The study met the performance criteria established by the protocol including control mortality of larvae on Day 8 of <15% averaged across replicates, control adult emergence on Day 22 of >70%, positive control responses in the dimethoate treatment of >50% larval mortality on Day 8 and in the fenoxycarb treatment of <20% emergence on Day 22.

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	Rimsulfuron
Purity:	98%
Description (physical state):	White, solid
Lot/batch no.:	20130126

Test System

Organism (<i>Species</i>):	Honey bee (<i>Apis mellifera</i>)
Study type:	Chronic Larval—repeated exposure
Study design:	Dose response test; duration 22 days; 3 replicates, each starting with 16 synchronized 1 st 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 from D4 to D9 prior to transfer to pupal incubation plate. Monitoring of pupal development and adult emergence (eclosion) until Day 22.
Test concentrations:	0 (control), 0.61, 1.8, 5.5, 16 and 49 μg a.s./larva equivalent to 0, 0.0043, 0.013, 0.039, 0.012 and 0.35 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 4 weeks prior to study initiation.
Analytical verification:	

Feeding method:	<p>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> <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:	<p>Temperature: 32.6–36.1°C Relative Humidity: 34.1–92.5% Photoperiod: The climate cabinet was kept dark.</p>
Reference toxicant:	<p>Dimethoate: 7.4 µg /larva, 48 mg/kg diet</p>

Methodology

1st instar larvae were grafted from combs from three different colonies on D1 and kept inside a desiccator in a climate cabinet. They were fed with the test item incorporated in the larval diet on D3, D4, D5 and D6 for repeated exposure. On D8 the grafting cells containing the larvae were transferred to a new sterile plate and relocated in a different desiccator with temperature and relative humidity conditions adapted for pupation. On D15 the plates were transferred into emergence boxes and put into a second climate cabinet with conditions adapted for adult emergence. Mortality was assessed at the time of feeding on D4, D5, D6, D7 and on D8 respectively. Immobile larvae were noted as dead. Dead larvae were removed and observed abnormal behaviour was recorded. Food consumption was recorded qualitatively from D4 to D8. Pupal mortality was assessed on D11, D13 and D15 and from D18 to D22. Adult emergence was assessed from D18 to D22. The test was terminated on D22. The mortality in the treatments was corrected for solvent control mortality using Abbots with improvements by Schneider Orelli. As the solvent control was not statistically significantly different from the control, the treatment values were compared to the solvent control. To determine the effect values, Fisher's Exact Binomial test with Bonferroni Correction (one sided greater $\alpha = 0.05$) was performed on mortality on D8, pupal mortality on D15 and D22 as well as on adult emergence on D22.

Duplicate samples of 1000 µL were taken from the controls and from all stock and freshly prepared feeding solutions on D3 and D6. The concentration of rimsulfuron in the test samples was determined by HPLC MS/MS using external calibration.

RESULTS AND DISCUSSION

The study is considered valid as the following validity criteria were met:

- In the controls, larval mortality from D3 to D8 was $\leq 15\%$ across replicates (actual control 2.1%, solvent control 0%)
- In the controls adult emergence rate was $\geq 70\%$ on D22 (actual control 85%, solvent control 81%)
- In the reference item treatment (positive control), larval mortality was $\geq 50\%$ on D8 (reference 98%)

Analytical determination of rimsulfuron in the stock solutions on D3 and D6 ranged from 98% to 117% of the nominal values in all treatments except for the 0.056 mg/kg treatment which was confirmed to be above the 80-120% range at 127%. The recoveries in the feeding solutions immediately after preparation ranged from 71-97%. The concentrations in feeding solutions sampled after being fed to larvae ranged from 39-67%. The treatment doses were therefore recalculated using mean measured values.

On D8 the mean percentage of uneaten food ranged from 6.3-23% without a clear dose response relationship.

No statistically significant effects on larval mortality were reported for any of the treatment groups compared to the control groups.

The D8 LD₅₀ and NOED were therefore calculated to be >32 µg a.s./larva equivalent to 0.23 mg a.s./kg diet, the highest dose tested.

No statistically significant effects on pupal mortality were reported for any of the treatment groups compared to the control groups.

The D15 and D22 LD₅₀ and NOED were therefore calculated to be >32 µg a.s./larva equivalent to 0.23 mg a.s./kg diet, the highest dose tested.

No statistically significant effects on adult emergence were reported for any of the treatment groups compared to the control groups.

The D22 adult emergence LD₅₀ and NOED were therefore calculated to be >32 µg a.s./larva equivalent to 0.23 mg a.s./kg diet, the highest dose tested.

Table A 23: Toxicity of rimsulfuron to honey bee larvae in a chronic exposure toxicity test

Mean Measured Treatment		Chronic larval exposure toxicity			Adult emergence rate
µg/larva	mg/kg	Mortality (%)			(%)
		Day 3-8	Day 8-15	Day 8-22	
Control (0)		2.1	6.3	13	85
Solvent control (0)		0.0	10	19	81
0.27	0.0019	8.3	22	25	71
1.0	0.0071	0.0	25	25	75
2.4	0.017	4.2	18	22	75
7.8	0.056	4.2	22	24	73
32	0.23	0.0	15	15	85
Reference item (7.4 µg dimethoate/larva)		98	100	100	0
8 day LD ₅₀ , mean measured treatment		>32 µg a.s./larva equivalent to 0.23 mg a.s./kg diet			
22 day NOED, mean measured treatment		32 µg a.s./larva equivalent to 0.23 mg a.s./kg diet			

Table A 24: Uneaten food, developmental and behavioural effects in the chronic exposure larval toxicity test for rimsulfuron

Mean Measured Treatment		Uneaten food observed on Day 8 (%)	Behavioural effects (day)	Developmental effects (day)
µg/larva	mg/kg			
Control (0)		6.3	None	None
Solvent control (0)		8.3	None	None
0.27	0.0019	12	None	None
1.0	0.0071	23	None	None
2.4	0.017	14	None	None
7.8	0.056	6.7	None	None
32	0.23	6.3	None	None
Reference item (7.4 µg dimethoate/larva)		100	-	-

CONCLUSION

No statistically significant effects on mortality adult emergence were reported for any of the treatment groups compared to the control groups.

The D8, D15 and D22 mortality and D22 adult emergence, LD₅₀ and NOED were therefore determined to be >32 µg a.s./larva equivalent to 0.23 mg a.s./kg diet, the highest dose tested.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Honey-bee	<i>Apis mellifera</i>	Rimsulfuron	8-day	LD ₅₀	>32 µg a.s./larva	µg/larva, mean measured
Honey-bee	<i>Apis mellifera</i>	Rimsulfuron	8-day	LC ₅₀	>0.23 mg a.s./kg diet	mg/kg, mean measured
Honey-bee	<i>Apis mellifera</i>	Rimsulfuron	22-day	NOED	32 µg a.s./larva	µg/larva, mean measured
Honey-bee	<i>Apis mellifera</i>	Rimsulfuron	22-day	NOEC	0.23 mg a.s./kg diet	mg/kg, mean measured

A 2.3.1.3.2 Study 2, 200438

<p>Comments of zRMS:</p>	<p>The study was performed in line with OECD 239 with following deviations:</p> <ol style="list-style-type: none"> 1. The average relative humidity of 78% during pupation was slightly below 80% recommended by the test guideline. It should be noted that the average humidity was calculated including the fluctuations observed during opening of desiccator, when drop in humidity and temperature is unavoidable. Observed deviation is considered to have no impact on the test results since all validity criteria were met. 2. The water content in the larval diet was modified in line with indications of Schmehl et al. (2016)¹⁴. Schmehl et al. (2016) investigated modifications of the larval diet since current rearing protocols have had variable success with immature bees survival and protocol repeatability. Based on performed trials a modified diet has been established resulting with high survival (>95%) in control and solvent-control groups. Details of the performed trials may be found in the publication. The zRMS is of the opinion that modification of the diet leading to increased survival success in control groups is considered to have no negative impact on the test results and may be thus accepted. <p>The measured concentrations of thifensulfuron-methyl in the larval diet were maintained at 80-120% of nominal. However, measured concentrations of rimsulfuron dropped below 80% and for this reason the endpoints must be expressed in terms of mean measured concentrations. Since only endpoints based on nominal concentrations were available in the study report, the Applicant was requested to calculate the endpoints in line with indications of Appendix J of EFSA Supporting publication 2019:EN-1673. Although method described in EFSA (2019) is recommended to be used for aquatic toxicity studies, the approach is relevant also for other media where the measured concentration of the test item is being determined.</p> <p>In addition to that it was noted that no endpoints for adult emergence were calculated by the study authors, although according to OECD 239, adult emergence is the primary parameter for derivation of the endpoints. Although visual inspection of the results of the below summarised study indicated that the NOED for adult emergence would be at the same level as NOED for mortality, the ECx values would be different. Taking this into account, the Applicant was requested to provide respective endpoints calculated with consideration of effects of GF-3969 on adult emergence.</p> <p>In the position paper with recalculations the Applicant indicated that the mean measured concentrations of rimsulfuron dropped below 80% of nominal only on day 5 and 6, while on days 3 and 4 were within 80-120% of nominal. The overall mean measured concentrations over the whole study period were calculated and these were >80% of nominal, indicating that in general, the endpoints could be expressed in term of the nominal concentrations. Results of the calculation of the overall mean measured concentrations for particular active compounds and the formulation (based on the sum of measured concentrations of active substances) are presented in tables below.</p>
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¹⁴ Daniel R Schmehl, Hudson V V Tomé, Ashley N Mortensen, Gustavo Ferreira Martins & James D Ellis (2016) Protocol for the *in vitro* rearing of honey bee (*Apis mellifera* L.) workers, Journal of Apicultural Research, 55:2, 113-129

Measured Isoxadifen-ethyl (mg a.i./kg)						
Nominal concentration (mg a.i./kg)	Day 3	Day 4	Day 5	Day 6	Geometric mean measured concentration (mg a.i./kg)	% of nominal based on a.i.
4.3	3.7	3.7	3.7	3.6	3.7	86
8.7	6.9	6.0	7.3	7.5	6.9	79
17.8	15.6	14.4	14.0	13.9	14.5	81
34.4	30.8	30.6	29.7	29.1	30.0	87
69.9	55.4	57.5	58.9	55.3	56.8	81
Measured Thifensulfuron-methyl (mg a.i./kg)						
Nominal concentration (mg a.i./kg)	Day 3	Day 4	Day 5	Day 6	Geometric mean measured concentration (mg a.i./kg)	% of nominal based on a.i.
3.6	3.3	3.8	3.5	3.4	3.5	97
7.2	6.5	6.8	6.8	6.6	6.7	93
14.8	13.8	13.5	13.2	13.6	13.5	91
29.7	26.7	28.3	27.5	27.6	28.0	97
59.3	56.2	56.9	58.2	54.2	56.4	97
Measured Rimsulfuron (mg a.i./kg)						
Nominal concentration (mg a.i./kg)	Day 3	Day 4	Day 5	Day 6	Geometric mean measured concentration (mg a.i./kg)	% of nominal based on a.i.
5.8	5.5	6.2	4.8	3.8	5.0	86
11.6	10.3	12.6	10.0	7.7	10.0	87
23.7	22.8	23.2	18.4	14.9	19.5	82
45.9	47.6	47.7	39.0	32.2	41.1	89
93.2	94.0	104.9	88.9	72.3	89.2	96

Sum of active ingredients (nominal mg a.i./kg)	Sum of active ingredients (measured mg a.i./kg)	Sum of actives % of nominal
13.7	12.2	89
27.4	23.6	86
56.3	47.5	84
109.0	99.1	91
221.4	202.3	91

The endpoints from the study were calculated by the Applicant based on the nominal and mean measured concentrations with consideration of effects on mortality and adult emergence.

Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment (ED20 and ED50 are not presented as being not used for ETR calculation):

Based on nominal concentrations:

20 day ED₁₀ = 16.0 µg product/larvae
 20 day NOEDD = 13.0 µg product/larvae

Based on mean measured concentrations:

20 day ED₁₀ = 13.48 µg product/larvae
 20 day NOEDD = 11.0 µg product/larvae

Reference:	KCP 10.3.1.3/02
Report:	Porch, J.R., Riles, B. (2021b); GF-3969 (DPX-V4B07) + DPX-KG691 (VIVOLT): A Chronic Larval Toxicity Study with the Honey Bee (<i>Apis mellifera</i>)
DAS Study No.:	200438
Testing Facility Report No.:	112H-130
Guidelines	OECD 239
Deviations:	Minor (see commenting box above)
GLP:	Yes
Acceptability:	Acceptable

EXECUTIVE SUMMARY

The purpose of this study was to determine the chronic toxicity of GF-3969 after repeated exposure to honey bee larvae (*A. mellifera* L.) followed by observations of mortality and adult emergence for a period of 22 days. Mortality of the larvae, developmental effects and emergence of adults were used as the toxic endpoints.

Nominal dietary concentrations were 39, 78, 160, 310 and 630 mg GF-3969/kg diet, and nominal larval dosages were 6.3, 13, 25, 50 and 100 µg GF-3969/bee.

The LD_{10,20,50} (22 day) were 16.0, 19.6 and 57.7 µg GF-3969/bee, respectively. The LC_{10, 20,50} (22 day) were 97.5, 122 and 359 mg GF-3969/kg diet, respectively.

The NOED and LOED (22 day) were 13 and 25 µg GF-3969/bee, respectively, and the NOEC and LOEC (22 day) were 78 and 160 mg GF-3969/kg diet, respectively.

The study met the performance criteria established by the protocol including control mortality of larvae on Day 8 of <15 % averaged across replicates, control adult emergence on Day 22 of >70 %, positive control responses in the dimethoate treatment of >50 % larval mortality on Day 8.

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-3969 (Isoxadifen Ethyl 50WG / Rimsulfuron 25SG / Thifensulfuron Methyl 50SG (DPX-V4B07) plus DPX-KG691
Purity:	11.11 % + 14.82 % + 9.26 %, respectively + adjuvant surfactant
Description (physical state):	Wettable granules (WG), soluble granules (SG) and adjuvant surfactant
Lot/batch no.:	GF-3966 [TSN315416], GF-3866 [TSN316738], GF-3968 [TSN315297], TSN401051

Test System

Organism (<i>Species</i>):	Honey bee (<i>Apis mellifera</i>)
Study type:	Chronic Larval – repeated exposure

Study design:	<p>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.</p> <p>Visual assessment of uneaten food at the end of Day 9 prior to transfer to pupal incubation plate.</p> <p>Monitoring of pupal development until adult emergence (eclosion).</p>
Test concentrations:	<p>0 (control), 6.3, 13, 25, 50 and 100 µg GF-3969/larva, equivalent to 0, 39, 78, 160, 310 and 630 mg GF-3969/kg diet.</p>
Information on bee colony (health etc):	<p>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.</p>
Analytical verification:	<p>The measured concentrations of isoxadifen-ethyl, rimsulfuron and thifensulfuron-methyl were determined using LC-MS/MS method.</p> <p>On days 3 and 6, three samples were collected from the lowest and highest levels. On days 3, 4, 5 and 6, a single sample was collected from all other test substance concentrations and the negative control. Samples were collected from stirring diets. Samples were not collected from the positive control diet.</p> <p>A duplicate set of samples, consisting of the same number and sample volume, was collected in order to provide back-up samples if needed for the verification of the GF-3969 (DPX-V4B07) + DPX-KG691 (VIVOLT) within the larval diet. Collected samples were stored in a freezer at ≤ -10°C.</p> <p>GF-3969 recoveries based on Isoxadifen-ethyl: 71 to 96 % of nominal</p> <p>GF-3969 recoveries based on Rimsulfuron: 58 to 91 % of nominal</p> <p>GF-3969 recoveries based on Thifensulfuron-methyl: 87 to 109 % of nominal</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 fresh royal jelly and yeast extract, glucose and fructose. The ratio of ingredients varied in the three diets. The feeding solutions were prepared as needed.

Diet A: 44.25 % weight of fresh royal jelly, 44.25 % weight of water, 0.90 % weight of yeast extract, 5.30 % weight of glucose and 5.30 % weight of fructose

Diet B: 42.95 % weight of fresh royal jelly, 42.95 % weight of water, 1.30 % weight of yeast extract, 6.40 % weight of glucose and 6.40 % weight of fructose

Diet C: 50 % weight of fresh royal jelly, 30 % weight of water, 2.0 % weight of yeast extract, 9.0 % weight of glucose and 9.0 % weight of fructose

Diets A and B (20 µL/larva, 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/larva. The test item was administered on Days 3, 4, 5 and 6 homogeneously dispersed in 20 to 50 µL/larva of diet B or C depending upon the day of incubation.

Environmental conditions:

Average Temperature: 34.5 °C

Relative Humidity: 96 % (larval stage), 78 % (pupal stage)

Photoperiod: The climate cabinet was kept dark.

Reference substance:

Dimethoate: 7.39 µg /larva, 46 mg/kg diet

Methodology

The queen from each of 3 hives was confined in an excluder for 26 hours on an empty frame of drawn comb in order to provide a uniform age of larvae. After the egg-laying period, the queens were released and the frames were kept in the hive for approximately 75 hours, until the larvae hatched and were transferred to the laboratory.

In the laboratory, larvae were grafted into plastic cell cups containing artificial diet and placed in 48-well tissue culture plates and covered with lids. Larvae were incubated for approximately 48 hours, at which time the appropriate number of healthy larvae were selected for use in testing.

Test diets (dosed and un-dosed) were administered with a micropipette directly into the cells containing the larvae. All bees received untreated diets on Day 1 (the day of grafting). The test plates were randomly assigned to treatment levels before the dosing procedure. On Days 3, 4, 5 and 6, treated diets were placed next to larvae. Each well plate was identified by study number and treatment group. After dosing, the plates were randomly assigned to locations in the desiccator until the end of the test. Larvae were observed daily at the time of feeding.

After all diet had been consumed, living larvae were transferred to clean pupal plates until adult bees emerged. Cells containing dead larvae or pupae were removed from well plates after mortality was recorded. The test duration was 14 days after the end of dosing. Mortality was assessed at test termination.

RESULTS AND DISCUSSION

Mean mortality in the negative and positive control were 17 and 97 %, respectively. Mean mortality in the 6.3, 13, 25, 50 and 100 µg GF-3969/bee groups was 14, 11, 42, 47 and 94 %, respectively.

The LD_{10,20,50} (22 day) were 16.0, 19.6 and 57.7 µg GF-3969/bee, respectively. The LC_{10, 20,50} (22 day) were 97.5, 122 and 359 mg GF-3969/kg diet, respectively.

The NOED and LOED (22 day) were 13 and 25 µg GF-3969/bee, respectively, and the NOEC and LOEC (22 day) were 78 and 160 mg GF-3969/kg diet, respectively.

Table A 25: Toxicity of GF-3969 to honey bee larvae in a chronic exposure toxicity test

Nominal treatment		Chronic larval exposure toxicity		
µg/bee	mg/kg	Mortality (%)		
		Day 8	Day 15	Day 22
Control	0	11	14	17
6.3	39	11	14	14
13	78	11	11	11
25	160	31	36	42
50	310	31	47	47
100	630	94	94	94
Reference item (7.39 µg dimethoate/ larva)	46.3	97	97	97
LD₁₀ (95 % CI)		16.0 (n/a – 19.9) µg GF-3969/bee		
LD₂₀ (95 % CI)		19.6 (10.1 – 29.6) µg GF-3969/bee		
LD₅₀ (95 % CI)		57.7 (10.2 – 77.2) µg GF-3969/bee		
22-day NOED, nominal treatment		13 µg GF-3969/bee		
22-day LOED, nominal treatment		25 µg GF-3969/bee		
LC₁₀ (95 % CI)		975 (n/a – 119) mg GF-3969/kg diet		
LC₂₀ (95 % CI)		122 (46.3 – 179) mg GF-3969/kg diet		
LC₅₀ (95 % CI)		359 (54.2 – 462) mg GF-3969/kg diet		
22-day NOEC, nominal treatment		78 mg GF-3969/kg diet		
22-day LOEC, nominal treatment		160 mg GF-3969/kg diet		

The mean survival of larvae, pupae and adults as well as adult emergence are given in the table below.

Nominal Cumulative Doses	Larval Survival ¹	Pupal Survival ²	Adult Emergence ³	Adult Mortality ⁴
	(%) Day 8	(%) Day 20	(%) Day 20	(%) Day 20
Negative Control	89	94	83	17
6.3 µg/bee	89	97	86	14
13 µg/bee	89	100	89	11
25 µg/bee	69	84	58	42*
50 µg/bee	69	76	53	47*
100 µg/bee	6	100	6	94*
Positive Control (dimethoate, 7.39 µg a.i./bee)	3	100	3	97

¹ Number of living larvae on day 8 / Initial number of larvae * 100 (per treatment group)

² Number of emerged adults at test termination / Number of living larvae on day 8 * 100 (per treatment group)

³ Number of emerged adults at test termination / Initial number of larvae * 100 (per treatment group)

⁴ Number of dead bees at test termination / Initial number of larvae * 100 (per treatment group)

* Significant statistical differences compared to the negative control according to Williams Multiple Comparison test ($p < 0.05$).

Table A 26: Uneaten food, developmental and behavioural effects in the chronic exposure larval toxicity test for GF-3969

Nominal treatment		Chronic larval exposure toxicity	
µg/larva	mg/kg	Uneaten food observed on Day 8 (%)	Developmental effects (upon adult eclosion) (%)
Control	0	0	0
6.3	39	6	0
13	78	3	0
25	160	14	0
50	310	22	3
100	630	80	0
Reference item (7.39 µg dimethoate/ larva)	46.3	88	0

The validity criteria for the test were met:

1. In the negative control plate(s), cumulative larval mortality (prior to pupal transfer) was less than 15 % across replicates (11 %).
2. In the negative control group, adult emergence was higher than 70 % on Day 19 of the test (83 %).
3. In the positive control (dimethoate) group, larval mortality was > 50 % by Day 8 (97 %).

CONCLUSION

The LD_{10,20,50} (22 day) were 16.0, 19.6 and 57.7 µg GF-3969/bee, respectively. The LC_{10, 20,50} (22 day) were 97.5, 122 and 359 mg GF-3969/kg diet, respectively. The NOED and LOED (22 day) were 13 and 25 µg GF-3969/bee, respectively, and the NOEC and LOEC (22 day) were 78 and 160 mg GF-3969/kg diet, respectively.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Honey bee	<i>Apis mellifera</i>	GF-3969	22 day	LD ₁₀	16.0	µg GF-3969/bee
Honey bee	<i>Apis mellifera</i>	GF-3969	22 day	LD ₂₀	19.6	µg GF-3969/bee
Honey bee	<i>Apis mellifera</i>	GF-3969	22 day	LD ₅₀	57.7	µg GF-3969/bee
Honey bee	<i>Apis mellifera</i>	GF-3969	22 day	LC ₁₀	97.5	mg GF-3969/kg diet
Honey bee	<i>Apis mellifera</i>	GF-3969	22 day	LC ₂₀	122	mg GF-3969/kg diet
Honey bee	<i>Apis mellifera</i>	GF-3969	22 day	LC ₅₀	359	mg GF-3969/kg diet
Honey bee	<i>Apis mellifera</i>	GF-3969	22 day	NOED	13	µg GF-3969/bee
Honey bee	<i>Apis mellifera</i>	GF-3969	22 day	LOED	25	µg GF-3969/bee
Honey bee	<i>Apis mellifera</i>	GF-3969	22 day	NOEC	78	mg GF-3969/kg diet
Honey bee	<i>Apis mellifera</i>	GF-3969	22 day	LOED	160	mg GF-3969/kg diet

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

No new or additional studies have been submitted.

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

No new or additional studies have been submitted.

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

No new or additional studies have been submitted.

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 1, DuPont-49935

Comments of zRMS:	<p>The study was performed in line with the respective guidelines with no deviations.</p> <p>All the validity criteria were met:</p> <ul style="list-style-type: none"> - in the control the arithmetic mean mortality should not exceed 20 % (actual was 16.7 %), - in the control the cumulative mean number of eggs per female should be ≥ 4 (actual was 7.8 eggs/female), - in the toxic reference treatment the cumulative mean corrected mortality should be between 50 and 100 % (actual was 100 %). <p>Overall the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>LR₅₀ > 135 g product/ha</p>
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Reference:	KCP 10.3.2.1/04
Report:	Moll, M., (2018); Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) plus DPX-KG691 surfactant: A laboratory rate-response test to evaluate the effects on the predatory mite <i>Typhlodromus pyri</i> (Acari, Phytoseiidae)
DuPont Report No.:	DuPont-49935
Testing Facility Report No.:	128711063
Guidelines	Blumel <i>et al.</i> 2000, Candolfi <i>et al.</i> 2001
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable

EXECUTIVE SUMMARY

An acute 7-day toxicity study, on the predatory mite, *Typhlodromus pyri*, was conducted in the laboratory according to Blümel *et al.* 2000 and Candolfi *et al.* 2001. The test organisms were exposed for 7 days to an untreated control and to fresh dried residues of GF-3969 plus DPX-KG691 adjuvant surfactant applied to glass plates at five nominal concentrations of 8.4375, 16.875, 33.75, 67.5 and 135 g GF-3969 plus 6.25, 12.5, 25, 50 and 100 mL DPX-KG691/ha. A toxic reference (Perfekthion (dimethoate a.s.)) was included with the test. The 7-day LR₅₀ for *Typhlodromus pyri* based on corrected mortality and nominal concentrations was greater than 135 g GF-3969/ha (equivalent to 20 g rimsulfuron + 12.5 g thifensulfuron methyl/ha, based on the nominal content of a.s.) plus 100 mL DPX-KG691/ha.

The ER₅₀ for *Typhlodromus pyri* based on reduction in reproduction (reproductive effects) could not be determined, because the effect on reproduction was always below 50% up to and including 135 g GF-3969 plus 100 mL DPX-KG691/ha compared to the control.

MATERIALS AND METHODS

Test Item(s)

Test item (Common name): GF-3969 (DPX-V4B07 24.08WG, DPX-V4B07-001), blend of three formulated components

Mixing Ratio of the Formulations: 59.26% DPX-E9636-227 25SG,

Nominal concentrations of active ingredients in the formulation: 18.52% DPX-M6316-323 50SG,
22.22% DPX-X4145-021 50WG
14.82% rimsulfuron active ingredient
9.26% thifensulfuron methyl active ingredient
11.11% isoxadifen ethyl safener

Formulation 1

Name: DPX-E9636-227 25SG
Batch #: MAR15EL004
Concentration of a.s.: 25.1% (w/w) rimsulfuron by analysis
CAS #: None for the formulation

Formulation 2

Name: DPX-M6316-323 50SG
Batch #: APR15EL002
Concentration of a.s.: 49.8% (w/w) thifensulfuron methyl by analysis
CAS #: None for the formulation

Formulation 3

Name: DPX-X4145-021 50WG
Batch #: DEC15EL001
Concentration of a.s.: 50.4% (w/w) isoxadifen ethyl by analysis
CAS #: None for the formulation

Adjuvant surfactant

Name: DPX-KG691
Batch #: JAN15CE035

Mixing Ratio: 100 mL of DPX-KG691 adjuvant surfactant was mixed to every 32.5 g a.s./ha, i.e. 135 g DPX-V4B07 24.08WG/ha of the dry mixed product

Stability of test compounds: Not determined in the test system
Control: Deionized water
Test vehicle: Deionized water
Toxic reference: Perfekthion (dimethoate a.s.)

Test System

Organism (*Species*): Predatory Mite (*Typhlodromus pyri*)
Age at dosing: Protonymphs (not older than 24 hours)
Source: Katz Biotech AG, An der Birkenpfluhheide 10, D-15837 Baruth

Diet: Pine (*Pinus sp.*) and birch (*Betula sp.*) pollen (3:1)

Water: Tap water, *ad libitum*

Test chamber: Two slides (glass, 24 mm × 60 mm) side by side fixed by gluing small cover slides (glass, 20 mm x 20 mm) to both side-ends. A non-drying glue barrier was placed on the test unit to keep the mites on this test arena.

Environmental conditions
Temperature: Minimum: 25°C
Maximum: 25°C
Mean: 25°C
Relative humidity: Minimum: 72%
Maximum: 75%
Mean: 74%
Photoperiod: 16 hour light, 8 hour dark, photoperiod (320 to 430 lux)

Methodology

- In life initiated/completed:
04-December-2017 to 06-February-2018
- Experimental treatments
In an acute toxicity laboratory study, predatory mites of the species *Typhlodromus pyri* were exposed to GF-3969 plus DPX-KG691 **adjuvant** ~~surfactant~~. The test organisms were exposed for 7 days to an untreated control and to fresh dried residues of GF-3969 plus DPX-KG691 **adjuvant** ~~surfactant~~ applied to glass plates at five nominal concentrations of GF-3969 plus DPX-KG691 of 8.4375, 16.875, 33.75, 67.5 and 135 g GF-3969 plus 6.25, 12.5, 25, 50 and 100 mL DPX-KG691/ha in a volume equivalent to 200 L water/ha. Test item application rates were based on the results of a GLP range finding study and consultation with the Sponsor's Study Monitor. A toxic reference, Perfekthion (dimethoate a.s.), was included in the test (3.2 g dimethoate/ha, based on nominal content of a.s.). The test was comprised of three replicates of 20 protonymphs for each treatment rate, control, and toxic reference. Reproduction was assessed from day 7 to day 14 of exposure for test rates where the corrected mortality was <50%.
- Observations
Assessments for mortalities (cumulative sum of dead and missing organisms) were carried out 3 and 7 days after treatment.
Reproduction was assessed in concentrations where the corrected mortality was <50%. To assess effects on reproduction, the number of eggs and juveniles produced per female were evaluated on day 10, 13 and 14.
- Statistics
Mortality data were analysed for significance by using Bonferroni Chi²-2x2 Test for the test item and Fisher's Exact Test for the reference item, respectively (alpha = 0.05).
Reproduction data were analysed for significance by using Williams t-test (alpha = 0.05)

RESULTS AND DISCUSSION

Mortality in the control and toxic reference groups was 16.7% and 100.0% (corrected mortality), respectively. All validation criteria were met. The reproductive capacity of *Typhlodromus pyri* was tested at all dose rates. The results for mortality and reduction in reproduction (reproductive effects) of *Typhlodromus pyri* are given in the table below.

Table A 27: The effects on mortality and reproduction of *Typhlodromus pyri* exposed to fresh dried residues of GF-3969 plus DPX-KG691 **adjuvant** ~~surfactant~~ on glass plates in the laboratory

Nominal GF-3969 (g/ha) + DPX-KG691 (mL/ha) rate	7-day mortality (%)	Corrected mortality (%) ^a	Mean cumulative reproduction (R) [eggs/female]	Reduction in reproduction (%) ^b
Untreated control (0.0)	16.7	-	7.8	-
Toxic standard (3.2 g dimethoate/ha)	100.0*	100.0	n.d. ^c	n.d. ^c
8.4375 + 6.25	13.3	-4.0	8.2	-5.5
16.875 + 12.5	8.3	-10.0	7.3	5.8
33.75 + 25	28.3	14.0	6.3	18.8
67.5 + 50	8.3	-10.0	6.7	14.2
135 + 100	26.7	12.0	5.1*	34.6

a Schneider-Orelli's Correction; negative values indicate better survivorship compared to control

b Negative values indicate better performance compared to the control

c n.d. = not determined

* Significantly different from the control (mortality: Fisher's Exact Test, reproduction: Williams t-Test; alpha = 0.05)

CONCLUSION

Under worst case laboratory conditions, the LR₅₀ of GF-3969 plus DPX-KG691 **adjuvant** ~~surfactant~~ is estimated to be greater than 135 g GF-3969/ha (equivalent to 20 g rimsulfuron + 12.5 g thifensulfuron methyl/ha, based on the nominal content of a.s.) plus 100 mL DPX-KG691/ha.

Reproduction was tested at all dose rates. There was no statistically significant effect on reproduction up to and including 67.5 g GF-3969 plus 50 mL DPX-KG691/ha compared to the control. At 135 g GF-3969 plus 100 mL DPX-KG691/ha reproduction was statistically significantly affected compared to the control.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
predatory mite	<i>Typhlodromus pyri</i>	GF-3969 plus adjuvant surfactant DPX-KG691	Tier-1	LR ₅₀	>135	g product/ha

A 2.3.2.1.2 Study 2, DuPont-49934

Comments of zRMS:	<p>The study was performed in line with the respective guidelines with no deviations.</p> <p>All the validity criteria were met:</p> <ul style="list-style-type: none"> - mortality in the control should not exceed 13 % (actual was 0.0 %), - corrected mortality in the toxic reference treatment should be > 50 % (actual was 100 %), - wasps in the control should produce ≥ 5 mummies per female (mean value; actual was 23.1), - in the control there should be no more than 2 parasitoids producing zero values (actual no parasitoid produced zero values). <p>Overall the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>LR₅₀ > 135 g product/ha</p>
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Reference:	KCP 10.3.2.1/03
Report:	Moll, M., (2018); Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isoxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) plus DPX-KG691 Surfactant: A laboratory rate-response test to evaluate the effects on the parasitoid <i>Aphidius rhopalosiphi</i> (Hymenoptera, Braconidae)
DuPont Report No.:	DuPont-49934
Testing Facility Report No.:	128711001
Guidelines	Mead-Briggs <i>et al.</i> 2000, Mead-Briggs <i>et al.</i> 2010, Candolfi <i>et al.</i> 2001
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable

EXECUTIVE SUMMARY

An acute 48-hour toxicity study on the parasitic wasp *Aphidius rhopalosiphi* was conducted according to Mead-Briggs *et al.* 2000, Mead-Briggs *et al.* 2010 and Candolfi *et al.* 2001. The test organisms were exposed for 48 hours to an untreated control and to fresh dried residues of GF-3969 plus DPX-KG691 ~~adjuvant surfactant~~ applied to glass plates at five nominal concentrations of 8.4375, 16.875, 33.75, 67.5 and 135 g GF-3969 plus 6.25, 12.5, 25, 50 and 100 mL DPX-KG691/ha. A toxic reference (Perfekthion (dimethoate a.s.)) was included with the test. Following the 48-hour exposure period, reproductive effects (parasitism rate) were evaluated for test rates where the corrected mortality was less than or equal to 50%. The 48-hour LR₅₀ for *Aphidius rhopalosiphi* based on corrected mortality and nominal concentrations was greater than 135 g GF-3969/ha (equivalent to 20 g rimsulfuron + 12.5 g thifensulfuron methyl/ha, based on the nominal content of a.s.) plus 100 mL DPX-KG691/ha. The ER₅₀ for *Aphidius rhopalosiphi* based on reduction in parasitisation rate (reproductive effects) could

not be determined, because the effect on reproduction was always below 50% up to and including 135 g GF-3969 plus 100 mL DPX-KG691/ha compared to the control.

MATERIALS AND METHODS

Test Item(s)

Test item (Common name): GF-3969 (DPX-V4B07 24.08WG, DPX-V4B07-001), blend of three formulated components

Mixing Ratio of the Formulations: 59.26% DPX-E9636-227 25SG,
18.52% DPX-M6316-323 50SG,
22.22% DPX-X4145-021 50WG

Nominal concentrations of active ingredients in the formulation: 14.82% rimsulfuron active ingredient
9.26% thifensulfuron methyl active ingredient
11.11% isoxadifen ethyl safener

Formulation 1

Name: DPX-E9636-227 25SG
Batch #: MAR15EL004
Concentration of a.s.: 25.1% (w/w) rimsulfuron by analysis
CAS #: None for the formulation

Formulation 2

Name: DPX-M6316-323 50SG
Batch #: APR15EL002
Concentration of a.s.: 49.8% (w/w) thifensulfuron methyl by analysis
CAS #: None for the formulation

Formulation 3

Name: DPX-X4145-021 50WG
Batch #: DEC15EL001
Concentration of a.s.: 50.4% (w/w) isoxadifen-ethyl by analysis
CAS #: None for the formulation

Adjuvant surfactant

Name: DPX-KG691
Batch #: JAN15CE035
Mixing Ratio: 100 mL of DPX-KG691 adjuvant surfactant was mixed to every 32.5 g a.s./ha, i.e. 135 g DPX-V4B07 24.08WG/ha of the dry mixed product

Stability of test compounds: Not determined in the test system
Control: Deionized water
Test vehicle: Deionized water
Toxic reference: Perfekthion (dimethoate a.s.)

Test System

Organism (*Species*): Parasitoid (*Aphidius rhopalosiphi*)
Age at dosing: Adults (not older than 48 hours)
Source: Katz Biotech AG, An der Birkenpfehlheide 10, D-15837 Baruth
Diet: A solution of fructose (10%)
Water: See diet

Test chamber:	Mortality: Cages built of two treated glass plates (13 cm × 13 cm) which were held apart by an untreated aluminum frame (13 cm × 1.5 cm × 1 cm per side) Reproduction: Untreated pots with barley seedlings infested with host aphids (<i>Rhopalosiphum padi</i>). The plants (14 - 26 seedlings, 10 days old) were enclosed within a clear polyacrylic cylinder (30 cm high and 10 cm in diameter) with a fine mesh gauze on the top.
Environmental conditions	Temperature: 18 °C to 22 °C, mean = 20 °C Relative humidity: Acclimatization, exposure: 72% - 77%, mean = 75% Post-exposure period, within the test units: 67% - 79%, mean = 73% Light intensity: Acclimatization, exposure, parasitisation period: 890 - 1200 lux Post-parasitisation period: 10270 - 12460 lux

Methodology

1. In life initiated/completed:
04-December-2017 to 06-February-2018
2. Experimental treatments
In an acute toxicity laboratory study, parasitic wasps of the species *Aphidius rhopalosiphii* were exposed to GF-3969 plus DPX-KG691 **adjuvant surfactant**. The test organisms were exposed for 48-hours to an untreated control and to fresh dried residues of GF-3969 plus DPX-KG691 **adjuvant surfactant** applied to glass plates at five nominal concentrations of 8.4375, 16.875, 33.75, 67.5 and 135 g GF-3969 plus 6.25, 12.5, 25, 50 and 100 mL DPX-KG691/ha in a volume equivalent to 200 L water/ha. Test item application rates were based on the results of a GLP range finding study and consultation with the Sponsor's Study Monitor. A toxic reference, Perfekthion (dimethoate a.s.), was included in the test (0.12 g dimethoate/ha, based on nominal content of a.s.). The test was comprised of four replicates of ten adult parasitoids (7 females and 3 males) for each treatment rate, control, and toxic reference.
After the 48-hour exposure period, reproduction (parasitisation rate) was evaluated by transferring living female wasps from treatments with corrected mortality ≤ 50% to individual test chambers containing 14 to 26 barley seedlings infested with adult and nymphal aphids (*Rhopalosiphum padi*). Each treatment level was comprised of maximum 20 replicates of single female wasps. After a 24-hour parasitisation period the wasps were discarded and the plants and aphids (parasitized) were held for an additional 10 - 11 days. At this time the number of parasitised aphids (aphid mummies) per female wasp was determined (n = 17 - 20).
3. Observations
Assessments for adult wasp mortality and behavioural abnormalities were carried out approximately 2, 24 and 48 hours after treatment.
Reproduction was assessed 10 - 11 days after the parasitisation period.
4. Statistics
Mortality data were analysed for significance by using Bonferroni-Holm Fisher's Exact Test for the test item and Fisher's Exact Test for the reference item, respectively (alpha = 0.05).
Reproduction data were analysed for significance by using Williams t-test (alpha = 0.05)

RESULTS AND DISCUSSION

Adult mortality in the control and toxic standard groups was 0.0% and 100%, respectively. All validation criteria were met. The reproductive capacity of *Aphidius rhopalosiphii* was tested at all dose

rates. The results for mortality and reduction in reproduction (parasitisation rate) of *Aphidius rhopalosiphi* are given in the table below.

Table A 28: The effects on mortality and reproduction of *Aphidius rhopalosiphi*, exposed to fresh dried residue of GF-3969 plus DPX-KG691 adjuvant surfactant under worst-case laboratory conditions

Nominal GF-3969 (g/ha) + DPX-KG691 (mL/ha) rate	48-hour mortality (%)	Corrected mortality (%) ^a	Parasitised aphid/female (mean)	Reduction in reproduction (%) ^b
Untreated control (0.0)	0.0	-	23.1	-
Toxic standard (0.12 g dimethoate/ha)	100.0*	100.0	n.d. ^c	n.d. ^c
8.4375 + 6.25	0.0	0.0	25.3	-9.7
16.875 + 12.5	2.5	2.5	41.3	-79.2
33.75 + 25	2.5	2.5	32.7	-42.0
67.5 + 50	12.5	12.5	39.8	-72.6
135 + 100	12.5	12.5	40.9	-77.6

a Schneider-Orelli's Correction

b Negative values indicate better performance compared to the control

c n.d. = not determined

* Significantly different from the control (mortality: Fisher's Exact Test; alpha = 0.05)

CONCLUSION

Under worst case laboratory conditions the LR₅₀ of GF-3969 plus DPX-KG691 adjuvant surfactant is estimated to be greater than 135 g GF-3969/ha (equivalent to 20 g rimsulfuron + 12.5 g thifensulfuron methyl/ha, based on the nominal content of a.s.) plus 100 mL DPX-KG691/ha.

The reproductive capacity of *A. rhopalosiphi* was tested at all dose rates. There were no effects on reproduction up to and including 135 g GF-3969 plus 100 mL DPX-KG691/ha compared to the control.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
parasitic wasp	<i>Aphidius rhopalosiphi</i>	GF-3969 plus adjuvant surfactant DPX-KG691	Tier-1	LR ₅₀	>135	g product/ha

A 2.3.2.1.3 Study 3, DuPont-49973

Comments of zRMS:	<p>The study was performed in line with the respective guidelines with no major deviations.</p> <p>It was noted that the sex ratio for reproduction testing was not achieved in one of the treatment groups. However, as the test item had no effect on the reproduction of the tested organisms this deviation is considered to have no impact on the outcome of the study.</p> <p>All the validity criteria were met:</p> <ul style="list-style-type: none"> - in the control the arithmetic mean mortality should not exceed 20 % (actual was 11.7 %), - in the control the cumulative mean number of eggs per female should be ≥ 4 (actual was 6.9 eggs/female), - in the toxic reference treatment the cumulative mean corrected mortality should be between 50 and 100 % (actual was 96.2 %). <p>Overall the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>LR₅₀ > 135 g product/ha</p>
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Reference:	KCP 10.3.2.1/02
Report:	Moll, M., (2018); Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) plus codacide: A laboratory rate-response test to evaluate the effects on the predatory mite, <i>Typhlodromus pyri</i> (Acari, Phytoseiidae)
DuPont Report No.:	DuPont-49973
Testing Facility Report No.:	128721063
Guidelines	Blumel <i>et al.</i> 2000, Candolfi <i>et al.</i> 2001
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable

EXECUTIVE SUMMARY

An acute 7-day toxicity study, on the predatory mite, *Typhlodromus pyri*, was conducted in the laboratory according to Blümel *et al.* 2000 and Candolfi *et al.* 2001. The test organisms were exposed for 7 days to an untreated control and to fresh dried residues of GF-3969 plus Codacide applied to glass plates at five nominal concentrations of 8.4375, 16.875, 33.75, 67.5 and 135 g GF-3969 plus Codacide 6.25, 12.5, 25, 50 and 100 mL product/ha. A toxic reference (Perfekthion (dimethoate a.s.)) was included with the test. The 7-day LR₅₀ for *Typhlodromus pyri* based on corrected mortality and nominal concentrations was greater than 135 g GF-3969/ha (equivalent to 20 g rimsulfuron + 12.5 g thifensulfuron methyl/ha, based on the nominal content of a.s.) plus 100 mL Codacide/ha.

The ER₅₀ for *Typhlodromus pyri* based on reduction in reproduction (reproductive effects) could not be determined, because the effect on reproduction was always below 50% up to and including 135 g GF-3969 plus 100 mL Codacide/ha compared to the control.

MATERIALS AND METHODS

Test Item(s)

Test item (Common name): GF-3969 (DPX-V4B07 24.08WG, DPX-V4B07-001) , blend of three formulated components

Mixing Ratio of the Formulations: 59.26% DPX-E9636-227 25SG,
18.52% DPX-M6316-323 50SG,
22.22% DPX-X4145-021 50WG

Nominal concentrations of active ingredients in the formulation: 14.82% rimsulfuron active ingredient
9.26% thifensulfuron methyl active ingredient
11.11% isoxadifen ethyl safener

Formulation 1

Name: DPX-E9636-227 25SG
Batch #: MAR15EL004
Concentration of a.s.: 25.1% (w/w) rimsulfuron by analysis
CAS #: None for the formulation

Formulation 2

Name: DPX-M6316-323 50SG
Batch #: APR15EL002
Concentration of a.s.: 49.8% (w/w) thifensulfuron methyl by analysis
CAS #: None for the formulation

Formulation 3

Name: DPX-X4145-021 50WG
Batch #: DEC15EL001
Concentration of a.s.: 50.4% (w/w) isoxadifen ethyl by analysis
CAS #: None for the formulation

Adjuvant surfactant

Name:	Codacide
Batch #:	FEB16CE020
Mixing Ratio	100 mL of Codacide will be mixed to every 32.5 g a.s./ha, i.e. 135 g DPX-V4B07 24.08WG/ha of the dry mixed product
Stability of test compounds:	Not determined in the test system
Control:	Deionized water
Test vehicle:	Deionized water
Toxic reference:	Perfekthion (dimethoate a.s.)

Test System

Organism (<i>Species</i>):	Predatory mite (<i>Typhlodromus pyri</i>)
Age at dosing:	Protonymphs (not older than 24 hours)
Source:	Katz Biotech AG, An der Birkenpfehlheide 10, D-15837 Baruth
Diet:	Pine (<i>Pinus sp.</i>) and birch (<i>Betula sp.</i>) pollen (3:1)
Water:	Tap water, <i>ad libitum</i>
Test chamber:	Two slides (glass, 24 mm × 60 mm) side by side fixed by gluing small cover slides (glass, 20 mm × 20 mm) to both side-ends. A non-drying glue barrier was placed on the test unit to keep the mites on this test arena.
Environmental conditions	Temperature: Minimum: 25 °C Maximum: 25 °C Mean: 25 °C Relative humidity: Minimum: 72% Maximum: 74% Mean: 73% Photoperiod: 16-hour light, 8-hour dark, photoperiod (320 to 420 lux)

Methodology

1. In life initiated/completed:
27-November-2017 to 23-January-2018
2. Experimental treatments
In an acute toxicity laboratory study, predatory mites of the species *Typhlodromus pyri* were exposed to GF-3969 plus Codacide. The test organisms were exposed for 7 days to an untreated control and to fresh dried residues of GF-3969 plus Codacide applied to glass plates at five nominal concentrations of GF-3969 plus Codacide of 8.4375, 16.875, 33.75, 67.5 and 135 g GF-3969 plus Codacide 6.25, 12.5, 25, 50 and 100 mL product/ha in a volume equivalent to 200 L water/ha. Test item application rates were based on the results of a GLP range finding study and consultation with the Sponsor's Study Monitor. A toxic reference, Perfekthion (dimethoate a.s.), was included in the test (3.2 g dimethoate/ha, based on nominal content of a.s.). The test was comprised of three replicates of 20 protonymphs for each treatment rate, control, and toxic reference. Reproduction was assessed from day 7 to day 14 of exposure for test rates where the corrected mortality was <50%.
3. Observations
Assessments for mortalities (cumulative sum of dead and missing organisms) were carried out 2 and 7 days after treatment.
Reproduction was assessed in concentrations where the corrected mortality was <50%. To assess effects on reproduction, the number of eggs and juveniles produced per female were evaluated on day 10, 13 and 14.
4. Statistics
Mortality data were analysed for significance by using Bonferroni Chi²-2x2 Test for the test item and Fisher's Exact Test for the reference item, respectively (alpha = 0.05).

Reproduction data were analysed for significance by using Dunnett's t-test ($\alpha = 0.05$)

RESULTS AND DISCUSSION

Mortality in the control and toxic reference groups was 11.7% and 96.2% (corrected mortality), respectively. All validation criteria were met. The reproductive capacity of *Typhlodromus pyri* was tested at all dose rates. The results for mortality and reduction in reproduction (reproductive effects) of *Typhlodromus pyri* are given in the table below.

Table A 29: The effects on mortality and reproduction of *Typhlodromus pyri* exposed to fresh dried residues of GF-3969 plus Codacide on glass plates in the laboratory

Nominal GF-3969 (g/ha) + Codacide (mL/ha) rate	7-day mortality (%)	Corrected mortality (%) ^a	Mean cumulative reproduction (R) [eggs/female]	Reduction in reproduction (%) ^b
Untreated control (0.0)	11.7	-	6.9	-
Toxic standard (3.2 g dimethoate/ha)	96.7*	96.2	n.d. ^c	n.d. ^c
8.4375 + 6.25	1.7	-11.3	7.6	-9.6
16.875 + 12.5	11.7	0.0	9.3	-34.6
33.75 + 25	6.7	-5.7	7.7	-12.0
67.5 + 50	11.7	0.0	7.6	-10.5
135 + 100	11.7	0.0	7.4	-7.9

a Schneider-Orelli's Correction; negative values indicate better survivorship compared to control

b Negative values indicate better performance compared to the control

c n.d. = not determined

* Significantly different from the control (mortality: Fisher's Exact Test; $\alpha = 0.05$)

CONCLUSION

Under worst case laboratory conditions, the LR₅₀ of GF-3969 plus Codacide is estimated to be greater than 135 g GF-3969/ha (equivalent to 20 g rimsulfuron + 12.5 g thifensulfuron methyl/ha, based on the nominal content of a.s.) plus 100 mL Codacide/ha.

The reproduction of *T. pyri* was tested at all dose rates. There were no effects on reproduction up to and including 135 g GF-3969 plus 100 mL Codacide/ha compared to the control.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
<i>Typhlodromus pyri</i>	predatory mite	GF-3969 plus adjuvant surfactant Codacide	Tier-1	LR ₅₀	>135	g product/ha

A 2.3.2.1.4 Study 4, DuPont-49972

Comments of zRMS:	<p>The study was performed in line with the respective guidelines with no deviations.</p> <p>All the validity criteria were met:</p> <ul style="list-style-type: none"> - mortality in the control should not exceed 13 % (actual was 0.0 %), - corrected mortality in the toxic reference treatment should be > 50 % (actual was 100 %), - wasps in the control should produce ≥ 5 mummies per female (mean value; actual was 22.8), - in the control there should be no more than 2 parasitoids producing zero values (actual no parasitoid produced zero values). <p>Overall the study is considered acceptable with the following endpoint relevant for the risk assessment: LR₅₀ > 135 g product/ha</p>
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Reference:	KCP 10.3.2.1/01
Report:	Moll, M., (2018); Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) plus codacide: A laboratory rate-response test to evaluate the effects on the parasitoid <i>Aphidius rhopalosiphi</i> (Hymenoptera, Braconidae)
DuPont Report No.:	DuPont-49972
Testing Facility Report No.:	128721001
Guidelines	Mead-Briggs <i>et al.</i> 2000, Mead-Briggs <i>et al.</i> 2010, Candolfi <i>et al.</i> 2001
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable

EXECUTIVE SUMMARY

An acute 48-hour toxicity study on the parasitic wasp *Aphidius rhopalosiphi* was conducted according to Mead-Briggs *et al.* 2000, Mead-Briggs *et al.* 2010 and Candolfi *et al.* 2001. The test organisms were exposed for 48 hours to an untreated control and to fresh dried residues of GF-3969 plus Codacide applied to glass plates at five nominal concentrations of 8.4375, 16.875, 33.75, 67.5 and 135 g GF-3969 plus Codacide 6.25, 12.5, 25, 50 and 100 mL product/ha. A toxic reference (Perfekthion (dimethoate a.s.)) was included with the test. Following the 48-hour exposure period, reproductive effects (parasitism rate) were evaluated for test rates where the corrected mortality was less than or equal to 50%. The 48-hour LR₅₀ for *Aphidius rhopalosiphi* based on corrected mortality and nominal concentrations was greater than 135 g GF-3969/ha (equivalent to 20 g rimsulfuron + 12.5 g thifensulfuron methyl/ha, based on the nominal content of a.s.) plus 100 mL Codacide/ha. The ER₅₀ for *Aphidius rhopalosiphi* based on reduction in parasitisation rate (reproductive effects) could not be determined, because the effect on reproduction was always below 50% up to and including 135 g GF-3969 plus 100 mL Codacide/ha compared to the control.

MATERIALS AND METHODS

Test Item(s)

Test item (Common name): GF-3969 (DPX-V4B07 24.08WG, DPX-V4B07-001), blend of three formulated components

Nominal concentrations of active ingredients in the formulation: 14.82% rimsulfuron active ingredient
 9.26% thifensulfuron methyl active ingredient
 11.11% isoxadifen ethyl safener

Mixing Ratio of the Formulations: 59.26% DPX-E9636-227 25SG,
 18.52% DPX-M6316-323 50SG
 22.22% DPX-X4145-021 50WG

Formulation 1

Name: DPX-E9636-227 25SG
 Batch #: MAR15EL004
 Concentration of a.s.: 25.1% (w/w) rimsulfuron by analysis
 CAS #: None for the formulation

Formulation 2

Name: DPX-M6316-323 50SG
 Batch #: APR15EL002
 Concentration of a.s.: 49.8% (w/w) thifensulfuron methyl by analysis
 CAS #: None for the formulation

Formulation 3

Name: DPX-X4145-021 50WG
 Batch #: DEC15EL001
 Concentration of a.s.: 50.4% (w/w) isoxadifen ethyl by analysis
 CAS #: None for the formulation

Adjuvant surfactant

Name: Codacide
Batch #: FEB16CE020
Mixing Ratio: 100 mL of Codacide mixed to every 32.5 g a.s./ha, i.e. 135 g DPX-V4B07 24.08WG/ha of the dry mixed product
Stability of test compounds: Not determined in the test system
Control: Deionized water
Test vehicle: Deionized water
Toxic reference: Perfekthion (dimethoate a.s.)

Test System

Organism (*Species*): Parasitoid (*Aphidius rhopalosiphi*)
Age at dosing: Adults (not older than 48 hours)
Source: Katz Biotech AG, An der Birkenpfehlheide 10, D-15837 Baruth
Diet: A solution of fructose (10%)
Water: See diet
Test chamber: Mortality: Cages built of two treated glass plates (13 cm × 13 cm) which were held apart by an untreated aluminum frame (13 cm × 1.5 cm × 1 cm per side)
Reproduction: Untreated pots with barley seedlings infested with host aphids (*Rhopalosiphum padi*). The plants (15-26 seedlings, 9 days old) were enclosed within a clear polyacrylic cylinder (30 cm high and 10 cm in diameter) with fine mesh gauze on the top.
Environmental conditions: Temperature: 18°C to 21°C, mean = 20°C
Relative humidity: Acclimatization, exposure: 72% - 74%, mean = 73%
Post-exposure period, within the test units: 70% - 81%, mean = 76%
Light intensity: Acclimatization, exposure, parasitisation period: 830 - 1110 lux
Post-parasitisation period: 10330 - 12400 lux

Methodology

1. In life initiated/completed:
27-November-2017 to 23-January-2018
2. Experimental treatments
In an acute toxicity laboratory study, parasitic wasps of the species *Aphidius rhopalosiphi* were exposed to GF-3969 plus Codacide. The test organisms were exposed for 48-hours to an untreated control and to fresh dried residues of GF-3969 plus Codacide applied to glass plates at five nominal concentrations of 8.4375, 16.875, 33.75, 67.5 and 135 g GF-3969 plus Codacide 6.25, 12.5, 25, 50 and 100 mL product/ha in a volume equivalent to 200 L water/ha. Test item application rates were based on the results of a GLP range finding study and consultation with the Sponsor's Study Monitor. A toxic reference, Perfekthion (dimethoate a.s.), was included in the test (0.12 g dimethoate/ha, based on nominal content of a.s.). The test was comprised of four replicates of ten adult parasitoids (7 females and 3 males) for each treatment rate, control, and toxic reference.
After the 48-hour exposure period, reproduction (parasitisation rate) was evaluated by transferring living female wasps from treatments with corrected mortality ≤ 50% to individual test chambers containing 15 to 26 barley seedlings infested with adult and nymphal aphids (*Rhopalosiphum padi*). Each treatment level was comprised of maximum 20 replicates of single female wasps. After a 24-hour parasitisation period the wasps were discarded and the plants and aphids (parasitized) were held for an additional 10 - 11 days.

At this time, the number of parasitised aphids (aphid mummies) per female wasp was determined (n = 17 - 20).

3. Observations

Assessments for adult wasp mortality and behavioural abnormalities were carried out approximately 2, 24 and 48 hours after treatment.

Reproduction was assessed 10 - 11 days after the parasitisation period.

4. Statistics

Mortality data were analysed for significance by using Bonferroni-Holm Fisher's Exact Test for the test item and Fisher's Exact Test for the reference item, respectively (alpha = 0.05).

Reproduction data were analysed for significance by using Bonferroni-Holm U-test (alpha = 0.05)

RESULTS AND DISCUSSION

Adult mortality in the control and toxic standard groups was 0.0% and 100%, respectively. All validation criteria were met. The reproductive capacity of *Aphidius rhopalosiphi* was tested at all dose rates. The results for mortality and reduction in reproduction (parasitisation rate) of *Aphidius rhopalosiphi* are given in the table below.

Table A 30: The effects on mortality and reproduction of *Aphidius rhopalosiphi*, exposed to fresh dried residue of GF-3969 plus Codacide under worst-case laboratory conditions

Nominal GF-3969 (g/ha) + Codacide (mL/ha) rate	48-hour mortality (%)	Corrected mortality (%) ^a	Parasitised aphid/female (mean)	Reduction in reproduction (%) ^b
Untreated control (0.0)	0.0	-	22.8	-
Toxic standard (0.12 g dimethoate/ha)	100.0*	100.0	n.d. ^c	n.d. ^c
8.4375 + 6.25	0.0	0.0	38.4	-68.5
16.875 + 12.5	5.0	5.0	38.9	-70.9
33.75 + 25	0.0	0.0	34.7	-52.4
67.5 + 50	0.0	0.0	36.6	-60.9
135 + 100	0.0	0.0	39.2	-72.2

a Schneider-Orelli's Correction

b Negative values indicate better performance compared to the control

c n.d. = not determined

* Significantly different from the control (mortality: Fisher's Exact Test; alpha = 0.05)

CONCLUSION

Under worst case laboratory conditions, the LR₅₀ of GF-3969 plus Codacide is estimated to be greater than 135 g GF-3969/ha (equivalent to 20 g rimsulfuron + 12.5 g thifensulfuron methyl/ha, based on the nominal content of a.s.) plus 100 mL Codacide/ha.

The reproductive capacity of *A. rhopalosiphi* was tested at all dose rates. There were no effects on reproduction up to and including 135 g GF-3969 plus 100 mL Codacide/ha compared to the control.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
parasitic wasp	<i>Aphidius rhopalosiphi</i>	GF-3969 plus adjuvant surfactant Codacide	Tier-1	LR ₅₀	>135	g product/ha

A 2.3.2.2 KCP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods

No new or additional studies have been submitted.

A 2.3.2.3 KCP 10.3.2.3 Semi-field studies with non-target arthropods

No new or additional studies have been submitted.

A 2.3.2.4 KCP 10.3.2.4 Field studies with non-target arthropods

No new or additional studies have been submitted.

A 2.3.2.5 KCP 10.3.2.5 Other routes of exposure for non-target arthropods

No new or additional studies have been submitted.

A 2.4 KCP 10.4 Effects on non-target soil meso- and macrofauna

A 2.4.1 KCP 10.4.1 Earthworms

No new or additional studies have been submitted.

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

A 2.4.1.1.1 Study 1, DuPont-49950

Comments of zRMS:	<p>The study was performed in line with OECD 222 with no deviations.</p> <p>All the validity criteria were met:</p> <ul style="list-style-type: none"> - In the controls adult mortality over the initial 4 weeks should be $\leq 10\%$ (was 0%), - In the controls each replicate containing 10 adults should produce ≥ 30 juveniles by the end of the test (was 188 to 317 juveniles per replicate), - In the controls the coefficient of variation of reproduction should be $\leq 30\%$ (was 16.3%). <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, a meaningful ECx value could not be calculated.</p> <p>Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>56d NOEC (mortality, body weight) > 720 mg product/kg dw soil 56d NOEC (reproduction) = 123 mg product/kg dw soil</p>
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Reference:	KCP 10.4.1.1/01
Report:	Pavic, B., (2018); Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isoxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) plus DPX-KG691 surfactant: Effects on reproduction and growth of the earthworm, <i>Eisenia andrei</i> , in artificial soil
DuPont Report No.:	DuPont-49950
Testing Facility Report No.:	128711022
Guidelines	OECD 222 (2016), ISO 11268-2 (2012)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable

EXECUTIVE SUMMARY

The sublethal toxicity of GF-3969 to earthworms, *Eisenia andrei*, was determined in a 56-day soil exposure laboratory study according to OECD 222, 2016 and ISO 11268-2, 2012. Adult earthworms were exposed to artificial soil (prepared according to OECD 222) treated with the test item to obtain the nominal concentrations of 11.8, 21.2, 38.1, 68.6, 123, 222, 400, and 720 mg GF-3969 /kg dry artificial soil plus 8.74, 15.7, 28.2, 50.8, 91.1, 164, 296, and 533 mg DPX-KG691/kg soil dry artificial soil and to an untreated control (deionized water only).

Mortality and growth (body weight) of the earthworms were assessed after 28 days and the effect on reproduction (number of juveniles produced) was assessed after 56 days. The LC₅₀ after 28 days was estimated to be greater than 720 mg GF-3969 + 533 mg DPX-KG691/kg dry artificial soil. The NOEC (No-Observed-Effect Concentration) for earthworms based on mortality, growth and nominal concentrations was ≥ 720 mg GF-3969 + 533 mg DPX-KG691/kg dry artificial soil, the highest

concentration tested. The NOEC based on reproduction was 123 mg GF-3969 + 91.1 mg DPX-KG691/kg dry artificial soil.

MATERIALS AND METHODS

Test Item(s)

Test item (Common name): GF-3969 (DPX-V4B07 24.08WG, DPX-V4B07-001) , blend of three formulated components

Lot #: DPX-V4B07-001

Composition: DPX-V4B07-001 is a formulation containing nominal concentrations (w/w):
14.82% rimsulfuron active ingredient
9.26% thifensulfuron methyl active ingredient
11.11% isoxadifen ethyl safener

Mixing Ratio: DPX-V4B07-001 is prepared by blending

59.26% DPX-E9636-227 25SG

18.52% DPX-M6316-323 50SG

22.22% DPX-X4145-021 50WG

GF-3969 and the DPX-KG691 **adjuvant surfactant** were mixed in a ratio of 135 g product (32.5 g a.s.) to 100 mL DPX-KG691, that means 135 g product to 100 g DPX-KG691 which is corresponding to 1: 0.7407, *i.e.* to 1 g GF-3969 0.7407 g DPX-KG691 was added

Formulation 1

Name: DPX-E9636 25SG

Batch #: MAR15EL004

Concentration of a.s.: 25% (w/w) Rimsulfuron
25.1% (w/w) Rimsulfuron, by analysis

CAS #: 122931-48-0

Formulation 2

Name: DPX-M6316 50SG

Batch #: APR15EL002

Concentration of a.s.: 50% Thifensulfuron methyl
49.8% Thifensulfuron methyl, by analysis
79277-27-3 for thifensulfuron methyl active ingredient

Safener

Name: DPX-X4145 50WG

Batch #: DEC15EL001

Concentration of a.s.: 50% (w/w) Isoxadifen-ethyl
50.4% (w/w) Isoxadifen-ethyl, by analysis

CAS #: 163520-33-0 for isoxadifen-ethyl active ingredient

Adjuvant surfactant

Name: DPX-KG691

Batch #: JAN15CE035

Stability of test compound: Not analysed in the test system

Control: Untreated (and moistened with deionized water)

Test vehicle: Deionized water

Toxic reference: Carbendazim, tested at least once a year in the test facility to ensure that laboratory test conditions are adequate and to verify that the response of the test organisms does not change significantly over time. Recently performed test resulted with NOEC of 1.44 mg carbendazim/kg dws and EC₁₀ of 0.85 mg carbendazim/kg dws (95% CL: 0.69-1.06

mg carbendazim/ kg dws) Dimethoate

Test System

Organism (<i>Species</i>):	Earthworm (<i>Eisenia andrei</i>)
Age at dosing:	Approximately 8 months (all within 4 weeks of the same age), with well-developed clitella
Weight at dosing:	301 to 600 mg
Source:	In-house laboratory culture (Laboratory: ibacon GmbH, Rossdorf, Germany)
Acclimatization period:	1 day
Test chamber:	Plastic boxes with perforated transparent lids (volume: 1 L), filled with ca. 500 g artificial soil dry weight
Test medium:	Artificial soil prepared according to OECD 222, maximum water holding capacity of the artificial soil, as measured: 61%
Diet:	Finely ground cattle manure
Water content of soil:	Initiation: 33.2% to 34.3% (equivalent to 54.5% to 56.3% of the maximum water holding capacity) Termination: 33.4 to 34.8% (equivalent to 54.7% to 57.0% of the maximum water holding capacity)
Soil pH:	5.5 to 5.7 at test start and 6.2 to 6.4 at test termination
Environmental conditions	Temperature: Within the range of 18 to 22°C Photoperiod: 16 hour light, 8 hour dark, photoperiod within the range of 400 to 800 lux

Methodology

1. In-life initiated/completed:
13-FEB-2018 to 11-APR-2018
2. Experimental treatments
The sublethal toxicity of GF-3969 to earthworms, *Eisenia andrei*, was estimated in a 56-day soil exposure GLP-compliant laboratory study based on OECD 222 (2016) and ISO 11268, Part 2 (2012). Eight replicates for the control and four replicates for the test item groups of ten clitellated adult earthworms each were exposed to artificial soil (prepared according to OECD 222) treated with the test item to obtain the nominal concentrations of 11.8, 21.2, 38.1, 68.6, 123, 222, 400, and 720 mg GF-3969/kg dry artificial soil plus 8.74, 15.7, 28.2, 50.8, 91.1, 164, 296, and 533 mg DPX-KG691/kg dry artificial soil and to an untreated control (deionized water only).
The reference item (active substance carbendazim) is tested at least once a year at five concentrations. The most recent test was conducted from July to September 2017, performed under ibacon Study Number 105683022.
3. Observations
Worms were assessed for mortality and sublethal (behavioural) effects after 28 days of exposure. Body weight change (adults) was assessed between test start (day 0) and 28 days after application. For reproduction, soil was replaced in the test container and juveniles were allowed to grow for another 28 days (day 56), at which time they were removed from soil, counted, and reproduction effects assessed.
4. Statistics
Mortality data were analysed for significance by using Fisher's Exact Test (one-sided greater, $\alpha = 0.05$).
Data of weight changes and reproduction were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ($\alpha = 0.05$). Because data of body weight changes were normally distributed and homogeneous but did not follow a monotonicity trend by contrasts, the further statistical evaluation was performed using Dunnett's t-test (multiple comparison, $\alpha = 0.05$, two-sided for weight changes).
Further statistical evaluation of the NOEC for reproduction was performed using Williams t-

test (multiple comparison, $\alpha = 0.05$, one-sided smaller).

The EC values for reproduction were not determined by a statistical analysis due to the lack of a concentration-response relationship.

RESULTS AND DISCUSSION

All study validity criteria were met. No mortality was observed in any treatment group. The LC_{50} after 28 days was estimated to be greater than 720 mg GF-3969 + 533 mg DPX-KG691/kg dry artificial soil.

The body weight changes were not statistically significantly different compared to the control up to and including the highest concentration of 720 mg GF-3969 + 533 mg DPX-KG691/kg dry artificial soil (Dunnett's t-test, $\alpha = 0.05$, two-sided).

No statistically significant effects on reproduction were observed up to and including the concentration of 123 mg GF-3969 + 91.1 mg DPX-KG691/kg dry artificial soil. At the concentration of 222 mg GF-3969 + 164 mg DPX-KG691/kg dry artificial soil and above reproduction was statistically significantly reduced when compared to the control (Williams t-test, $\alpha = 0.05$, one-sided smaller). It was not possible to calculate meaningful EC_{10} or EC_{20} values. No behavioural abnormalities were observed in any treatment group in this study.

Cumulative mortality and weight change (of adults) at 28 days, and reproduction at 56 days are reported in the summary table below.

Table A 31: Sublethal toxicity of GF-3969 to earthworms

Nominal GF-3969 + DPX-KG691 concentration (mg/kg dry soil)	28-day mortality (%) mean	28-day weight change (%) mean	56-day reproduction (# of juveniles) mean	% of control
Control (0.0)	0	40.2	251	-
11.8 + 8.74	0 n.s.	40.9 n.s.	228 n.s.	90.8
21.2 + 15.7	0 n.s.	41.0 n.s.	218 n.s.	86.7
38.1 + 28.2	0 n.s.	40.9 n.s.	218 n.s.	86.9
68.6 + 50.8	0 n.s.	37.6 n.s.	249 n.s.	99.2
123 + 91.1	0 n.s.	38.6 n.s.	224 n.s.	89.3
222 + 164	0 n.s.	42.4 n.s.	186*	74.0
400 + 296	0 n.s.	43.2 n.s.	207*	82.3
720 + 533	0 n.s.	42.0 n.s.	212*	84.5

n.s. Not statistically significant

* Statistically significant

Mortality: Fisher's Exact Test, one-sided greater, $\alpha = 0.05$

Weight change: Dunnett's t-test, two-sided, $\alpha = 0.05$

Reproduction: Williams t-test, one-sided smaller, $\alpha = 0.05$

CONCLUSION

The LC_{50} for GF-3969 plus DPX-KG691 to the earthworm *Eisenia andrei* after 28 days was estimated to be greater than 720 mg GF-3969+ 533 mg DPX-KG691/kg dry artificial soil.

The No-Observed-Effect Concentration (NOEC) for mortality and body weight changes was determined to be ≥ 720 mg GF-3969 + 533 mg DPX-KG691/kg dry artificial soil and the Lowest-Observed-Effect Concentration (LOEC) was estimated to be >720 mg GF-3969 + 533 mg DPX-KG691/kg dry artificial soil.

The NOEC for reproduction was determined to be 123 mg GF-3969 + 91.1 mg DPX-KG691/kg dry artificial soil and the LOEC was determined to be 222 mg GF-3969 + 164 mg DPX-KG691/kg dry artificial soil.

The EC_x values for reproduction could not be analysed by statistical evaluation but EC_{50} was estimated to be >720 mg GF-3969 + 533 mg DPX-KG691/kg dry artificial soil, the highest concentration tested.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Earthworm	<i>Eisenia andrei</i>	GF-3969 plus adjuvant surfactant DPX-KG691	56 d	LC ₅₀ NOEC (mortality, growth and nominal concentrations) NOEC (reproduction)	>720 ≥720 123	mg product/kg dw

A 2.4.1.1.2 Study 2, DuPont-49980

Comments of zRMS:	<p>The study was performed in line with OECD 222 with no deviations.</p> <p>All the validity criteria were met:</p> <ul style="list-style-type: none"> - In the controls adult mortality over the initial 4 weeks should be ≤ 10% (was 0%), - In the controls each replicate containing 10 adults should produce ≥ 30 juveniles by the end of the test (was 172 to 248 juveniles per replicate), - In the controls the coefficient of variation of reproduction should be ≤ 30% (was 13.9%). <p>The test design was relevant to derive both NOEC and EC_x values (8 concentrations, 8 replicates for control, 4 replicates per treatment group). However, a meaningful EC_x value could not be calculated.</p> <p>Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>56d NOEC (mortality, body weight, reproduction) > 180 mg product/kg dw soil</p>
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Reference:	KCP 10.4.1.1/02
Report:	Pavic, B., (2018); Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isoxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) plus codacide: Effects on reproduction and growth of the earthworm, <i>Eisenia andrei</i> , in artificial soil
DuPont Report No.:	DuPont-49980
Testing Facility Report No.:	128721022
Guidelines	OECD 222 (2016), ISO 11268-2 (2012)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable

EXECUTIVE SUMMARY

The sublethal toxicity of GF-3969 to earthworms, *Eisenia andrei*, was determined in a 56-day soil exposure laboratory study according to OECD 222, 2016 and ISO 11268-2, 2012. Adult earthworms were exposed to artificial soil (prepared according to OECD 222) treated with the test item to obtain the nominal concentrations of 2.94, 5.29, 9.53, 17.1, 30.9, 55.6, 100 and 180 mg GF-3969 /kg dry artificial soil + 1.99, 3.57, 6.43, 11.5, 20.9, 37.5, 67.5, and 121.5 mg Codacide/kg dry artificial soil and to an untreated control (deionized water only).

Mortality and growth (body weight) of the earthworms were assessed after 28 days and the effect on reproduction (number of juveniles produced) was assessed after 56 days. The LC₅₀ after 28 days was estimated to be greater than 180 mg GF-3969 + 121.5 mg Codacide/kg soil dry artificial soil. The NOEC (No-Observed-Effect Concentration) for earthworms based on mortality, growth, reproduction

and nominal concentrations was ≥ 180 mg GF-3969 + 121.5 mg Codacide/kg soil dry artificial soil, the highest concentration tested.

MATERIALS AND METHODS

Test item (Common name): GF-3969 (DPX-V4B07 24.08WG, DPX-V4B07-001) , blend of three formulated components

Lot #: DPX-V4B07-001

Composition: DPX-V4B07-001 is a formulation containing nominal concentrations (w/w):
14.82% rimsulfuron active ingredient
9.26% thifensulfuron methyl active ingredient
11.11% isoxadifen ethyl safener

DPX-V4B07-001 is prepared by blending
59.26% DPX-E9636-227 25SG
18.52% DPX-M6316-323 50SG
22.22% DPX-X4145-021 50WG

Mixing Ratio: GF-3969 and the Codacide were mixed in a ratio of 135 g product (32.5 g a.s.) to 100 mL Codacide, that means 135 g product to 91.15 g Codacide which is corresponding to 1: 0.6752, *i.e.* to 1 g GF-3969 0.6752 g Codacide was added

Formulation 1

Name: DPX-E9636 25SG
Batch #: MAR15EL004
Concentration of a.s.: 25% (w/w) Rimsulfuron
25.1% (w/w) Rimsulfuron, by analysis
CAS #: 122931-48-0

Formulation 2

Name: DPX-M6316 50SG
Batch #: APR15EL002
Concentration of a.s.: 50% Thifensulfuron methyl
49.8% Thifensulfuron methyl, by analysis
79277-27-3 for thifensulfuron methyl active ingredient
CAS #: 79277-27-3

Safener

Name: DPX-X4145 50WG
Batch #: DEC15EL001
Concentration of a.s.: 50% (w/w) Isoxadifen-ethyl
50.4% (w/w) Isoxadifen-ethyl, by analysis
CAS #: 163520-33-0 for isoxadifen-ethyl active ingredient

Adjuvant surfactant

Name: Codacide
Batch #: FEB16CE020
Stability of test compound: Not analysed in the test system

Control: Untreated (and moistened with deionized water)
Test vehicle: Deionized water
Toxic reference: Carbendazim tested at least once a year in the test facility to ensure that laboratory test conditions are adequate and to verify that the response of the test organisms does not change significantly over time. Recently performed test resulted with NOEC of 1.44 mg carbendazim/kg dws and EC₁₀ of 0.85 mg carbendazim/kg dws (95% CL: 0.69-1.06 mg carbendazim/ kg dws) Dimethoate

Test System

Organism (<i>Species</i>):	Earthworm (<i>Eisenia andrei</i>)
Age at dosing:	Approximately 8 months (all within 4 weeks of the same age), with well-developed clitella
Weight at dosing:	303 to 599 mg
Source:	In-house laboratory culture (Laboratory: ibacon GmbH, Rossdorf, Germany)
Acclimatization period:	1 day
Test chamber:	Plastic boxes with perforated transparent lids (volume: 1 L), filled with ca. 500 g artificial soil dry weight
Test medium:	Artificial soil prepared according to OECD 222, maximum water holding capacity of the artificial soil, as measured: 72%
Diet:	Finely ground cattle manure
Water content of soil:	Initiation: 39.8% to 41.6% (equivalent to 55.3% to 57.8% of the maximum water holding capacity) Termination: 36.4 to 40.2% (equivalent to 50.6% to 55.8% of the maximum water holding capacity)
Soil pH:	5.8 to 6.0 at test start and 6.2 to 6.3 at test termination
Environmental conditions	Temperature: Within the range of 18 to 22°C Photoperiod: 16 hour light, 8 hour dark, photoperiod within the range of 400 to 800 lux

Methodology

1. In-life initiated/completed:
16-JAN-2018 to 14-MAR-2018
2. Experimental treatments
The sublethal toxicity of GF-3969 to earthworms, *Eisenia andrei*, was estimated in a 56-day soil exposure GLP-compliant laboratory study based on OECD 222 (2016) and ISO 11268, Part 2 (2012). Eight replicates for the control and four replicates for the test item groups of ten clitellated adult earthworms each were exposed to artificial soil (prepared according to OECD 222) treated with the test item to obtain the nominal concentrations of 2.94, 5.29, 9.53, 17.1, 30.9, 55.6, 100, and 180 mg GF-3969 /kg dry artificial soil plus 1.99, 3.57, 6.43, 11.5, 20.9, 37.5, 67.5, and 121.5 mg Codacide/kg dry artificial soil and to an untreated control (deionized water only).
The reference item (active substance carbendazim) is tested at least once a year at five concentrations. The most recent test was conducted from July to September 2017, performed under ibacon Study Number 105683022.
3. Observations
Worms were assessed for mortality and sublethal (behavioural) effects after 28 days of exposure. Body weight change (adults) was assessed between test start (day 0) and 28 days after application. For reproduction, soil was replaced in the test container and juveniles were allowed to grow for another 28 days (day 56), at which time they were removed from soil, counted, and reproduction effects assessed.
4. Statistics
Mortality data were analysed for significance by using Fisher's Exact Test (one-sided greater, $\alpha = 0.05$).
Data of weight changes and reproduction were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test and Cochran's test, respectively ($\alpha = 0.05$). Because data of body weight changes were normally distributed and homogeneous the further statistical evaluation was performed using Williams t-test (multiple comparison, $\alpha = 0.05$, one-sided greater).
Further statistical evaluation of the NOEC for reproduction was performed using Dunnett's t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller).
The EC values for reproduction were not determined by a statistical analysis due to the lack

of a concentration-response relationship.

RESULTS AND DISCUSSION

All study validity criteria were met. No mortality was observed in any treatment group. The LC₅₀ after 28 days was estimated to be greater than 180 mg GF-3969 + 121.5 mg Codacide/kg dry artificial soil. The body weight changes were not statistically significantly different compared to the control up to and including the highest concentration of 180 mg GF-3969 + 121.5 mg Codacide/kg dry artificial soil (Williams t-test, $\alpha = 0.05$, one-sided greater).

No statistically significant effects on reproduction were observed up to and including the concentration of 180 mg GF-3969 + 121.5 mg Codacide/kg dry artificial soil. (Dunnett's t-test, $\alpha = 0.05$, one-sided smaller). It was not possible to calculate meaningful EC₁₀ or EC₂₀ values. No behavioural abnormalities were observed in any treatment group in this study.

Cumulative mortality and weight change (of adults) at 28 days, and reproduction at 56 days are reported in the summary table below.

Table A 32: Sublethal toxicity of GF-3969 to earthworms

Nominal GF-3969 + Codacide concentration (mg/kg dry soil)	28-day mortality (%) mean	28-day weight change (%) mean	56-day reproduction (# of juveniles) mean	% of control
Control (0.0)	0	30.1	216	-
2.94 + 1.99	0 n.s.	28.5 n.s.	219 n.s.	102
5.29 + 3.57	0 n.s.	29.7 n.s.	212 n.s.	98.1
9.53 + 6.43	0 n.s.	28.6 n.s.	211 n.s.	97.9
17.1 + 11.5	0 n.s.	33.0 n.s.	247 n.s.	115
30.9 + 20.9	0 n.s.	33.3 n.s.	207 n.s.	95.8
55.6 + 37.5	0 n.s.	33.4 n.s.	235 n.s.	109
100 + 67.5	0 n.s.	33.7 n.s.	201 n.s.	93.2
180 + 121.5	0 n.s.	37.7 n.s.	198 n.s.	92.0

n.s. not statistically significant

Mortality: Fisher's Exact Test, one-sided greater, $\alpha = 0.05$

Weight change: Williams t-test, one-sided greater, $\alpha = 0.05$

Reproduction: Dunnett's t-test, one-sided smaller, $\alpha = 0.05$

CONCLUSION

The LC₅₀ for GF-3969 plus Codacide to the earthworm *Eisenia andrei* after 28 days was estimated to be greater than 180 mg GF-3969+ 121.5 mg Codacide/kg dry artificial soil.

The No-Observed-Effect Concentration (NOEC) for mortality and body weight changes was determined to be ≥ 180 mg GF-3969 + 121.5 mg Codacide/kg dry artificial soil and the Lowest-Observed-Effect Concentration (LOEC) was estimated to be >180 mg GF-3969 + 121.5 mg Codacide/kg dry artificial soil.

The NOEC for reproduction was determined to be ≥ 180 mg GF-3969 + 121.5 mg Codacide/kg dry artificial soil and the LOEC was determined to be >180 mg GF-3969 + 121.5 mg Codacide/kg dry artificial soil.

The EC_x values for reproduction could not be analysed by statistical evaluation but EC₅₀ was estimated to be >180 mg GF-3969 + 121.5 mg Codacide/kg dry artificial soil, the highest concentration tested.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
earthworm	<i>Eisenia andrei</i>	GF-3969 plus adjuvant surfactant Codacide	56 d	LC ₅₀ NOEC (mortality, growth, reproduction and nominal concentrations)	>180 ≥ 180	mg product/kg dw

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

No new or additional studies have been submitted.

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 2.4.2.1.1 Study 1, DuPont-49954

Comments of zRMS:	<p>The study was performed in line with OECD 232 with no deviations.</p> <p>All the validity criteria were met:</p> <ul style="list-style-type: none"> - In the controls the mean adult mortality should not exceed 20% at the end of the test (was 9%), - In the controls the mean number of juveniles per vessel should be ≥ 100 at the end of the test (was 574; range 433-715), - In the controls the coefficient of variation calculated for the number of juveniles should be $< 30\%$ at the end of the test (was 16.9%). <p>The study design (5 concentrations, 8 replicates for control, 4 replicates per treatment group) was relevant to derive only the NOEC values and not the EC_x values. Therefore, in the opinion of the zRMS, the calculated EC_x values are not reliable and should not be used in the risk assessment, especially confidence intervals could not be determined.</p> <p>Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>28d NOEC (reproduction) = 125 mg product/kg soil dw</p>
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Reference:	KCP 10.4.2.1/02
Report:	Pavic, B., (2018); Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isoxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) plus DPX-KG691 surfactant: Effects on the collembola <i>Folsomia candida</i> in artificial soil
DuPont Report No.:	DuPont-49954
Testing Facility Report No.:	128711016
Guidelines	OECD 232 (2016), ISO 11267 (2014)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable

EXECUTIVE SUMMARY

The effects of GF-3969 on the mortality and reproduction of Collembola (*Folsomia candida*) were determined in a 28-day soil exposure laboratory study according to OECD 232, 2016 and ISO 11267, 2014. Ten to twelve days old Collembola were exposed for 28 days to artificial soil (prepared according to OECD 232) treated with five nominal concentrations of GF-3969 of 31.25, 62.5, 125, 250 and 500 mg GF-3969 /kg dry weight soil plus 23.1, 46.3, 92.6, 185.2 and 370.4 mg DPX-KG691/kg dry weight soil and to an untreated control (deionized water only). Mortality and reproduction (number of juveniles produced) were assessed after 28 days. The 28-day NOEC (No-Observed-Effect Concentration) based on mortality was determined to be 500 mg GF-3969 + 370.4 mg DPX-KG691/kg dry artificial soil. The 28-day NOEC based on reproduction was determined to be 125 mg GF-3969 + 92.6 mg DPX-KG691/kg dry artificial soil.

MATERIALS AND METHODS

Test Item(s)

Test item (Common name): GF-3969 (synonym: DPX-V4B07 24.08WG, DPX-V4B07-001), blend of three formulated components

Lot #: DPX-V4B07-001

Composition: DPX-V4B07-001 is a formulation containing nominal concentrations (w/w):
14.82% rimsulfuron active ingredient
9.26% thifensulfuron methyl active ingredient
11.11% isoxadifen ethyl safener

Mixing Ratio: DPX-V4B07-001 is prepared by blending
59.26% DPX-E9636-227 25SG
18.52% DPX-M6316-323 50SG
22.22% DPX-X4145-021 50WG

GF-3969 and the DPX-KG691 adjuvant surfactant were mixed in a ratio of 135 g product (32.5 g a.s.) to 100 mL DPX-KG691, that means 135 g product to 100 g DPX-KG691 which is corresponding to 1: 0.7407, i.e. to 1 g GF-3969 0.7407 g DPX-KG691 was added

Formulation 1

Name: DPX-E9636 25SG

Batch #: MAR15EL004

Concentration of a.s.: 25% (w/w) Rimsulfuron

CAS #: 25.1% (w/w) Rimsulfuron, by analysis

122931-48-0

Formulation 2

Name: DPX-M6316 50SG

Batch #: APR15EL002

Concentration of a.s.: 50% Thifensulfuron methyl
49.8% Thifensulfuron methyl, by analysis

CAS #: 79277-27-3 for thifensulfuron methyl active ingredient

Safener

Name: DPX-X4145 50WG

Batch #: DEC15EL001

Concentration of a.s.: 50% (w/w) Isoxadifen-ethyl
50.4% (w/w) Isoxadifen-ethyl, by analysis

CAS #: 163520-33-0 for isoxadifen-ethyl active ingredient

Adjuvant surfactant

Name: DPX-KG691

Batch #: JAN15CE035

Stability of test compounds: Not analysed in the test system

Control: Untreated (and moistened with deionized water)

Test vehicle: Deionized water

Reference item: Boric acid tested at least once a year in the test facility to ensure that laboratory test conditions are adequate and to verify that the response of the test organisms does not change significantly over time. Recently performed test resulted with NOEC for reproduction of 30.5 mg test item/kg dws and EC₁₀ of 49.0 mg test item/kg dws (95% CL: 41.1-55.7 mg test item/ kg dws)

Test System

Organism (*Species*): Collembola (*Folsomia candida*, Willem (Collembola: Isotomidae))

Age at dosing: 10 to 12 days

Weight at dosing:	Not determined
Source:	In-house laboratory culture
Acclimation period:	12 days
Test chamber:	Glass containers (volume: 100 mL; diameter: 5.0 cm), closed, filled with 30 ± 1.0 g artificial soil fresh weight
Test medium:	Artificial soil prepared according to OECD 232, maximum water holding capacity of the artificial soil, as measured: 39%
Diet:	Granulated dry yeast
Water content of soil:	Initiation: 20.5% to 20.8% equivalent to 52.5% to 53.4% of the maximum water holding capacity Termination: 18.5% to 20.2% equivalent to 47.3% to 51.9% of the maximum water holding capacity
Soil pH:	5.9 to 6.1 at test start; 5.6 to 5.8 at test termination
Environmental conditions	Temperature: Within the range of 18 to 22°C Photoperiod: 16 h light, 8 h dark, photoperiod within the range of 400 to 800 lux

Methodology

1. In-life initiated/completed
22-JAN-2018 to 20-FEB-2018
2. Experimental treatments
A study was conducted to determine the effects of GF-3969 on the mortality and reproduction of Collembola (*Folsomia candida*). Eight replicates for the control and four replicates per test item group, containing ten Collembola each (total 80 per control and 40 individuals per test item group) were each exposed for 28 days to the nominal concentrations of 31.25, 62.5, 125, 250 and 500 mg GF-3969/kg dry weight soil plus 23.1, 46.3, 92.6, 185.2 and 370.4 mg DPX-KG691/kg dry weight soil and to an untreated control (deionized water only). A reference item (boric acid, at a concentration range of 30.5 to 200 mg/kg artificial soil dry weight) is tested at least once per year to ensure sensitivity of the test system. The most recent test to this study was conducted in August/September 2017.
3. Observations
After the 28-day exposure period, adult Collembola were counted and the mean number of adults in each treatment group was determined. The number of juveniles produced in each treatment group over 28 days exposure period was also determined and the percent reduction in juveniles produced relative to the untreated control group was calculated.
4. Statistics
Mortality data were analysed for significance by using Fisher's Exact Test (one-sided greater, $\alpha = 0.05$).
Reproduction data were tested for normal distribution and homoscedasticity using Shapiro-Wilk's test and Levene's test ($\alpha = 0.05$). Further statistical evaluation of the NOEC for reproduction was performed using Williams t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller). The EC values for reproduction and the 95% confidence limits were determined by applying Probit-Analysis.

RESULTS AND DISCUSSION

All validity criteria were met. The EC_{50} for reproduction of the reference item (boric acid) in the most recent test was 104.9 mg boric acid/kg dry artificial soil.
A summary of the results is provided in the table below.

Table A 33: The effects on mortality and reproduction of Collembola, *Folsomia candida*, exposed to GF-3969 in artificial soil for 28 days

Nominal GF-3969 + DPX-KG691 concentration (mg/kg soil dry weight)	Mean % mortality	Reproduction	
		Mean juveniles per replicate	% of control
Untreated control (0.0)	9	574	-
31.25 + 23.1	8 n.s.	508 n.s.	88.6
62.5 + 46.3	5 n.s.	584 n.s.	102
125 + 92.6	10 n.s.	536 n.s.	93.4
250 + 185.2	18 n.s.	432 *	75.3
500 + 370.4	23 n.s.	326 *	56.8

n.s. There were no significant differences from the control

* Statistically significant

mortality: Fisher's Exact Test, alpha = 0.05, one-sided greater;

number of juveniles: Williams t-test, alpha = 0.05, one-sided smaller

CONCLUSION

The 28-day EC₁₀ for reproduction was determined to be 133.1 mg GF-3969 + 98.6 mg DPX-KG691/kg dry artificial soil, the EC₂₀ was determined to be 223.4 mg GF-3969 + 165.5 mg DPX-KG691/kg dry artificial soil and the EC₅₀ was determined to be 602.1 mg GF-3969 + 446.0 mg DPX-KG691/kg dry artificial soil (95% confidence limits could not be determined).

The Lowest-Observed-Effect Concentration (LOEC) for reproduction was determined to be 250 mg GF-3969 + 185.2 mg DPX-KG691/kg dry artificial soil. The No-Observed-Effect Concentration (NOEC) for reproduction was determined to be 125 mg GF-3969 + 92.6 mg DPX-KG691/kg dry artificial soil.

The NOEC for mortality was determined to be 500 mg GF-3969 + 370.4 mg DPX-KG691/kg dry artificial soil.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Collembola	<i>Folsomia candida</i>	GF-3969 plus adjuvant surfactant DPX-KG691	28 d	NOEC (mortality) NOEC (reproduction)	500 125	mg product/kg dw

A 2.4.2.1.2 Study 2, DuPont-49955

Comments of zRMS:	<p>The study was performed in line with OECD 226 with no deviations.</p> <p>All the validity criteria were met:</p> <ul style="list-style-type: none"> - In the controls the mean adult female mortality should not exceed 20% at the end of the test (was 1%), - In the controls the mean number of juveniles per replicate should be ≥ 50 at the end of the test (was 194; range 143-217), - In the controls the coefficient of variation calculated for the number of juveniles per replicate should be $\leq 30\%$ at the end of the test (was 12.4%). <p>The study design (5 concentrations, 8 replicates for control, 4 replicates per treatment group) was relevant to derive only the NOEC values and not the EC_x values. However, effects $\geq 10\%$ were not observed at any of the concentrations tested.</p> <p>Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>14d NOEC (reproduction) > 1000 mg product/kg soil dw</p>
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Reference:	KCP 10.4.2.1/01
Report:	Pavic, B., (2018); Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isoxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) plus DPX-KG691 surfactant: Effects on the reproduction of the predatory mite <i>Hypoaspis aculeifer</i> in artificial soil
DuPont Report No.:	DuPont-49955
Testing Facility Report No.:	128711089
Guidelines	OECD 226 (2016)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable

EXECUTIVE SUMMARY

A study was conducted to determine the effect of GF-3969 on the mortality and reproduction of the soil mite (*Hypoaspis aculeifer*) according to OECD 226. The soil mites were exposed for 14 days to artificial soil (prepared according to OECD 226) treated with the test item to obtain the nominal concentrations of 62.5, 125, 250, 500 and 1000 mg GF-3969 /kg dry artificial soil plus 46.3, 92.6, 185.2, 370.4 and 740.7 mg DPX-KG691/kg dry artificial soil and to an untreated control (deionized water only). GF-3969 had no statistically significant lethal or reproductive effects on the Predatory Mite *Hypoaspis aculeifer* when exposed to concentrations up to and including 1000 mg GF-3969 + 740.7 mg DPX-KG691/kg dry artificial soil for 14 days, the highest dose tested.

The 14-day EC₅₀ and the overall Lowest-Observed-Effect Concentration (LOEC) for GF-3969 were estimated to be greater than 1000 mg GF-3969 + 740.7 mg DPX-KG691/kg dry artificial soil. The 14-day No-Observed-Effect Concentration (NOEC) based on mortality and reproduction was determined to be 1000 mg GF-3969 + 740.7 mg DPX-KG691/kg dry artificial soil.

MATERIALS AND METHODS

Test Item(s)

Test item (Common name): GF-3969 (synonym: DPX-V4B07 24.08WG, DPX-V4B07-001), blend of three formulated components

Lot #: DPX-V4B07-001

Composition: DPX-V4B07-001 is a formulation containing nominal concentrations (w/w):
 14.82% rimsulfuron active ingredient
 9.26% thifensulfuron methyl active ingredient
 11.11% isoxadifen ethyl safener

Mixing Ratio: DPX-V4B07-001 is prepared by blending
 59.26% DPX-E9636-227 25SG
 18.52% DPX-M6316-323 50SG
 22.22% DPX-X4145-021 50WG
 GF-3969 and the DPX-KG691 adjuvant surfactant were mixed in a ratio of 135 g product (32.5 g a.s.) to 100 mL DPX-KG691, that means 135 g product to 100 g DPX-KG691 which is corresponding to 1: 0.7407, i.e. to 1 g GF-3969 0.7407 g DPX-KG691 was added

Formulation 1

Name: DPX-E9636 25SG
 Batch #: MAR15EL004
 Concentration of a.s.: 25% (w/w) Rimsulfuron
 25.1% (w/w) Rimsulfuron, by analysis
 CAS #: 122931-48-0

Formulation 2

Name: DPX-M6316 50SG
Batch #: APR15EL002
Concentration of a.s.: 50% Thifensulfuron methyl
49.8% Thifensulfuron methyl, by analysis
CAS #: 79277-27-3 for thifensulfuron methyl active ingredient

Safener

Name: DPX-X4145 50WG
Batch #: DEC15EL001
Concentration of a.s.: 50% (w/w) Isoxadifen-ethyl
50.4% (w/w) Isoxadifen-ethyl, by analysis
CAS #: 163520-33-0 for isoxadifen-ethyl active ingredient

Adjuvant surfactant

Name: DPX-KG691
Batch #: JAN15CE035
Stability of test compound: Not analysed in the test system
Control: Untreated (and moistened with deionized water)
Test vehicle: Deionized water
Toxic reference: Dimethoate tested at least once a year in the test facility to ensure that laboratory test conditions are adequate and to verify that the response of the test organisms does not change significantly over time. Recently performed test resulted with NOEC for reproduction of 2.24 mg a.i./kg dws and EC₁₀ of 3.05 mg a.i./kg dws (95% CL: 2.73-3.28 mg a.i./ kg dws)

Test System

Organism (*Species*): Predatory soil mites (adult females) (*Hypoaspis aculeifer*)
Age at dosing: Adults, approximately 14 days after reaching the adult stage (35 days after placing adult females in clean rearing vessels over a period of 3 days)
Source: Cultured by ibacon
Acclimation period: 35 days
Test chamber: Glass containers (volume: 100 mL; diameter: 5 cm), closed, filled with 20 ± 1.0 g artificial soil dry weight
Test medium: Artificial soil prepared according to OECD 226, maximum water holding capacity of the artificial soil, as measured: 39%
Diet: Cheese mites (*Tyrophagus putrescentiae*)
Water content of soil: Initiation: 20.5% to 20.8% equivalent to 52.6% to 53.4% of the maximum water holding capacity
Termination: 19.3% to 20.6% equivalent to 49.6% to 52.9% of the maximum water holding capacity
Soil pH: 5.9 to 6.1 at test start; 5.9 to 6.0 at test termination
Environmental conditions: Temperature: Within a range of 18 to 22°C
Photoperiod: 16 hour light, 8 hour dark, photoperiod within a range of 400 to 800 lux

Methodology

1. In-life dates
22-JAN-2018 to 07-FEB-2018
2. Experimental treatments
A study was conducted to determine the effect of GF-3969 on the mortality and reproduction of the predatory soil mite (*Hypoaspis aculeifer*). Eight replicates for the control and four replicates per test item group, containing ten predatory mites each (total 80 individuals per control and 40 individuals per test item group) were each exposed for 14 days to

nominal concentrations of 62.5, 125, 250, 500 and 1000 mg GF-3969/kg dry artificial soil plus 46.3, 92.6, 185.2, 370.4 and 740.7 mg DPX-KG691/kg dry weight soil and to an untreated control (deionized water only). A reference item (dimethoate) is tested at least once a year to ensure sensitivity of the test system at five concentrations. The most recent test relevant to this study was conducted in September/October 2017.

3. Observations

After the 14-day exposure period, surviving soil mites were extracted by a heat gradient. The mean number of adults in each treatment group was determined. The mean number of juveniles produced in each treatment group over 14-day exposure period was also determined and the percent reduction in juveniles produced relative to the untreated control group was calculated.

4. Statistics

Mortality data were analysed for significance by using Fisher's Exact Test (one-sided greater, alpha = 0.05). Reproduction data were tested for normal distribution and homoscedasticity using Kolmogorov-Smirnov test and Levene's test (alpha = 0.05).

Further statistical evaluation of the NOEC for reproduction was performed using Dunnett's t-test (multiple comparison, alpha = 0.05, one-sided smaller).

The EC values for reproduction were not determined by a statistical analysis due to the lack of a concentration-response relationship.

RESULTS AND DISCUSSION

All validity criteria were met. The reference item caused statistically significant effects on reproduction at a concentration of 3.25 mg dimethoate/kg dry artificial soil and above; the EC₅₀ for reproduction was 4.12 mg dimethoate/kg dry artificial soil.

A summary of the results is provided in the table below.

Table A 34: The effects on mortality and reproduction of the soil mite, *Hypoaspis aculeifer*, exposed to GF-3969 in artificial soil for 14 days

Nominal GF-3969 + DPX-KG691 concentration (mg/kg soil dry weight)	Mean % mortality ^a	Reproduction	
		Mean juveniles per replicate ^a	% of control
Untreated control (0.0)	1	194	-
62.5 + 46.3	0	204	105
125 + 92.6	0	204	105
250 + 185.2	0	196	101
500 + 370.4	0	190	98.0
1000 + 740.7	0	190	98.3

^a There were no significant differences compared to the control (mortality: Fisher's Exact Test, one-sided greater, alpha = 0.05; number of juveniles: Dunnett's t-test, one-sided smaller, alpha = 0.05)

CONCLUSION

The 14-day EC₁₀, EC₂₀ and EC₅₀ based on reproduction could not be determined by a statistical analysis due to the lack of a concentration-response relationship but were estimated to be greater than 1000 mg GF-3969 + 740.7 mg DPX-KG691/kg dry artificial soil.

The Lowest-Observed-Effect Concentration (LOEC) for GF-3969 based on mortality and reproduction was estimated to be greater than 1000 mg GF-3969 + 740.7 mg DPX-KG691/kg dry artificial soil. The 14-day No-Observed-Effect Concentration (NOEC) based on mortality and reproduction was determined to be 1000 mg GF-3969 + 740.7 mg DPX-KG691/kg dry artificial soil.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Predatory mite	<i>Hypoaspis aculeifer</i>	GF-3969 plus adjuvant surfactant DPX-KG691	14 d	EC ₅₀ LOEC NOEC	>1000 >1000 1000	mg product/kg dry soil

A 2.4.2.1.3 Study 3, DuPont-49981

Comments of zRMS:	<p>The study was performed in line with OECD 232 with no deviations.</p> <p>All the validity criteria were met:</p> <ul style="list-style-type: none"> - In the controls the mean adult mortality should not exceed 20% at the end of the test (was 5%), - In the controls the mean number of juveniles per vessel should be ≥ 100 at the end of the test (was 386; range 357-429), - In the controls the coefficient of variation calculated for the number of juveniles should be $< 30\%$ at the end of the test (was 7%). <p>The study design (5 concentrations, 8 replicates for control, 4 replicates per treatment group) was relevant to derive only the NOEC values and not the EC_x values. Therefore, in the opinion of the zRMS, the calculated EC_x values are not reliable and should not be used in the risk assessment, especially the confidence interval for EC₁₀ was very wide (38.3-283.1 mg/kg dws) and median EC₁₀ was higher than lower limit EC₂₀.</p> <p>Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>28d NOEC (reproduction) = 250 mg product/kg soil dw</p>
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Reference:	KCP 10.4.2.1/04
Report:	Pavic, B., (2018); Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isoxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) plus codacide: Effects on the collembola <i>Folsomia candida</i> in artificial soil with 5% peat
DuPont Report No.:	DuPont-49981
Testing Facility Report No.:	128721016
Guidelines	OECD 232 (2016), ISO 11267 (2014)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable

EXECUTIVE SUMMARY

The effects of GF-3969 on the mortality and reproduction of Collembola (*Folsomia candida*) were determined in a 28-day soil exposure laboratory study according to OECD 232, 2016 and ISO 11267, 2014. Nine to eleven days old Collembola were exposed for 28 days to artificial soil (prepared according to OECD 232) treated with five nominal concentrations of GF-3969 of 62.5, 125, 250, 500 and 1000 mg GF-3969 /kg dry weight soil plus 42.2, 84.4, 168.8, 337.6 and 675.2 mg Codacide/kg dry weight soil and to an untreated control (deionized water only). Mortality and reproduction (number of juveniles produced) were assessed after 28 days. The 28-day NOEC (No-Observed-Effect Concentration) based on mortality was determined to be 1000 mg GF-3969 + 675.2 mg Codacide/kg dry artificial soil. The 28-day NOEC based on reproduction was determined to be 250 mg GF-3969 + 168.8 mg Codacide/kg dry artificial soil.

MATERIALS AND METHODS

Test Item(s)

Test item (Common name): GF-3969 (DPX-V4B07 24.08WG, DPX-V4B07-001), blend of three formulated components

Lot #: DPX-V4B07-001

Composition: DPX-V4B07-001 is a formulation containing nominal concentrations (w/w):
14.82% rimsulfuron active ingredient

9.26% thifensulfuron methyl active ingredient
11.11% isoxadifen ethyl safener

Mixing Ratio: DPX-V4B07-001 is prepared by blending
59.26% DPX-E9636-227 25SG
18.52% DPX-M6316-323 50SG
22.22% DPX-X4145-021 50WG
GF-3969 and the Codacide were mixed in a ratio of 135 g product (32.5 g a.s.) to 100 mL Codacide, that means 135 g product to 91.15 g Codacide which is corresponding to 1: 0.6752, *i.e.* to 1 g GF-3969 0.6752 g Codacide was added

Formulation 1

Name: DPX-E9636 25SG
Batch #: MAR15EL004
Concentration of a.s.: 25% (w/w) Rimsulfuron
25.1% (w/w) Rimsulfuron, by analysis
CAS #: 122931-48-0

Formulation 2

Name: DPX-M6316 50SG
Batch #: APR15EL002
Concentration of a.s.: 50% Thifensulfuron methyl
49.8% Thifensulfuron methyl, by analysis
CAS #: 79277-27-3 for thifensulfuron methyl active ingredient

Safener

Name: DPX-X4145 50WG
Batch #: DEC15EL001
Concentration of a.s.: 50% (w/w) Isoxadifen-ethyl
50.4% (w/w) Isoxadifen-ethyl, by analysis
CAS #: 163520-33-0 for isoxadifen-ethyl active ingredient

Adjuvant surfactant

Name: Codacide
Batch #: FEB16CE020
Stability of test compound: Not analysed in the test system

Control: Untreated (and moistened with deionized water)

Test vehicle: Deionized water

Reference item: Boric acid tested at least once a year in the test facility to ensure that laboratory test conditions are adequate and to verify that the response of the test organisms does not change significantly over time. Recently performed test resulted with NOEC for reproduction of 30.5 mg test item/kg dws and EC₁₀ of 49.0 mg test item/kg dws (95% CL: 41.1-55.7 mg test item/ kg dws)

Test System

Organism (*Species*): Collembola (*Folsomia candida*, Willem (Collembola: Isotomidae))
Age at dosing: 9 to 11 days
Weight at dosing: Not determined
Source: In-house laboratory culture
Acclimation period: 11 days
Test chamber: Glass containers (volume: 100 mL; diameter: 5.0 cm), closed, filled with 30 ± 1.0 g artificial soil fresh weight
Test medium: Artificial soil prepared according to OECD 232, maximum water holding capacity of the artificial soil, as measured: 39%
Diet: Granulated dry yeast
Water content of soil: Initiation: 19.9% to 21.2% equivalent to 51.1% to 54.2% of the

maximum water holding capacity
Termination: 16.4% to 17.6% equivalent to 42.1% to 45.1% of the maximum water holding capacity
Soil pH: 5.9 to 6.0 at test start; 5.4 to 5.7 at test termination
Environmental conditions Temperature: Within the range of 18 to 22°C
Photoperiod: 16 h light, 8 h dark, photoperiod within the range of 400 to 800 lux

Methodology

- In-life initiated/completed
19-JAN-2018 to 19-FEB-2018
- Experimental treatments
A study was conducted to determine the effects of GF-3969 on the mortality and reproduction of *Collembola (Folsomia candida)*. Eight replicates for the control and four replicates per test item group, containing ten *Collembola* each (total 80 per control and 40 individuals per test item group) were each exposed for 28 days to the nominal concentrations of 62.5, 125, 250, 500 and 1000 mg GF-3969/kg dry weight soil plus 42.2, 84.4, 168.8, 337.6 and 675.2 mg Codacide/kg dry weight soil and to an untreated control (deionized water only). A reference item (boric acid, at a concentration range of 30.5 to 200 mg/kg artificial soil dry weight) is tested at least once per year to ensure sensitivity of the test system. The most recent test to this study was conducted in August/September 2017.
- Observations
After the 28-day exposure period, adult *Collembola* were counted and the mean number of adults in each treatment group was determined. The number of juveniles produced in each treatment group over 28 days exposure period was also determined and the percent reduction in juveniles produced relative to the untreated control group was calculated.
- Statistics
Mortality data were analysed for significance by using Fisher's Exact Test (one-sided greater, alpha = 0.05).
Reproduction data were tested for normal distribution and homoscedasticity using Shapiro-Wilk's test and Levene's test (alpha = 0.05). Further statistical evaluation of the NOEC for reproduction was performed using Williams t-test (multiple comparison, alpha = 0.05, one-sided smaller). The EC values for reproduction and the 95% confidence limits were determined by applying Probit-Analysis.

RESULTS AND DISCUSSION

All validity criteria were met. The EC₅₀ for reproduction of the reference item (boric acid) in the most recent test was 104.9 mg boric acid/kg dry artificial soil.

A summary of the results is provided in the table below.

Table A 35: The effects on mortality and reproduction of *Collembola, Folsomia candida*, exposed to GF-3969 in artificial soil for 28 days

Nominal GF-3969 + Codacide concentration (mg/kg soil dry weight)	Mean % mortality	Reproduction	
		Mean juveniles per replicate	% of control
Untreated control (0.0)	5	386	-
62.5 + 42.2	8 n.s.	406 n.s.	105
125 + 84.4	8 n.s.	345 n.s.	89.5
250 + 168.8	15 n.s.	355 n.s.	91.9
500 + 337.6	18 n.s.	274 *	71.1
1000 + 675.2	15 n.s.	229 *	59.3

n.s. There were no significant differences from the control

* Statistically significant

mortality: Fisher's Exact Test, alpha = 0.05, one-sided greater;

number of juveniles: Williams t-test, alpha = 0.05, one-sided smaller

CONCLUSION

The EC₁₀ for reproduction was determined to be 169.6 mg GF-3969 + 114.5 mg Codacide/kg dry artificial soil, the EC₂₀ was determined to be 353.7 mg GF-3969 + 238.8 mg Codacide/kg dry artificial soil and the EC₅₀ was determined to be 1442.7 mg GF-3969 + 974.1 mg Codacide/kg dry artificial soil.

The Lowest-Observed-Effect Concentration (LOEC) for reproduction was determined to be 500 mg GF-3969 + 337.6 mg Codacide/kg dry artificial soil. The No-Observed-Effect Concentration (NOEC) for reproduction was determined to be 250 mg GF-3969 + 168.8 mg Codacide/kg dry artificial soil. The NOEC for mortality was determined to be 1000 mg GF-3969 + 675.2 mg Codacide/kg dry artificial soil.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Collembola	<i>Folsomia candida</i>	GF-3969 plus adjuvant surfactant Codacide	28 d	NOEC (mortality) NOEC (reproduction)	1000 250	mg product/kg dw

A 2.4.2.1.4 Study 4, DuPont-49982

Comments of zRMS:	<p>The study was performed in line with OECD 226 with no deviations.</p> <p>All the validity criteria were met:</p> <ul style="list-style-type: none"> - In the controls the mean adult female mortality should not exceed 20% at the end of the test (was 3%), - In the controls the mean number of juveniles per replicate should be ≥ 50 at the end of the test (was 194; range 183-206), - In the controls the coefficient of variation calculated for the number of juveniles per replicate should be $\leq 30\%$ at the end of the test (was 4.1%). <p>The study design (5 concentrations, 8 replicates for control, 4 replicates per treatment group) was relevant to derive only the NOEC values and not the EC_x values. However, effects >10% were not observed at any of the concentrations tested.</p> <p>Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>14d NOEC (reproduction) > 1000 mg product/kg soil dw</p>
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Reference:	KCP 10.4.2.1/03
Report:	Pavic, B., (2018); Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isoxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) plus codacide: Effects on reproduction of the predatory mite <i>Hypoaspis aculeifer</i> in artificial soil
DuPont Report No.:	DuPont-49982
Testing Facility Report No.:	128721089
Guidelines	OECD 226 (2016)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable

EXECUTIVE SUMMARY

A study was conducted to determine the effect of GF-3969 on the mortality and reproduction of the soil mite (*Hypoaspis aculeifer*) according to OECD 226. The soil mites were exposed for 14 days to

artificial soil (prepared according to OECD 226) treated with the test item to obtain the nominal concentrations of 62.5, 125, 250, 500 and 1000 mg GF-3969 /kg dry artificial soil plus 42.2, 84.4, 168.8, 337.6 and 675.2 mg Codacide/kg dry artificial soil and to an untreated control (deionized water only). GF-3969 had no statistically significant lethal or reproductive effects on the Predatory Mite *Hypoaspis aculeifer* when exposed to concentrations up to and including 1000 mg GF-3969 + 675.2 mg Codacide/kg dry artificial soil for 14 days, the highest dose tested.

The 14-day EC₅₀ and the overall Lowest-Observed-Effect Concentration (LOEC) for GF-3969 were estimated to be greater than 1000 mg GF-3969 + 675.2 mg Codacide/kg dry artificial soil. The 14-day No-Observed-Effect Concentration (NOEC) based on mortality and reproduction was determined to be 1000 mg GF-3969 + 675.2 mg Codacide/kg dry artificial soil.

MATERIALS AND METHODS

Test Item(s)

Test item (Common name): GF-3969 (synonym: DPX-V4B07 24.08WG, DPX-V4B07-001), blend of three formulated components

Lot #: DPX-V4B07-001

Composition: DPX-V4B07-001 is a formulation containing nominal concentrations (w/w):
14.82% rimsulfuron active ingredient
9.26% thifensulfuron methyl active ingredient
11.11% isoxadifen ethyl safener

Mixing Ratio: DPX-V4B07-001 is prepared by blending
59.26% DPX-E9636-227 25SG
18.52% DPX-M6316-323 50SG
22.22% DPX-X4145-021 50WG

GF-3969 and the Codacide were mixed in a ratio of 135 g product (32.5 g a.s.) to 100 mL Codacide, that means 135 g product to 100 g Codacide which is corresponding to 1: 0.6752, *i.e.* to 1 g GF-3969 0.6752 g Codacide was added

Formulation 1

Name: DPX-E9636 25SG
Batch #: MAR15EL004
Concentration of a.s.: 25% (w/w) Rimsulfuron
25.1% (w/w) Rimsulfuron, by analysis

CAS #: 122931-48-0

Formulation 2

Name: DPX-M6316 50SG
Batch #: APR15EL002
Concentration of a.s.: 50% Thifensulfuron methyl
49.8% Thifensulfuron methyl, by analysis

CAS #: 79277-27-3 for thifensulfuron methyl active ingredient

Safener

Name: DPX-X4145 50WG
Batch #: DEC15EL001
Concentration of a.s.: 50% (w/w) Isoxadifen-ethyl
50.4% (w/w) Isoxadifen-ethyl, by analysis

CAS #: 163520-33-0 for isoxadifen-ethyl active ingredient

Adjuvant surfactant

Name: Codacide
Batch #: FEB16CE020
Stability of test compound: Not analysed in the test system
Control: Untreated (and moistened with deionized water)
Test vehicle: Deionized water

Toxic reference: Dimethoate tested at least once a year in the test facility to ensure that laboratory test conditions are adequate and to verify that the response of the test organisms does not change significantly over time. Recently performed test resulted with NOEC for reproduction of 2.24 mg a.i./kg dws and EC₁₀ of 3.05 mg a.i./kg dws (95% CL: 2.73-3.28 mg a.i./ kg dws)

Test System

Organism (*Species*): Predatory soil mites (adult females) (*Hypoaspis aculeifer*)
Age at dosing: Adults, approximately 14 days after reaching the adult stage (35 days after placing adult females in clean rearing vessels over a period of 3 days)
Source: Cultured by ibacon
Acclimation period: 35 days
Test chamber: Glass containers (volume: 100 mL; diameter: 5 cm), closed, filled with 20 ± 1.0 g artificial soil dry weight
Test medium: Artificial soil prepared according to OECD 226, maximum water holding capacity of the artificial soil, as measured: 39%
Diet: Cheese mites (*Tyrophagus putrescentiae*)
Water content of soil: Initiation: 19.9% to 21.2% equivalent to 51.1% to 54.2% of the maximum water holding capacity
Termination: 18.6% to 19.6% equivalent to 47.7% to 50.4% of the maximum water holding capacity
Soil pH: 5.9 to 6.0 at test start; 5.7 to 5.9 at test termination
Environmental conditions: Temperature: Within a range of 18 to 22°C
Photoperiod: 16 hour light, 8 hour dark, photoperiod within a range of 400 to 800 lux

Methodology

1. In-life dates
19-JAN-2018 to 05-FEB-2018
2. Experimental treatments
A study was conducted to determine the effect of GF-3969 on the mortality and reproduction of the predatory soil mite (*Hypoaspis aculeifer*). Eight replicates for the control and four replicates per test item group, containing ten predatory mites each (total 80 individuals per control and 40 individuals per test item group) were each exposed for 14 days to nominal concentrations of 62.5, 125, 250, 500 and 1000 mg GF-3969/kg dry artificial soil plus 42.2, 84.4, 168.8, 337.6 and 675.2 mg Codacide/kg dry artificial soil and to an untreated control (deionized water only). A reference item (dimethoate) is tested at least once a year to ensure sensitivity of the test system at five concentrations. The most recent test relevant to this study was conducted in September/October 2017.
3. Observations
After the 14-day exposure period, surviving soil mites were extracted by a heat gradient. The mean number of adults in each treatment group was determined. The mean number of juveniles produced in each treatment group over 14-day exposure period was also determined and the percent reduction in juveniles produced relative to the untreated control group was calculated.
4. Statistics
Mortality data were analysed for significance by using Fisher's Exact Test (one-sided greater, alpha = 0.05). Reproduction data were tested for normal distribution and homoscedasticity using Shapiro-Wilk's test and Levene's test (alpha = 0.05).
Further statistical evaluation of the NOEC for reproduction was performed using Williams t-test (multiple comparison, alpha = 0.05, one-sided smaller).

The EC values for reproduction were not determined by a statistical analysis due to the lack of a concentration-response relationship.

RESULTS AND DISCUSSION

All validity criteria were met. The reference item caused statistically significant effects on reproduction at a concentration of 3.25 mg dimethoate/kg dry artificial soil and above; the EC₅₀ for reproduction was 4.12 mg dimethoate/kg dry artificial soil.

A summary of the results is provided in the table below.

Table A 36: The effects on mortality and reproduction of the soil mite, *Hypoaspis aculeifer*, exposed to GF-3969 in artificial soil for 14 days

Nominal GF-3969 + Codacide concentration (mg/kg soil dry weight)	Mean % mortality ^a	Reproduction	
		Mean juveniles per replicate ^a	% of control
Untreated control (0.0)	3	194	-
62.5 + 42.2	3	201	103
125 + 84.4	0	196	101
250 + 168.8	0	215	110
500 + 337.6	0	207	106
1000 + 675.2	3	208	107

a There were no significant differences compared to the control (mortality: Fisher's Exact Test, one-sided greater, alpha = 0.05; number of juveniles: Williams t-test, one-sided smaller, alpha = 0.05)

CONCLUSION

The 14-day EC₁₀, EC₂₀ and EC₅₀ based on reproduction could not be determined by a statistical analysis due to the lack of a concentration-response relationship but were estimated to be greater than 1000 mg GF-3969 + 675.2 mg Codacide/kg dry artificial soil.

The Lowest-Observed-Effect Concentration (LOEC) for GF-3969 based on mortality and reproduction was estimated to be greater than 1000 mg GF-3969 + 675.2 mg Codacide/kg dry artificial soil. The 14-day No-Observed-Effect Concentration (NOEC) based on mortality and reproduction was determined to be 1000 mg GF-3969 + 675.2 mg Codacide/kg dry artificial soil.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
soil mite	<i>Hypoaspis aculeifer</i>	GF-3969 plus adjuvant surfactant Codacide	14 d	EC ₅₀ LOEC NOEC	>1000 >1000 1000	mg product/kg dry soil

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

No new or additional studies have been submitted.

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

A 2.5.1 Study 1, DuPont-49938

Comments of zRMS:	<p>The study was performed fully in line with OECD 216 with no deviations.</p> <p>Information regarding effects on carbon mineralisation is no longer a data requirement and for this reason the part of the study pertaining to carbon mineralisation was not validated by the zRMS and is struck through.</p> <p>All the validity criteria were met:</p> <ul style="list-style-type: none"> - The variation between replicate control samples should be $\pm 15\%$ (was 1.20% on day 43). <p>Overall the study is considered acceptable.</p> <p>It may be concluded that the effects of the test item on soil nitrogen formation rates were < 25 % at the end of the study period (43 days) up to 10.4 mg product/kg soil dw</p>
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Reference:	KCP 10.5/01
Report:	Hammesfahr, U., (2018); Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isoxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) plus KG691 surfactant: Assessment of the effects on soil microflora
DuPont Report No.:	DuPont-49938
Testing Facility Report No.:	128711080
Guidelines	OECD 216 (2000), OECD 217 (2000)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable

EXECUTIVE SUMMARY

A laboratory soil microflora study was conducted in a loamy sand soil to determine the effects of GF-3969 plus **adjuvant** ~~surfactant~~ DPX-KG691 on nitrogen transformation ~~and soil respiration~~. This study was conducted according to OECD-Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation Test, Guideline 216 ~~and OECD-Guideline for the Testing of Chemicals, Soil Microorganisms: Carbon Transformation Test, Guideline 217, (21 January 2000)~~. GF-3969 was dissolved in pure water by weighing out 59.3 mg DPX-E9636-227, 18.47 mg DPX-M6316 -323 and 22.20 mg DPX-X4145-021; after addition of 74 μL **adjuvant** ~~surfactant~~ DPX-KG691, the solution was filled up with pure water to a combined stock solution of 100 mL and applied to the soil at nominal test concentrations of 1.04 mg test item plus 0.77 μL **adjuvant** ~~surfactant~~ and 10.4 mg test item plus 7.70 μL **adjuvant** ~~surfactant~~ /kg soil dry weight. The control consisted of soil mixed with pure water. At the end of ~~28 days for soil respiration and~~ 43 days for nitrogen transformation, deviations in soil containing up to 10.4 mg GF-3969 plus 7.7 μL **adjuvant** ~~surfactant~~ /kg soil dry weight were <25%, the effect threshold specified by the OECD test guidelines, when compared to the control.

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-3969 plus adjuvant surfactant DPX-KG691
Synonym:	Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isoxadifen ethyl 50WG (GF-3969) A Blend of Paste Extruded Granules (14.82% + 9.26% Active)
Uninverted CAS Name:	None for the formulation
CAS Registry Number:	None for the formulation

Stability of test compound: The test item is considered to be stable under test conditions.

Control: Untreated soil
Test vehicle: Pure water
Reference item: Sodium chloride (tested once per year). Recent test performed at 16 g/kg dws resulted with inhibitory effect on nitrogen transformation of >60% on day 28 and >80% on day 97.

Test System

Test organism: Soil microflora in a natural soil
Source: Fallow land near Mechtersheim, Germany
Test chambers: Nitrogen transformation test: 500 mL plastic boxes with perforated plastic lids containing approximately 400 g soil dry weight
~~Respiration test: 1000 mL plastic boxes, with perforated plastic lids containing approximately 800 grams of soil dry weight~~
Substrates: Lucerne meal: 5 g/kg soil dry weight (nitrogen determination),
~~Glucose: 3 g/kg soil wet weight (short term respiration study)~~
Acclimation period: ~~28 days for soil respiration~~
43 days for nitrogen transformation
Environmental conditions
Temperature: 20°C ± 2°C
Photoperiod: Continuous darkness
Soil: Natural soil
Soil type: Loamy sand soil
Soil pH: 7.2-7.4
% total organic carbon: 1.01
CEC (meq / 100 g dry weight): 15.7
Water holding capacity (%): 41.6
Soil moisture range during test % of water holding capacity: 43-46

Purity and composition

The certificates of analysis include all necessary specification of purity and composition of GF-3969 plus adjuvant surfactant DPX-KG691 and are provided by the Sponsor.

Methodology

1. In-life initiated/completed
20 NOV 2017 to 04 JAN 2018
2. Experimental treatments
A laboratory study was conducted in a loamy sand soil to determine the effects of GF-3969 plus adjuvant surfactant DPX-KG691 on nitrogen transformation and soil respiration. GF-3969 was dissolved in pure water by weighing out 59.3 mg DPX-E9636-227, 18.47 mg DPX-M6316 -323 and 22.20 mg DPX-X4145-021; after addition of 74 µL adjuvant surfactant DPX-KG691, the solution was filled up with pure water to a combined stock solution of 100 mL and mixed with the soil at nominal test concentrations of 1.04 mg test item plus 0.77 µL adjuvant surfactant and 10.4 mg test item plus 7.70 µL adjuvant surfactant /kg soil dry weight. The control consisted of soil treated with pure water. The reference item (positive control), sodium chloride, is tested once a year at a concentration of 16 g/kg dry weight. Samples for soil respiration and nitrogen determination were incubated for 28 and 43 days, respectively.

3. Observations
Samples were collected for determination of nitrogen transformation ~~and soil respiration~~ at days 0, 7, 14, and 28 following application of the test item and additionally at day 43 for nitrogen transformation.
4. Statistics
R/S-Test and Levene's-Test ($\alpha = 0.01$): normality and homogeneity of variance
Student t-test, two sided, $\alpha = 0.05$: test for significant differences between the treatment groups and the control group.

Calculations

% deviation from the control = (value of test item * 100 / value of control) - 100;

Nitrate formation rate cumulative (mg/day) = the difference between the NO₃-N (mg/kg soil dry weight) content between the sampling day and day 0, divided by the number of sampling days;

Nitrate formation rate incremental (mg/day) = the difference between the NO₃-N (mg/kg soil dry weight) content between the sampling days in intervals, divided by the difference of the number of sampling days.

RESULTS AND DISCUSSION

Effects on the nitrate content of GF-3969 plus **adjuvant** ~~surfactant~~ DPX-KG691 at concentrations of 1.04 mg test item plus 0.77 µL **adjuvant** ~~surfactant~~ and 10.4 mg test item plus 7.70 µL **adjuvant** ~~surfactant~~ /kg soil dry weight were significant for both test item concentrations at days 28 and 43. At the end of the nitrate study (day 43), the deviations of the test item compared to the control soil were 8.07%, and 11.17% and therefore in the trigger range of 25% specified in the OECD guideline 216. Both nitrate formation rates were in the trigger range of 25% according to the OECD guideline 216 at the end of the study. On day 43, the deviations to control were 11.00% and 16.86% for the cumulative nitrate formation rate (statistically significant) and -6.91% and -15.97% for the incremental nitrate formation rate (statistically not significant) for the test concentrations of 1.04 mg test item plus 0.77 µL **adjuvant** ~~surfactant~~ and 10.4 mg test item plus 7.70 µL **adjuvant** ~~surfactant~~ /kg soil dry weight, respectively.

~~At the end of the soil respiration study (day 28), deviations of the test item concentration compared to the control were within the 25% range specified by the OECD guidelines up to and including 10.4 mg test item/kg soil (dry weight equivalent). At day 28, the short term respiration rates in soil treated with GF 3969 plus surfactant DPX KG691 was statistically not significantly different from the control for both test concentrations and differed from the control by 0.65% and 8.95% in the 1.04 mg test item plus 0.77 µL surfactant and 10.4 mg test item plus 7.70 µL surfactant/kg soil dry weight treatments, respectively.~~

Table A 37: Summary of effects of GF-3969 plus adjuvant surfactant DPX-KG691 on nitrate formation and short-term respiration in soil

GF-3969 plus adjuvant surfactant DPX-KG691 concentration ^a	NO ₃ -N levels (day 43)		Nitrate formation rate (day 0 to 43)		Nitrate formation rate (day 28 to 43)		Respiration rate (day 28)	
	mg/kg sdw ^c	% Dev. from control ^b	mg/kg sdw/d ^c	% Dev. from control ^b	mg/kg sdw/d ^c	% Dev. from control ^b	mg CO ₂ /h/sdw ^c	% Dev. from control ^b
Control	47.413	---	0.700	---	0.883	---	40.858	---
1.04 mg GF-3969 plus 0.77 µL adjuvant surfactant DPX-KG691 /kg sdw	51.241*	8.07	0.777*	11.00	0.822	-6.91	40.787	-0.65
10.4 mg GF-3969 plus 7.70 µL adjuvant surfactant DPX-KG691 /kg sdw	52.707*	11.17	0.818*	16.86	0.742	-15.97	9.886	-8.95

a Test item concentrations correspond to 1 time and 10 time the application rate

b Negative value =% inhibition, positive value =% stimulation

c Statistical evaluation (Student t-test, two sided, $\alpha = 0.05$): *significant differences from the control
sdw: soil dry weight

Results for the whole study period and particular sampling intervals are presented below.

	control		test rate 1			test rate 2		
Interval ^a sampling days	mean mg NO ₃ -N/kg soil dry weight per day ^b							
	mg/day	CV %	mg/day	Dev. % ^d	sig. ^e	mg/day	Dev. % ^d	sig. ^e
0 - 7	-0.600	-12.17	-0.112	-81.33	*	0.041	-106.83	*
0 - 14	0.352	12.78	0.673	91.19	*	0.842	139.20	*
0 - 28	0.603	10.95	0.754	25.04	*	0.859	42.45	*
0 - 43	0.700	2.14	0.777	11.00	*	0.818	16.86	*
Interval ^a sampling days	mean mg NO ₃ -N/kg soil dry weight per day ^c							
	mg/day	CV %	mg/day	Dev. % ^d	sig. ^e	mg/day	Dev. % ^d	sig. ^e
0 - 7	-0.600	-12.17	-0.112	-81.33	*	0.041	-106.83	*
7 - 14	1.303	6.75	1.457	11.82	*	1.642	26.02	*
14 - 28	0.853	16.18	0.834	-2.23	n.s.	0.876	2.70	n.s.
28 - 43	0.883	13.25	0.822	-6.91	n.s.	0.742	-15.97	n.s.

^a: time interval

^b: calculated from the mean values of NO₃-N content between the sampling date and day 0

^c: calculated from the mean values of NO₃-N content between each sampling date

^d: deviation from control

^e: sig.: significance according Student-t-test, two sided, $\alpha = 0.05$ (* = significant; n. s.: not significant)

CV: coefficient of variation (calculated as SD / mean value * 100)

CONCLUSION

GF-3969 plus adjuvant surfactant DPX-KG691 has no effect on nitrogen transformation or respiration at concentrations up to and including 10.4 mg GF-3969 plus 7.70 µL adjuvant surfactant DPX-KG691/kg soil dry weight (deviations between treatments and controls for both nitrogen and carbon transformation tests were in the range of 25% deviation as specified by the OECD guidelines at days 43 and 28, respectively).

	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
N-mineralisation	GF-3969 plus adjuvant surfactant DPX-KG691	43 d	Nitrate formation rate	10.4 ±25%	mg product/kg soil dw
C-mineralisation	GF-3969 plus surfactant DPX-KG691	28 d	CO₂-formation	10.4 ±25%	mg product/kg soil dw

A 2.5.2 Study 2, DuPont-49976

Comments of zRMS:	<p>The study was performed fully in line with OECD 216 with no deviations.</p> <p>Information regarding effects on carbon mineralisation is no longer a data requirement and for this reason the part of the study pertaining to carbon mineralisation was not validated by the zRMS and is struck through.</p> <p>All the validity criteria were met:</p> <ul style="list-style-type: none"> - The variation between replicate control samples should be ± 15% (was 1.20% on day 43). <p>Overall the study is considered acceptable.</p> <p>It may be concluded that the effects of the test item on soil nitrogen formation rates were < 25 % at the end of the study period (43 days) up to 10.4 mg product/kg soil dw</p>
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Reference:	KCP 10.5/02
Report:	Hammesfahr, U., (2018); Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isoxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) plus codacide: Assessment of the effects on soil microflora
DuPont Report No.:	DuPont-49976
Testing Facility Report No.:	128721080
Guidelines	OECD 216 (2000), OECD 217 (2000)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable

EXECUTIVE SUMMARY

A laboratory soil microflora study was conducted in a loamy sand soil to determine the effects of GF-3969 on nitrogen transformation ~~and soil respiration~~. This study was conducted according to OECD-Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation Test, Guideline 216 ~~and OECD Guideline for the Testing of Chemicals, Soil Microorganisms: Carbon Transformation Test, Guideline 217, (21 January 2000)~~. GF-3969 was dissolved in pure water by weighing out 59.31 mg DPX-E9636-227, 18.45 mg DPX-M6316 -323 and 22.17 mg DPX-X4145-021; after addition of 74 µL **adjuvant** surfactant Codacide, the solution was filled up with pure water to a combined stock solution of 100 mL and applied to the soil at nominal test concentrations of 1.04 mg test item plus 0.77 µL **adjuvant** surfactant and 10.4 mg test item plus 7.70 µL **adjuvant** surfactant /kg soil dry weight. The control consisted of soil mixed with pure water. At the end of ~~28 days for soil respiration and~~ 43 days for nitrogen transformation, deviations in soil containing up to 10.4 mg GF-3969 plus 7.70 µL **adjuvant** surfactant /kg soil dry weight were <25%, the effect threshold specified by the OECD test guidelines, when compared to the control.

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):

GF-3969 plus **adjuvant** surfactant Codacide

Synonym:

Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isoxadifen

ethyl 50WG (GF-3969) A Blend of Paste Extruded Granules (14.82% + 9.26% Active)
Uninverted CAS Name: None for the formulation
CAS Registry Number: None for the formulation
Stability of test compound: The test item is considered to be stable under test conditions.
Control: Untreated soil
Test vehicle: Pure water
Reference item: Sodium chloride (tested once per year). Recent test performed at 16 g/kg dws resulted with inhibitory effect on nitrogen transformation of >60% on day 28 and >80% on day 97.

Test System

Test organism: Soil microflora in a natural soil
Source: Fallow land near Mechtersheim, Germany
Test chambers: Nitrogen transformation test: 500 mL plastic boxes with perforated plastic lids containing approximately 400 g soil dry weight
~~Respiration test: 1000 mL plastic boxes, with perforated plastic lids containing approximately 800 grams of soil dry weight~~
Substrates: Lucerne meal: 5 g/kg soil dry weight (nitrogen determination),
~~Glucose: 3 g/kg soil wet weight (short term respiration study)~~
Acclimation period: ~~28 days for soil respiration~~
43 days for nitrogen transformation
Environmental conditions
Temperature: 20°C ± 2°C
Photoperiod: Continuous darkness
Soil: Natural soil
Soil type: Loamy sand soil
Soil pH: 7.2-7.4 ~~7.3~~
% total organic carbon: 1.01
CEC (meq / 100 g dry weight): 15.7
Water holding capacity (%): 41.6
Soil moisture range during test % of water holding capacity: 42-46

Purity and composition

The certificate of analysis includes all necessary specification of purity and composition of GF-3969 and is provided by the Sponsor.

Methodology

1. In-life initiated/completed
20 NOV 2017 to 04 JAN 2018
2. Experimental treatments
A laboratory study was conducted in a loamy sand soil to determine the effects of GF-3969 plus adjuvant surfactant Codacide on nitrogen transformation and soil respiration. GF-3969 was dissolved in pure water by weighing out 59.31 mg DPX-E9636-227, 18.45 mg DPX-M6316 -323 and 22.17 mg DPX-X4145-021; after addition of 74 µL adjuvant surfactant Codacide, the solution was filled up with pure water to a combined stock solution of 100 mL and mixed with the soil at nominal test concentrations of 1.04 mg test item plus 0.77 µL

adjuvant ~~surfactant~~ and 10.4 mg test item plus 7.70 μL **adjuvant** ~~surfactant~~ /kg soil dry weight. The control consisted of soil treated with pure water. The reference item (positive control), sodium chloride, is tested once a year at a concentration of 16 g/kg dry weight. Samples for ~~soil respiration and~~ nitrogen determination were incubated for ~~28 and~~ 43 days, ~~respectively.~~

3. Observations

Samples were collected for determination of nitrogen transformation ~~and soil respiration~~ at days 0, 7, 14, and 28 following application of the test item and additionally at day 43 for nitrogen transformation.

4. Statistics

R/S-Test and Levene's-Test ($\alpha = 0.01$): normality and homogeneity of variance

Student t-test, two sided, $\alpha = 0.05$: test for significant differences between the treatment groups and the control group.

Calculations

% deviation from the control = (value of test item * 100 / value of control) - 100;

Nitrate formation rate cumulative (mg/day) = the difference between the $\text{NO}_3\text{-N}$ (mg/kg soil dry weight) content between the sampling day and day 0, divided by the number of sampling days;

Nitrate formation rate incremental (mg/day) = the difference between the $\text{NO}_3\text{-N}$ (mg/kg soil dry weight) content between the sampling days in intervals, divided by the difference of the number of sampling days.

RESULTS AND DISCUSSION

Effects on the nitrate content of GF-3969 at concentrations of 1.04 mg test item plus 0.77 μL **adjuvant** ~~surfactant~~ and 10.4 mg test item plus 7.70 μL **adjuvant** ~~surfactant~~ /kg soil dry weight were significant for both test item concentrations at days 28 and 43. At the end of the nitrate study (day 43), the deviations of the test item compared to the control soil were 9.28%, and 12.52% and therefore in the trigger range of 25% specified in the OECD guideline 216.

Both nitrate formation rates were in the trigger range of 25% according to the OECD guideline 216 at the end of the study. On day 43, the deviations to control were 13.57% and 19.14% for the cumulative nitrate formation rate (statistically significant) and -17.89% and -8.38% for the incremental nitrate formation rate (statistically significant for the low test rate) for the test concentrations of 1.04 mg test item plus 0.77 μL **adjuvant** ~~surfactant~~ and 10.4 mg test item plus 7.70 μL **adjuvant** ~~surfactant~~ /kg soil dry weight, respectively.

~~At the end of the soil respiration study (day 28), deviations of the test item concentration compared to the control were within the 25% range specified by the OECD guidelines up to and including 10.4 mg test item/kg soil (dry weight equivalent). At day 28, the short term respiration rates in soil treated with GF 3969 plus surfactant Codacide was statistically not significantly different from the control for both test rates and differed from the control by 2.25% and 2.64% in the 1.04 mg test item plus 0.77 μL surfactant and 10.4 mg test item plus 7.70 μL surfactant/kg soil dry weight treatments, respectively.~~

Table A 38: Summary of effects of GF-3969 plus adjuvant surfactant Codacide on nitrate formation and short-term respiration in soil

GF-3969 concentration ^a	NO ₃ -N levels (day 43)		Nitrate formation rate (day 0 to 43)		Nitrate formation rate (day 28 to 43)		Respiration rate (day 28)	
	mg/kg sdw ^c	% Dev. from control ^b	mg/kg sdw/d ^c	% Dev. from control ^b	mg/kg sdw/d ^c	% Dev. from control ^b	mg CO ₂ /h/sdw ^c	% Dev. from control ^b
Control	47.413	---	0.700	---	0.883	---	40.858	---
1.04 mg GF-3969 plus 0.77 µL adjuvant surfactant Codacide /kg sdw	51.812*	9.28	0.795*	13.57	0.725*	-17.89	40.641	-2.25
10.4 mg GF-3969 plus 7.70 µL adjuvant surfactant Codacide /kg sdw	53.349*	12.52	0.834*	19.14	0.809	-8.38	40.571	-2.64

a Test item concentrations correspond to 1 time and 10 time the application rate

b Negative value =% inhibition, positive value =% stimulation

c Statistical evaluation (Student t-test, two sided, $\alpha = 0.05$): *significant differences from the control
sdw: soil dry weight

Results for the whole study period and particular sampling intervals are presented below.

Interval ^a sampling days	control		test rate 1			test rate 2		
	mg/day	CV %	mg/day	Dev. % ^d	sig. ^e	mg/day	Dev. % ^d	sig. ^e
mean mg NO ₃ -N/kg soil dry weight per day ^b								
0 - 7	-0.600	-12.17	0.076	-112.67	*	-0.073	-87.83	*
0 - 14	0.352	12.78	0.757	115.06	*	0.812	130.68	*
0 - 28	0.603	10.95	0.832	37.98	*	0.848	40.63	*
0 - 43	0.700	2.14	0.795	13.57	*	0.834	19.14	*
mean mg NO ₃ -N/kg soil dry weight per day ^c								
0 - 7	-0.600	-12.17	0.076	-112.67	*	-0.073	-87.83	*
7 - 14	1.303	6.75	1.437	10.28	n.s.	1.696	30.16	*
14 - 28	0.853	16.18	0.908	6.45	n.s.	0.884	3.63	n.s.
28 - 43	0.883	13.25	0.725	-17.89	*	0.809	-8.38	n.s.

^a: time interval

^b: calculated from the mean values of NO₃-N content between the sampling date and day 0

^c: calculated from the mean values of NO₃-N content between each sampling date

^d: deviation from control

^e: sig.: significance according Student-t-test, two sided, $\alpha = 0.05$ (* = significant; n. s.: not significant)

CV: coefficient of variation (calculated as SD / mean value * 100)

CONCLUSION

GF-3969 plus adjuvant surfactant Codacide has no effect on nitrogen transformation or respiration at concentrations up to and including 10.4 mg GF-3969 plus 7.70 µL adjuvant surfactant Codacide/kg soil dry weight (deviations between treatments and controls for both nitrogen and carbon transformation tests were in the range of 25% deviation as specified by the OECD guidelines at days 43 and 28, respectively).

	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
N-mineralisation	GF-3969 plus adjuvant surfactant Codacide	43 d	Nitrate formation rate	10.4 ±25%	mg product/kg soil dw
C-mineralisation	GF-3969 plus surfactant Codacide	28 d	CO ₂ -formation	40.4 ±25%	mg product/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

No new or additional studies have been submitted.

A 2.6.2 KCP 10.6.2 Testing on non-target plants

A 2.6.2.1 Study 1, DuPont-49939

Comments of zRMS:	<p>The study was performed in line with OECD 208 with minor deviations.</p> <p>It was noted that the maximum recorded air humidity (97%) during the study slightly exceed the recommended maximum of 95% but the mean humidity was 71%. The minimum recorded light intensity (202 $\mu\text{E}/\text{m}^2/\text{s}$) as well as the mean light intensity during the study (288 $\mu\text{E}/\text{m}^2/\text{s}$) were below the recommended minimum of 300 $\mu\text{E}/\text{m}^2/\text{s}$. The maximum recorded light intensity was 400 $\mu\text{E}/\text{m}^2/\text{s}$. It should be noted that measurements of light intensity included activating/deactivating of the lights in the growth chamber (starting phase and ending phase) and this the reason why the mean light intensity was below the minimum recommended by OECD 208. According to the study authors, deviations from the test conditions recommended by the test guideline were short-term (<2 hours). Since all validity criteria were met, they are considered to have no impact on the study results.</p> <p>Additionally, the visual injury rating system (e.g. for chlorosis, necrosis, wilting, leaf and stem deformations) was not provided but no phytotoxic effects were observed in the control plants so in the opinion of zRMS this deficiency should not invalidate the outcome of the study.</p> <p>It was also noted that in the analytical part of the study due to a human error the mixing ratio of the test item and the adjuvant surfactant for the fortified samples was changed from 1:0.7407 to 1:0.6173 for the highest concentration and to 1:0.9259 for the lowest concentration. As the recovery rates were within the acceptance criteria for both fortification levels, this error is considered to have no impact on the outcome of the analytical results.</p> <p>The analytical measurements of stock solutions showed that the concentrations of both active substances (rimsulfuron and thifensulfuron methyl) were within 80-120% of nominal concentrations.</p> <p>All the validity criteria were met:</p> <ul style="list-style-type: none">- the seedling emergence is at least 70% in the control (was 85-100%),- the seedlings do not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations) in the control and the control plants exhibit only normal variation in growth and morphology for particular species (yes),- the mean survival of emerged control seedlings is at least 90% for the duration of the study (was 95-100%),- environmental conditions for a particular species are identical and growing media contained the same amount of soil matrix, support media, or substrate from the same source (yes). <p>Overall the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>ER_{50, shoot fresh weight} = 5.07 g product/ha (onion)</p> <p>It is noted that endpoints for phytotoxicity were not calculated, although they are</p>
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currently required at the Central Zone level. The Applicant is thus requested to provide respective calculations in the course of the commenting period.

During the commenting period the Applicant provided phytotoxicity endpoints from the study calculated on the basis of the raw data available in the study report. The data has been evaluated and for species were effects of $\geq 50\%$ were observed, Probit analysis with linear maximum likelihood regression was applied on the visual injury data from test end (14 or 21 days) using ToxRat to derive the ER50 values based on visual injury. Due to the lack of dose response, ER₅₀ values could not be calculated for Sorghum, tomato, pea, soybean or oat. The visual injury ER₅₀ for these species is therefore considered to be greater than the highest concentration tested. The ER₅₀ values which could be determined are summarized in the following table.

Seedling emergence – visual injury ER₅₀ values

Species	Visual injury ER ₅₀ [g product/ha]	95% CI [g product/ha]
<i>Brassica napus</i>	33.66	12.27 - 437.51 *
<i>Glycine max</i>	>135	n.a.
<i>Pisum sativum</i>	>135	n.a.
<i>Cucumis sativus</i>	100.43	50.69 - 588.43 *
<i>Beta vulgaris</i>	28.61	16.71 - 50.73
<i>Solanum lycopersicum</i>	>45.0	n.a.
<i>Sorghum bicolor</i>	>45.0	n.a.
<i>Allium cepa</i>	26.1	12.19 - 69.96
<i>Avena sativa</i>	>135	n.a.
<i>Lolium perenne</i>	53.71	26.66 - 177.28 *

* Values considered not reliable since upper CI are outside the tested concentration range

Phytotoxic endpoints reported above are higher than the endpoints determined for fresh weight, which are thus more relevant for the risk assessment.

Reference:	KCP 10.6.2/01
Report:	Spatz, B., (2018); Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isoxadifen ethyl 50WG (DPX-V4B07) A blend of paste extruded granules (14.82% + 9.26% active) plus DPX-KG691 surfactant: Effects on terrestrial (non-target) plants: Seedling emergence and seedling growth test
DuPont Report No.:	DuPont-49939
Testing Facility Report No.:	128711086
Guidelines	OECD 208 (2006)
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable

EXECUTIVE SUMMARY

GF-3969 plus DPX-KG691 **adjuvant** ~~surfactant~~ was tested in a laboratory study for effects of the seedling emergence and seedling growth of ten non-target terrestrial plants species, representing seven families according to OECD test guideline 208 (2006). The species were: *Brassica napus*, *Glycine max*, *Pisum sativum*, *Cucumis sativus*, *Beta vulgaris*, *Solanum lycopersicum*, *Sorghum bicolor*, *Allium cepa*, *Avena sativa* and *Lolium perenne*.

Application was done with a laboratory sprayer. After application the pots were exposed in a growth chamber for 14 or 21 days after 50% germination in the control group. Germination, mortality and phytotoxicity were determined weekly. At the end of the test fresh weight per pot was determined.

The results were analysed with ToxRat Professional, Version 3.2.1, © ToxRat Solutions GmbH.

The most sensitive species in terms of fresh weight were *Allium cepa*, *Beta vulgaris* and *Brassica napus* with ER₅₀ values of 5.07, 9.26 and 9.74 g GF-3969 plus DPX-KG691/ha, respectively. They were followed by *Lolium perenne*, *Cucumis sativus* and *Pisum sativum*. (ER₅₀ values of 22.1, 48.1 and 129 g GF-3969 plus DPX-KG691/ha, respectively).

The least sensitive species were *Sorghum bicolor*, *Solanum lycopersicum*, *Glycine max* and *Avena sativa* for which ER₅₀ values could not be determined due to low effects of GF-3969 plus DPX-KG691.

MATERIALS AND METHODS

Test Item(s)

Test item (Common name): GF-3969
Prepared by blending:
59.26% DPX-E9636-227 25SG,
18.52% DPX-M6316-323 50SG,
22.22% DPX-X4145-021 50WG

Formulation 1

Name: DPX-E9636-227 25SG
Batch #: MAR15EL004
Concentration of a.s.: 25.1% (w/w) rimsulfuron by analysis
CAS #: None for the formulation

Formulation 2

Name: DPX-M6316-323 50SG
Batch #: APR15EL002
Concentration of a.s.: 49.8% (w/w) thifensulfuron methyl by analysis
CAS #: None for the formulation

Formulation 3

Name: DPX-X4145-021 50WG
Batch #: DEC15EL001
Concentration of a.s.: 50.4% (w/w) isoxadifen-ethyl by analysis
CAS #: None for the formulation

Adjuvant surfactant

Name: DPX-KG691
Batch #: JAN15CE035

Stability of test compounds: Not determined in the test system

Control: Deionized water
Test vehicle: Deionized water

Test System

Test organism: Plant species
 Species: *Brassica napus*
Glycine max
Pisum sativum
Cucumis sativus
Beta vulgaris
Solanum lycopersicum
Sorghum bicolor
Allium cepa
Avena sativa
Lolium perenne

Test chamber: Growth chamber
 Environmental conditions: Temperature: 15.2 to 24.7°C
 Relative humidity: 55% - 97%
 Light intensity: 16 h light, 8 h night, minimum 202 µE/m²/s,
 maximum 400 µE/m²/s, mean 288 µE/m²/s

Methodology

- In life initiated/completed:
 11-APR-2018 to 07-MAY-2018
- Experimental treatments
 The day after sowing and watering, the different treatments were applied onto the soil.
 The rates are given in the table below.

Species	Rate [g test item/ha]*								
	0.021	0.062	0.185	0.556	1.67	5.00	15.0	45.0	135
<i>Brassica napus</i>		X	X	X	X	X	X	X	
<i>Glycine max</i>					X	X	X	X	X
<i>Pisum sativum</i>					X	X	X	X	X
<i>Cucumis sativus</i>					X	X	X	X	X
<i>Beta vulgaris</i>					X	X	X	X	X
<i>Solanum lycopersicum</i>	X	X	X	X	X	X	X	X	
<i>Sorghum bicolor</i>				X	X	X	X	X	
<i>Allium cepa</i>					X	X	X	X	X
<i>Avena sativa</i>					X	X	X	X	X
<i>Lolium perenne</i>				X	X	X	X	X	X

* referring to GF-3969

After 50% of the control seeds were germinated, the exposure time of 14 or 21 days started, depending on the development speed of each species.

- Observations
 During the time of exposure, germination, mortality and phytotoxicity were determined weekly. At test end, fresh weight was determined, additionally.
- Statistics
 The results were analysed with ToxRat Professional, Version 3.2.1, © ToxRat Solutions GmbH.
 Fresh weight data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk's test ($\alpha = 0.05$) and the Levene's test ($\alpha = 0.05$). If the data were normally distributed and homogeneous the Dunnett's t-test (multiple comparison, one-sided smaller, $\alpha = 0.05$) or if the data showed a monotonic dose response the Williams t-test (multiple

comparison, one-sided smaller, $\alpha = 0.05$) was used for comparing treatment groups and control. If the data were not normally distributed the Bonferroni-Holm U-test (multiple comparison, one-sided smaller, $\alpha = 0.05$) was used.

RESULTS AND DISCUSSION

Following ER₅₀ values were determined based on fresh weight.

	NOER [g test item/ha]	LOER	Statistical Analysis	ER ₁₀ [g test item/ha]	ER ₂₀	ER ₅₀	Statistical Analysis
<i>Brassica napus</i>	5.00	15.0	¹	1.08	2.29	9.74	⁴
<i>Glycine max</i> **	135	>135	¹	73.5	251*	n.d.	⁴
<i>Pisum sativum</i> **	15.0	45.0	³	2.71	10.2	129	⁴
<i>Cucumis sativus</i>	5.00	15.0	³	7.43	14.1	48.1	⁴
<i>Beta vulgaris</i>	1.67	5.00	³	1.23	2.46	9.26	⁴
<i>Solanum lycopersicum</i>	45.0	>45.0	¹	not calculated due to low effects			
<i>Sorghum bicolor</i> **	45.0	>45.0	¹	15.7	47.2	n.d.	⁴
<i>Allium cepa</i>	1.67	5.00	²	0.600*	1.25	5.07	⁴
<i>Avena sativa</i>	45.0	135	¹	not calculated due to low effects			
<i>Lolium perenne</i>	5.00	15.0	¹	6.91	10.3	22.1	⁴

All values refer to the test item GF-3969. The amount of **adjuvant** ~~surfactant~~ DPX-KG691 arises out of the mixing ratio of 135 g product (35.2 g a.s.) to 100 mL DPX-KG691 and is not mentioned in detail.

results represent rounded values based on exact data

n.d. not determined due to mathematical reasons

* the ER_x-value is extrapolated

** poor dose-response fitting, therefore the values could be not valid

¹ multiple comparison Dunnett's t-test, $\alpha = 0.05$

² multiple comparison Williams t-test, $\alpha = 0.05$

³ multiple comparison Bonferroni-Holm U-test, $\alpha = 0.05$

⁴ Probit Analysis, cl = confidence limits

CONCLUSION

In the following paragraph the reported values refer to the test item GF-3969. The amount of the **adjuvant** ~~surfactant~~ DPX-KG691 arises out of the mixing ratio of 135 g product (35.2 g a.s.) to 100 mL DPX-KG691 and is not mentioned in detail.

GF-3969 plus DPX-KG691 was tested for effects on seedling emergence and seedling growth of ten plant species out of seven different plant families.

The analytical recovery rate of the active ingredient Rimsulfuron in the stock solution was 94% of the nominal value. The analytical recovery rate for the active ingredient Isoxadifen ethyl was 78% of the nominal value and 94% of the nominal value for the active ingredient Thifensulfuron methyl respectively.

The most sensitive species in terms of fresh weight were *Allium cepa*, *Beta vulgaris* and *Brassica napus* with ER₅₀ values of 5.07, 9.26 and 9.74 g GF-3969 plus DPX-KG691/ha, respectively. They were followed by *Lolium perenne*, *Cucumis sativus* and *Pisum sativum*. (ER₅₀ values of 22.1, 48.1 and 129 g GF-3969 plus DPX-KG691/ha, respectively).

The least sensitive species were *Sorghum bicolor*, *Solanum lycopersicum*, *Glycine max* and *Avena sativa* for which ER₅₀ values could not be determined due to low effects of GF-3969 plus DPX-KG691.

The germination rate was not statistically significantly reduced for all plant species except for *Lolium perenne*. For this species a statistically significantly reduced germination of 40% was observed at 135 g GF-3969 plus DPX-KG691/ha.

Statistically significant mortality was observed for *Allium cepa* (47% at 135 g GF-3969 plus DPX-KG691/ha). Mortality which was not statistically significant was observed for *Lolium perenne* at 15.0

g GF-3969 plus DPX-KG691/ha and at higher rates (6 to 13%) and in the control of *Glycine max* (5%). No mortality was observed for the other species tested.

Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
<i>Brassica napus</i> ¹⁾ <i>Glycine max</i> ²⁾ <i>Pisum sativum</i> ³⁾ <i>Cucumis sativus</i> ⁴⁾ <i>Beta vulgaris</i> ⁵⁾ <i>Solanum lycopersicum</i> ⁶⁾ <i>Sorghum bicolor</i> ⁷⁾ <i>Allium cepa</i> ⁸⁾ <i>Avena sativa</i> ⁹⁾ <i>Lolium perenne</i> ¹⁰⁾	GF-3969 plus adjuvant surfactant DPX-KG691	21 d	1) ER ₅₀ 2) ER ₅₀ 3) ER ₅₀ 4) ER ₅₀ 5) ER ₅₀ 6) ER ₅₀ 7) ER ₅₀ 8) ER ₅₀ 9) ER ₅₀ 10) ER ₅₀	1) 9.74 2) n.d. 3) 129 4) 48.1 5) 9.26 6) not calculated due to low effects 7) n.d. 8) 5.07 9) not calculated due to low effects 10) 22.1	g product/ha

A 2.6.2.2 Study 2, DuPont-49942

Comments of zRMS:	<p>The study was performed in line with OECD 227 with deviations.</p> <p>It was noted that the pots for onion were 11 cm in diameter which is smaller than the recommended minimum pot diameter of 15 cm.</p> <p>During the test with corn, oat, sorghum, cucumber, and oilseed rape on one occasion the temperature slightly exceed the recommended maximum of 32°C and was 34°C but the mean temperature over the study period was 25°C which is within the recommended range of 12-32°C. The relative humidity fell below the recommended minimum of 45% on several occasions down to a minimum of 33% but the mean humidity over the study period was 68% which is within the recommended range of 45-95%.</p> <p>During the test with onion, pean, soybean, sugar beet, and tomato the relative humidity also fell well below the recommended minimum on two occasions to a minimum of 39% but the mean humidity over the study period was 74%.</p> <p>The analytical measurements of stock solutions showed that the concentrations of both active substances (rimsulfuron and thifensulfuron methyl) were within 80-120% of nominal concentrations.</p> <p>Not all the validity criteria were met: Even though the mean control plants survival was >90% for the duration of the study and the score of 100 or 99 was calculated for all species on day 21 (test termination), on days 7 and 14 the control (both, negative and adjuvant surfactant) plants exhibited phytotoxic effects such as chlorosis, necrosis, leaf curl, lodging or wilting. Some slight phytotoxic effects in single replicates could have been accepted, however in this study for some species phytotoxic effects were seen in majority of replicates with effects comparable with lower rates test item groups in case of sorghum and oilseed rape (e.g. mean score for visual effects on sorghum in adjuvant surfactant control on day 14 was 90 with phytotoxicity scores ranging from 76 to 98; in case of oilseed rape the mean phytotoxicity score in adjuvant surfactant control was 88 with scores ranging from 74 to 94, no mean score was calculated for the negative controls, but the scores in particular replicates ranged from 72 to 100). In opinion of the zRMS phytotoxic effects observed on day 14 could have impact on growth of the control plants and it cannot be excluded that shoot height and dry weight could be lower comparing to not affected control</p>
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replicates, even if recovery from phytotoxic effects was seen on day 21. Analysis of the shoot dry weight data for oilseed rape indicates that this possible, since the lowest shoot dry weight on day 21 was observed in replicate in which most pronounced phytotoxic effects were observed. This could have impact on endpoints calculated for the test item, since reduced shoot weight of control plants could lead to lower deviation of this parameter from control in test item groups.

It should be noted that the validity criteria for lack of phytotoxic effects in control are not indicated to be relevant for the test termination only and for this reason no phytotoxic effects in controls should be observed over the whole study period.

Furthermore, no information on seedling emergence was provided (seedling emergence at $\geq 70\%$ is one of the validity criteria indicated in OECD 227 for all control and test item groups).

The phytotoxic effects in control groups could be potentially explained by too low light intensity. According to the study report the light intensity was in range of 9.7 to 17.8 mol/m²/d, which gives range of 168 to 309 $\mu\text{mol}/\text{m}^2/\text{s}$. It is to be noted that at majority of days the light intensity was below the recommended minimum of 300 $\mu\text{mol}/\text{m}^2/\text{s}$. On the other hand, measurements of light intensity included activating/deactivating of the lights in the growth chamber, which reduced the daily light intensity and in case validity criteria were met, the deviation in light intensity would not be considered to have impact on the test results. However, in this test it cannot be excluded that phytotoxic symptoms of the control plants were caused by too low light intensity.

During the commenting period the Applicant referred to the concerns of the zRMS regarding symptoms of phytotoxicity observed in some control plants of sorghum and oilseed rape. The position paper is presented under the study summary for the reference of the concerned Member States. In the position paper it was proposed by the Applicant to exclude the oilseed rape and sorghum control replicates with visible phytotoxic symptoms. After exclusion of these replicates and merging data for water and adjuvant control (which was justified since adjuvant have not induced phytotoxic effects) there was 45 and 35 sorghum and oilseed rape control plants, respectively, which is considered sufficient for statistical evaluation (OECD 227 indicates minimum 20 control plants to be sufficient). The endpoints for the 2 plants were recalculated and resulted with only slightly lower ER₅₀ values, showing that the phytotoxic effects observed in some control replicates of sorghum and oilseed rape had marginal impact on the mean growth of control plants and in consequence – the study results. Of all tested plants, sugar beet remained the species with the lowest endpoint. The zRMS agrees with the approach proposed by the Applicant, since after exclusion of the control replicates with phytotoxic symptoms, only healthy control plants were included for comparison with the treatment groups.

After recalculation performed by the Applicant, the study is considered acceptable with following endpoint relevant for the risk assessment:

Lowest ER₅₀ = 1.61 g product/ha (sugar beet)

~~Therefore, the study is considered not acceptable.~~

Reference:	KCP 10.6.2/02
Report:	Arnie, J.R., AufderheidieMcKelvey, R.A., Aufderheide, J.A., Lockard, L.A., Zhang, L. (2020); Isoxadifen ethyl 50WG/Rimsulfuron 25SG/Thifensulfuron methyl 50SG (DPX-V4B07), A Blend of Paste Extruded Granules Plus Isodecylalcohol Ethoxylated (DPX-KG691) Surfactant: A Greenhouse Study to Investigate the Effects on Vegetative Vigor of Ten Terrestrial Plants Following Foliar Exposure
DuPont Report No.:	DuPont-49942
Testing Facility Report No.:	112P-292
Guidelines	OECD 227 and OCSPP 850.4150
Deviations:	Yes (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable after exclusion of the control replicates of sorghum and oilseed rape with phytotoxic symptoms Unacceptable

EXECUTIVE SUMMARY

A glasshouse study was conducted to generate dose response data for GF-3969 when applied post-emergence to monocotyledon and dicotyledon species.

The methodology for the study was based on OECD Guideline 227 (July 2006) Terrestrial (Non-Target) Plant Test: Vegetative Vigour Test.

The test species consisted of four monocotyledon species (onion, oat, sorghum, and corn) and six dicotyledon species (sugar beet, oilseed rape, cucumber, soybean, tomato, and pea). Species tested represented the plant families of Liliaceae, Poaceae, Chenopodiaceae, Brassicaceae, Cucurbitaceae, Fabaceae, and Solanaceae.

GF-3969 was applied at six different rates (136, 45.5, 15.2, 5.05, 1.68, and 0.561 g product/ha) to oat, corn, sorghum, cucumber, oilseed rape, and tomato, at seven different rates (136, 45.5, 15.2, 5.05, 1.68, 0.561, and 0.187 g product/ha) to soybean, at seven different rates (45.5, 15.2, 5.05, 1.68, 0.561, 0.187, and 0.0624 g product/ha) to onion, at six different rates (15.2, 5.05, 1.68, 0.561, 0.187, and 0.0624 g product/ha) to pea and sugar beet, and compared with a pooled control group of untreated water only and **adjuvant** ~~surfactant~~ and water at a rate of 3.077 mL DPX-KG691 per gram of GF-3969. Applications were made post-emergence to all ten species at growth stage BBCH 12-14 (2 to 4 true leaves).

All species displayed visual injury.

Based on ER₅₀ values for shoot dry weight reduction the most sensitive monocotyledon species to post-emergence application of GF-3969 was sorghum with an ER₅₀ value of 3.00 g product/ha.

Based on ER₅₀ values for ~~shoot dry weight reduction~~ ~~visual response~~ the most sensitive dicotyledon species to post-emergence application of GF-3969 was sugar beet with an ER₅₀ value of 1.61 g product/ha.

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-3969 42.1% Rimsulfuron 25WG (14.82% active) 26.3% Thifensulfuron methyl 50SG (9.26% active) 31.6% Isoxadifen ethyl 50WG (11.11% safener)
Purity:	DPX-E9636-227, Rimsulfuron 25SG: 25.1% (w/w) rimsulfuron DPX-M6316323, Thifensulfuron methyl 50SG: 49.8% thifensulfuron methyl DPX-X4145-021, Isoxadifen ethyl 50WG: 50.4% isoxadifen-ethyl
Description (physical state):	Blend of paste extruded granules
Lot/batch no.:	DPX-E9636-227: MAR15EL004 DPX-M6316-323: APR15EL002 DPX-X4145-021: DEC15EL001

Test system

Monocotyledonous species:	<i>Allium cepa</i> , <i>Avena sativa</i> , <i>Sorghum bicolor</i> , and <i>Zea mays</i>														
Dicotyledonous species:	<i>Beta vulgaris</i> , <i>Brassica napus</i> , <i>Cucumis sativus</i> , <i>Glycine max</i> , <i>Lycopersicon esculentum</i> , and <i>Pisum sativum</i>														
Study type:	Greenhouse study assessing Vegetative Vigour														
Parameters measured:	Number of dead plants: 21 days after application Replicate Shoot Dry weight: 21 days after application Phytotoxicity rating system: Frans and Talbert (1977)														
	<table border="1"><thead><tr><th>Phytotoxicity Score (Frans and Talbert)</th><th>RSCAB R-Score</th></tr></thead><tbody><tr><td>0</td><td>0</td></tr><tr><td>10 – 20</td><td>1</td></tr><tr><td>30 – 40</td><td>2</td></tr><tr><td>50 – 60</td><td>3</td></tr><tr><td>70 – 80</td><td>4</td></tr><tr><td>90 – 100</td><td>5</td></tr></tbody></table>	Phytotoxicity Score (Frans and Talbert)	RSCAB R-Score	0	0	10 – 20	1	30 – 40	2	50 – 60	3	70 – 80	4	90 – 100	5
Phytotoxicity Score (Frans and Talbert)	RSCAB R-Score														
0	0														
10 – 20	1														
30 – 40	2														
50 – 60	3														
70 – 80	4														
90 – 100	5														
Growth conditions:	Temperature (range): 17 to 34°C Photoperiod: 16 hours with natural light augmented with HPS lamps Light intensity (range): 10 and 18 moles per square meter per day (Photosynthetically active radiation, PAR); measured: approx. 168-309 $\mu\text{mol}/\text{m}^2/\text{s}$ Relative humidity: 33 to 94% Water regime and schedules: Sub-irrigation as needed to maintain plant growth Water source/type: Spray Solutions: Municipal water purified by reverse osmosis Sub-irrigation: Well water Pest control method /fertilisation, if used: Slow-release fertilizer														
Growth medium:	Soil type: Artificial greenhouse soil Details of nutrient medium, if used:														
Test concentrations:	pH: 7.0 (in 0.01 M CaCl_2) Nominal: 136, 45.5, 15.2, 5.05, 1.68, and 0.561 g product/ha for oat, corn, sorghum, cucumber, oilseed rape and tomato; 136, 45.5, 15.2, 5.05, 1.68, 0.561, and 0.187 g product/ha for soybean, 45.5, 15.2, 5.05, 1.68, 0.561, 0.187, and 0.0624 g product/ha for onion, 15.2, 5.05, 1.68, 0.561, 0.187, and 0.0624 g product/ha for pea and sugar beet														
Analytical verification:	Samples collected from spray solutions for the highest application rate for verification of each active prior to application.														
Test material application:	Method: Automated research track sprayer equipped with a Teejet 8002 E nozzle Application interval: Single application Reference chemical (if used): None														
Seeds:	Source: not applicable Method of seeding: Planted into standard plastic pots (11 cm diameter for onion, 16 cm diameter for the remaining species, 10 cm deep for onion, 12 cm deep for the remaining species) filled with soil Prior seed treatment/sterilisation: None Number of seeds per replicate pot: 2 or 3 depending on species and plants were thinned as needed to one plant per pot prior to application														
Number of control replicates:	Growth stage at application: 2-5 true leaves, 10 to 56 cm tall 6 per control group (5 individually potted plants per replicate; 30 plants total per control group)														

Number of test concentration 6 per test concentration (5 individually potted plants per replicate; 30 replicates: plants total per test concentration group)

Methodology

Non-target terrestrial plant response to GF-3969 was evaluated on ten terrestrial plant species. For each species, test groups of six replicates containing five individually potted plants (a total of 30 plants per test group of a species) were treated with spray mixtures via foliar application for treatment rates or the control groups. The plants were arranged according to a random block design by species to minimize experimental variation and control bias. Tests were conducted in an artificial soil matrix under greenhouse conditions. Shoot height and numeric visual response of plant condition (including survival) were recorded on days 7, 14, and 21. Shoot dry weight was determined from plant shoots collected at test termination on day 21. The rates producing the NOER, ER₂₅, and ER₅₀ were determined, when possible. The NOER was determined using Jonckheere-Terpstra Step-Down Trend test, William's Multiple Comparison test, or Cochran-Armitage Trend Step-Down test ($\alpha = 0.05$). The NOER for visual response was determined using Rao-Scott Cochran-Armitage by Slices test (RSCABS, $\alpha=0.05$). The ER₂₅, and ER₅₀ estimates were calculated using non-linear regression, where possible. In instances, where non-linear regression models failed to converge linear interpolation was used to provide those estimates. Samples were collected prior to application from spray solution samples for analytical verification (HPLC-VWD) of active ingredient concentrations.

RESULTS AND DISCUSSION

The mean measured concentrations of thifensulfuron methyl in the stock solutions prepared on May 17 and 30 were 94.6% and 95.8% of nominal concentrations, respectively. The mean measured concentrations of rimsulfuron in the stock solutions prepared on May 17 and 30 were 95.7%, and 97.2% of nominal concentrations, respectively. This primary stock solution was the maximum application rate and was used to prepare the 0.0624, 0.187, 0.561, 1.68, 5.05, 15.2, and 45.5 g product/ha spray solutions for onion, sugar beet, soybean, pea, and tomato, where applicable. Visual responses less than 100 in the control replicates for the species tested were considered to be within normal variation for that particular species. Environmental conditions for a particular species were identical and the growth media were from the same source. Mean plant survival was at least 90% in the control groups for the duration of the study. Mortality was observed for onion, oat, sorghum, corn, sugar beet, oilseed rape, and tomato after 21 days. No significant differences were noted between the control group means for any of the species tested. Means comparisons for all species and parameters were based on the pooled control means (n=60 plants). All species displayed visual injury. Sugar beet was the most sensitive species based on shoot dry weight with an estimated ER₅₀ of 1.61 g product/ha. Biological Results are summarized in the tables below.

Table A 39: Observations of % survival, mean visual injury score, shoot dry weight (g), and shoot height (cm) 21 days after application: Monocotyledonous species

Treatment (g prod/ha)	Onion			
	Survival	Visual injury	Shoot dry weight	Shoot height
Pooled Controls	100	99	0.560	34.4
0.0624	100	99	0.559	34.9
0.187	100	99	0.571	34.4
0.561	100	100	0.611	36.2
1.68	100	99	0.592	34.5
5.05	100	80	0.241	25.8
15.2	67	33	0.122	21.6
45.5	40	6	0.055	19.9

Treatment (g prod/ha)	Oat				Sorghum			
	Survival	Visual injury	Shoot dry weight	Shoot height	Survival	Visual injury	Shoot dry weight	Shoot height
Pooled Controls	100	100	4.34	91.8	100	100	8.52	92.5
0.561	100	100	4.34	90.9	100	96	8.50	91.9
1.68	100	99	4.39	91.7	100	100	7.73	97.9
5.05	100	97	4.26	90.7	100	43	1.00	47.5
15.2	100	83	2.10	71.0	20	3	0.50	33.3
45.5	69	20	0.66	38.3	0	0	.	.
136	7	1	0.23	29.5	0	0	.	.

Table A 40: Observations of % survival, mean visual injury score, shoot dry weight (g), and shoot height (cm) 21 days after application: Monocotyledonous species

Treatment (g prod/ha)	Corn			
	Survival	Visual injury	Shoot dry weight	Shoot height
Pooled Controls	100	100	18.34	166.7
0.561	100	99	17.61	164.5
1.68	100	98	17.58	160.7
5.05	100	99	17.13	166.7
15.2	100	99	15.92	165.3
45.5	97	95	17.91	165.1
136	93	93	15.62	162.8

Table A 41: Observations of % survival, mean visual injury score, mean shoot dry weight (g), and mean shoot height (cm) 21 days after application: Dicotyledonous species

Treatment (g product/ha)	Sugar beet				Pea			
	Survival	Visual injury	Shoot dry weight	Shoot height	Survival	Visual injury	Shoot dry weight	Shoot height
Pooled Control	100	99	10.42	42.4	100	97	6.66	37.9
0.0624	100	99	10.49	42.0	100	92	6.26	35.4
0.187	100	99	10.33	42.2	100	100	7.38	40.4
0.561	100	99	10.32	42.0	100	93	6.34	36.4
1.68	100	67	4.96	34.7	100	95	6.77	39.3
5.05	87	23	2.41	22.4	100	94	5.36	35.5
15.2	47	8	1.70	22.1	100	63	2.28	20.7

Treatment (g product/ha)	Cucumber				Oilseed Rape				Tomato			
	Survival	Visual injury	Shoot dry weight	Shoot height	Survival	Visual injury	Shoot dry weight	Shoot height	Survival	Visual injury	Shoot dry weight	Shoot height
Pooled Control	100	100	13.60	138.7	100	99	14.59	50.1	100	98	15.78	83.4
0.561	100	100	13.56	138.9	100	96	13.29	49.6	100	99	13.81	81.8
1.68	100	100	13.70	136.0	100	94	12.02	47.4	100	100	16.00	82.5
5.05	100	95	12.85	113.8	100	82	5.49	39.9	100	97	13.77	80.1
15.2	100	83	10.68	80.5	100	52	1.74	27.4	100	94	13.35	78.2
45.5	100	61	8.60	54.8	17	2	0.39	15.3	100	95	13.42	76.0
136	100	46	7.36	47.3	17	2	0.28	14.4	97	81	10.52	72.5

Table A 42: Observations of % survival, mean visual injury score, shoot dry weight (g), and shoot height (cm) 21 days after application: Dicotyledonous species

Treatment (g product/ha)	Soybean			
	Survival	Visual injury	Shoot dry weight	Shoot height
Pooled Control	100	98	15.4	112.3
0.187	100	100	13.19	112.4
0.561	100	98	12.74	116.6
1.68	100	97	12.24	121.0
5.05	100	99	12.71	99.5
15.2	100	72	5.90	61.4
45.5	100	33	2.09	33.0
136	100	27	1.80	32.0

Table A 43: Reported ER₅₀ values for each species based on g product/ha

Plant	Family	Species	ER50 (g product/ha)	Parameter
<i>Monocots</i>				
Onion	Liliaceae	<i>Allium cepa</i>	5.80 ^a	Shoot dry weight
Oat	Poaceae	<i>Avena sativa</i>	15.9 ^a	Shoot dry weight
Sorghum	Poaceae	<i>Sorghum bicolor</i>	2.98 ^a	Shoot dry weight
Corn	Poaceae	<i>Zea mays</i>	>136 ^b	All parameters
<i>Dicots</i>				
Sugar beet	Chenopodiaceae	<i>Beta vulgaris</i>	1.61 ^c	Shoot dry weight
Oilseed rape	Brassicaceae	<i>Brassica napus</i>	3.82 ^a	Shoot dry weight
Cucumber	Cucurbitaceae	<i>Cucumis sativa</i>	31.4 ^a	Shoot height
Soybean	Fabaceae	<i>Glycine max</i>	11.1 ^a	Shoot dry weight
Tomato	Solanaceae	<i>Lycopersicon esculentum</i>	>136 ^b	All parameters
Pea	Fabaceae	<i>Pisum sativum</i>	10.6 ^a	Shoot dry weight

a Non-linear regression (Bruce-Versteeg, 3P Cum Log-Normal (Probit).
 b Empirically estimated. Inhibition in the treatments was <50%, when compared to the pooled control.
 c Linear interpolation (ICPIN).

Endpoints from the study were recalculated by the Applicant excluding control replicates of oilseed rape and sorghum with visible signs of phytotoxicity. Following endpoints were derived for all species and parameters investigated:

Species	ER ₅₀ [g product.ha]			
	Shoot height	Shoot dry weight	Survival	Visual response
Onion	>45.5	5.8	30.9	10.6
Oat	37.8	15.9	60.4	30.0
Sorghum	6.11	2.98	10.2	4.51
Corn	>136	>136	>136	>136
Sugar beet	11.0	1.61	13.8	2.63
Oilseed rape	16.08	3.82	40.5	11.39
Cucumber	31.4	>136	>136	103
Soybean	23.4	11.1	>136	29.1
Tomato	>136	>136	>136	>136
Pea	16.6	10.6	>15.2	>15.2

CONCLUSION

Visual injury was observed in all species. The most sensitive species based on visual response was sugar beet which had an ER₅₀ of 2.63 g product/ha. The most sensitive species based on shoot height was sorghum with an ER₅₀ of 7.07 g product/ha. The most sensitive species based on shoot dry weight was sugar beet with an ER₅₀ of 1.61 g product/ha. Effects on survival of seedlings were observed in onion, oat, sorghum, sugar beet, and oilseed rape.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Sugar beet	<i>Beta vulgaris</i> (dicot)	GF-3969	21 days	Shoot Dry Weight	1.61	g/ha

A 2.6.2.3 Position paper to Study 2 (DuPont-49942)

Comments of zRMS:	Please, refer to zRMS comments to study 2 (DuPont-49942) summarised above under KCP 10.6.2/02.
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Reference:	KCP 10.6.2/03
Report:	Ellis, S. (2022); Position paper to address zRMS comments on the risk to non-target plants from GF-3969
DuPont Report No.:	-
Testing Facility Report No.:	Not relevant, position paper
Guidelines	Not relevant, position paper
Deviations:	Not relevant, position paper
GLP:	Not relevant, position paper
Acceptability:	See zRMS comments to study 2 (DuPont-49942 summarised above)

Introduction

In the evaluation of GF-3969 the zRMS concluded 'the vegetative vigour test (Arnie et al., 2020, Report No 49942) was considered not valid due to phytotoxic effects observed in controls (especially on day 14), while lack of phytotoxic effects is one of the validity criteria of the OECD 227. It is noted that at test termination on day 21 the control plants recovered, but OECD 227 does not indicate that this validity criterion is relevant only for test termination and for this reason no phytotoxic effects should be observed during the entire study. In the study report no explanation of the phytotoxicity observed in

controls is given and it is thus not known if it was due to nutrient deficiency, overcrowding, unfavourable conditions or accidental exposure to the test item. Nevertheless, growing conditions do not seem to be the reason for these effects, since all plants were kept at the same conditions while chlorosis, necrosis or wilting were observed only in some replicates.

In opinion of the zRMS phytotoxic effects observed on day 14 could have impact on growth of the control plants and it cannot be excluded that shoot height and dry weight could be lower comparing to not affected control replicates, even if recovery from phytotoxic effects was seen on day 21. Analysis of the shoot dry weight data for oilseed rape indicates that this possible, since the lowest shoot dry weight on day 21 was observed in replicate in which most pronounced phytotoxic effects were observed. This could have impact on endpoints calculated for the test item, since reduced shoot weight of control plants could lead to lower deviation of this parameter from control in test item groups'

The study has been assessed based on the validity criteria in accordance with OECD guideline 227 Terrestrial plant test: Vegetative vigour test.

The guideline validity criteria states that for the control plants:

The plants do not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations). Plants exhibit only normal variation in growth and morphology for that particular species;

The applicant agrees with the zRMS comment that it is not clear in the OECD 227 guideline if this criteria is valid only for plants at test termination (day 21) or throughout the study. By day 21 there was no notable effect on visual injury in the control plants, but at day 14 in two species sorghum and oilseed rape visual effects were reported.

To address this concern of the potential effects on visual injury on the growth of the control plants, the applicant has;

1. Provided an evaluation from the CRO, who conducted the study, to ensure that contamination of the controls was avoided during application which may have resulted in the observed effect in the controls and to further clarify their methods for assessing the plants
2. Re-calculated the effect concentrations for the affected species to account for the control replicates with notable visual injury

CRO evaluation

The CRO have provided a statement on the conduct of the study and the evaluation – see Appendix 1 for the full statement.

In summary the CRO is confident there was no potential for the controls to be contaminated with the test item.

1. Water controls were sprayed first then surfactant controls
2. After application the plants are removed from the spray booth. Treated plants are transferred to a different greenhouse.
3. Spacing between trays is exacerbated to ensure foliage could not transfer material by contact.

The CRO have also noted' Visual injury scores are subjective and dependent upon the observations of the biologist evaluating the plants. There is potential for high variability in the scoring of a qualitative metric like plant visual injury, relative to other quantitative parameters (i.e. dry weight). Phytotoxic observations observed should collaborate with the responses of the quantitative parameters. In the Report No 49942 study, dry weight was the most sensitive parameter for all species in which adverse effects were noted, except cucumber, where the lowest ER50 estimate was based on shoot height. Visual injury effects noted in the control plants at test termination were infrequent, not consistent with symptoms noted in treated plants, and represent normal plant growth patterns for plants grown under greenhouse conditions. The more pronounced visual injury scores noted on day 14 for sorghum and

oilseed rape were also not aligned with the symptoms noted in the treated plants at this observation interval'

Seedling emergence validity criteria

In the review the zRMS noted '*Furthermore, no information on seedling emergence was provided (seedling emergence at $\geq 70\%$ is one of the validity criteria indicated in OECD 227 for all control and test item groups)*'.

The CRO have commented on their study design and have highlighted that while seedling emergence is not reported for the controls or treated groups, this is because the study is conducted using only seedlings which have emerged to ensure the required number of plants are available for each replicate. As a result the seedling emergence can be considered to be 100 % and the criteria fulfilled 'Prior to test start for vegetative vigor studies, 2 to 3 seeds are planted and the emerged plants are thinned (within 48 hours prior to study initiation) to a single plant per pot to produce a uniform stand of plants at the 2-4 true leaf stage for testing. The seedlings selected for use are assigned to experimental groups by randomization.

Statistical re-evaluation to account for visual injury in affected species

By the end of the study at 21 days, in seven of the nine species assessed, the overall mean score for visual response in the control groups ranged from 97 -100 (score for lack of any visual injury: 100) and so indicates a very minor deviation from the normal growth expected for the species. Based on these scores, the description would range from 'No noticeable effect' to 'Effect barely noticeable'.

Visual response was however apparently more pronounced in sorghum and oilseed rape at day 14. In the sorghum controls, four replicates were given a lower score of 76, 82, 84 and 88 which would be categorized as 'some effect, not apparently detrimental' and 'effect more pronounced, not obviously detrimental'. These effects were observed as leaf curl and chlorosis. These effects were shown to be short-lived. By the assessment at day 21 the visual response score in each replicate was ≥ 96 , indicating effects were barely noticeable. Similarly, in oilseed rape at day 14 five replicates were scored 72 – 86, with an overall mean of 88. By day 21 all replicates, with the exception of two, were scored as 100. The two other replicates scored 94 and 98. The overall mean for the controls was calculated to be 99. The applicant understands the zRMS concern that the effects observed at day 14 may have had a negative impact on the growth of the control plants. While it is shown that the oilseed rape replicate with the lowest visual response score (replicate E, negative control) had the lowest shoot dry weight at day 21, lower visual response scores did not consistently correlate with a lower dry weight in other replicates.

However, to mitigate any potential influence on the visual injury observed in the control groups on the analysis of the treatment, the applicant has removed those replicates with lowest scores from the statistical analysis i.e. those with a score below 90 and so only include any effects which were classed from 'No noticeable effect' to 'Effect barely noticeable' in the assessment of the control data.

The following table summarizes the reported visual injury in each of the control replicates the replicates for sorghum and oilseed rape.

Visual response data for control replicates of sorghum and oilseed rape

Species	Control group	Sampling day	A	B	C	D	E	F	Mean	Stdev
Sorghum	Negative control	7	100	100	100	100	98CL	100	-	-
	Surfactant control	7	100	100	98 CL	100	100	98 CL	100	1.0
	Negative control	14	92 CL, L	90 CL	82 CL, LC	90 CL, LC	88 CL	94 CL, L	-	-
	Surfactant control	14	84 CL, LC	76 CL, N, LC	98 N	94 CL	98 L	90 CL, LC	90	6.5
	Negative control	21	100	100	96 CL, LC	98 C, LC	100	100	-	-
	Surfactant control	21	100	100	100	100	100	100	100	1.2
Oilseed rape	Negative control	7	98 LC	92 N, LC	100	100	90 CL, N, LC	98 CL	-	-
	Surfactant control	7	98 LC	98 LC	100	96 CL, N, LC	96 CL, LC	98 CL	97	3.1
	Negative control	14	100	78 CL, N	96 CL, N	94 CL, N	72 CL, N, CL	92 CL, N, LC	-	-
	Surfactant control	14	92 CL, LC	84 CL, N, LC	94 CL, LC	92 CL, N	74 CL, N, LC	86 CL, N, LC	88	9.0
	Negative control	21	100	100	100	100	98 CL, LC	100	-	-
	Surfactant control	21	100	100	100	100	94 N, LC	100	99	1.8

A score of 100 indicates normal seedlings, while a score of 0 indicates complete mortality in the replicate. Intermediate scores are assigned to indicate the relative severity of observed signs of toxicity. Visual rating determined by subtracting visual assessment from 100. CL – Chlorosis, N - Necrosis, LC – Leaf Curl.

Endpoints in bold are the maximum recorded visual injury

Due to the higher effect on visual injury reported at day 14 for sorghum the negative control replicates C and E and solvent control replicates A and B were removed from the statistical analysis. For oilseed rape negative control replicates B and E and solvent control replicates B, E and F were removed.

As the negative and solvent controls can be pooled, the number of remaining replicates for sorghum is 9 and 7 replicates for oilseed rape.

A replicate consists of 5 individually potted plants so 45 individual plants would be available for assessment of sorghum controls and 35 for oilseed rape controls.

Based on the remaining replicates for the control, the ER50 values have been recalculated for sorghum and oilseed rape using ToxRat.

The re-calculated effect concentrations for sorghum and oil seed rape are summarized in the following table.

Re-calculated effect rates for sorghum and oilseed rape based on control replicates with no/minimal visual injury only

Species	Critical concentrations (g/ha) 21 days		
	Shoot length ER50 (95 % CL)	Shoot dry weight ER50 (95 % CL)	Visual injury ER50 (95 % CL)
Sorghum	6.11 (4.44 – 8.52)	2.98 (2.58 – 3.45)	4.51 (2.78 – 7.32)
Oilseed rape	16.08 (10.81 – 24.43)	3.822 (2.64 – 5.63)	11.39 (6.37 – 20.55)

These recalculated effect rates are lower, but similar to the original values derived in the study report (ER₅₀ for sorghum = 3.00 g/ha and ER₅₀ for oilseed rape = 3.99 g/ha, both based on shoot dry weight). The visual injury reported at day 14 for these control replicates therefore does not appear to have significantly affected the growth of the plants or impacted the evaluation of effect concentrations. The lowest reported endpoint is the ER₅₀ for sugar beet (1.61 g product/ha based on shoot dry weight)

APPENDIX 1 to the position paper by Ellie, 2022 (position of the study author)

TEST FACILITY STUDY NO: 112P-292
 SPONSOR STUDY NO. 49942

Joshua Arnie
 Manager of Plant and Invertebrate Toxicology
 Eurofins EAG Agrosience, LLC

To Whom it May Concern:

The Sponsor has informed us of concerns regarding visual injury in controls plants in the study: “DPX- V4B07 24 WG: Isoxadifen ethyl 50WG/Rimsulfuron 25SG/Thifensulfuron methyl 50SG (DPX-V4B07), A Blend of Paste Extruded Granules Plus Isodecylalcohol Ethoxylated (DPX-KG691) Surfactant: A Greenhouse Study to Investigate the Effects on Vegetative Vigor of Ten Terrestrial Plants Following Foliar Exposure” (Arnie, 2020; DuPont 49942). The following letter is provided to explain procedures employed to prevent potential contamination, methods for scoring plants at the testing facility, and to discuss the responses observed in the control plants in greater detail.

Prior to test start for vegetative vigor studies, 2 to 3 seeds are planted and the emerged plants are thinned (within 48 hours prior to study initiation) to a single plant per pot to produce a uniform stand of plants at the 2-4 true leaf stage for testing. The seedlings selected for use are assigned to experimental groups by randomization. In this study, onion, oat, sorghum, corn, and oilseed rape were treated on 17 May 2018 and sugarbeet, cucumber, soybean, tomato, and pea were treated on 30 May 2018. On each day of application, the plants were arranged on carts and transported from the propagation greenhouse to the head-house containing the research spray booth. Plants were arranged such that species of similar height were treated simultaneously to maintain the desired distance from the nozzle to the top of the plant (41cm). Negative control plants are treated first (RO water only), followed by the surfactant control plants, and then the treatment plants starting at the lowest rate and increasing until the highest rate was applied. Immediately following application, the plants are removed from the research spray booth and placed on carts for transport. Treated plants are transferred to a different greenhouse and set on trays for watering according to a random block design. Plants are sub irrigated via plastic trays, as needed, throughout the course of the study. Pots are positioned in the trays to prevent contact amongst plants as best as possible depending on the size of the plants and foliage. In addition, the spacing between the trays is exacerbated to avoid any potential transfer. In the subject study, but the spacing between trays was sufficient to ensure foliage could not transfer material by contact.

Visual injury scores are subjective and dependent upon the observations of the biologist evaluating the plants. There is potential for high variability in the scoring of a qualitative metric like plant visual injury, relative to other quantitative parameters (i.e. dry weight). Phytotoxic observations observed should collaborate with the responses of the quantitative parameters. In the DuPont 49942 study, dry weight was the most sensitive parameter for all species in which adverse effects were noted, except cucumber, where the lowest ER50 estimate was based on shoot height.

In the subject study, a replicate consisted of 5 individually potted plants and six replicates were included per treatment and control group, for a total of 30 plants per cohort. The phytotoxic index developed by Frans and Talbert (F-T)¹⁵ was used to score the severity of one or more phytotoxic effects. A single score was assigned to a plant, regardless of the number of signs or phytotoxic effects observed, and the observed effect or effects were recorded. The index assigns a numerical score ranging from 0 (no observable effect) to 100 (mortality). Plants were scored in increments of 10, with a score of 10 indicating the threshold of visible effects. Descriptions for each rating are provided in the table below.

Rating	Category	Description
0	No Effect	No noticeable effect
10		Effect barely noticeable
20	Slight Effect	Some effect, not apparently detrimental
30		Effect more pronounced, not obviously detrimental
40		Effect moderate, plants appear able to recover
50	Moderate Effect	Probable lasting effect, recovery doubtful
60		Lasting effect, recovery doubtful
70		Heavy injury, loss of individual leaves
80	Severe Effect	Plant nearly destroyed, a few surviving leaves
90		Occasional surviving leaves
100	Complete Effect	Death of entire plant

The visual condition scores presented in Appendix XIII as ‘percent visual response’ as 100 minus the visual condition as expressed in the raw data (F-T score). After this correction, 100 indicates no visual response and 0 indicates plant mortality. The visual conditions scores presented in the report are the mean plant values (n=5) by replicate (n=6). Individual plant scores (F-T score) are available in the raw data, but are not included in the final report.

In the DuPont 49942 study, symptoms observed in the control plants at test termination were not considered to be treatment related as evidenced by the different symptoms noted in the control plants and higher treatments. All observations noted were equivalent to slight injury and were considered to represent normal plant growth patterns for plants grown under greenhouse conditions. Mean percent visual response values less than 90 were noted for only soybean and pea at test termination. The scores greater than 90 were for a single replicate (replicate E for pea and replicate F for soybean) and the remaining 5 replicates either exhibited no visual injury or minor scores noted for symptoms

¹⁵ Frans, Robert E. and Ronald E. Talbert. 1977. *Design of Field Experiments and the Measurement and Analysis of Plant Responses*. Pages 15- 23 in B. Truelove, ed. *Research Methods in Weed Science*. Southern Weed Science Society, Auburn University, Alabama

not consistent with symptoms documented in the treated plants. Chlorosis, necrosis, and leaf curl were noted in all replicates of the 45.5 and 136 g product/ha treatment groups and 4 out of the five replicates of the 15.2 g product/ha treatment group, which was not consistent with the signs observed in the control plants. In the soybean control plants only necrosis (7% the total number of plants) and chlorosis (3% the total number of plants) were observed. Severity scores for these symptoms were slight, ≤ 30 . For pea, the replicate with a visual response score of 86 only exhibited necrosis (3% of the total number of control plants) and necrosis and leaf curl were noted in all replicates of the highest treatment group, 15.2 g product/ha.

Severity scores for visual responses noted in the control were greater for sorghum and oilseed rape on the day 14 observation interval, relative to the responses noted for these species at test termination. Although plants can recover over time, this discrepancy may be directly related to different individuals performing observations at these intervals. Whenever possible we strive to have the same biologist perform observations for a given study or at a minimum the same species within a study. The individuals conducting observations in this study were trained by experienced personnel and approved by test facility management to conduct this task.

The symptoms observed in the control plants for soybean and sorghum on day 14 were not consistent with the symptoms observed in higher treatment plants where severe phytotoxic symptoms were noted. Color change was prevalent in the 15.2, 45.5, and 136 g product/ha treatments for oilseed rape on day 14, but this was not noted in the control replicates. Color change was not noted for any oilseed rape plant on day 21. There is a clear rate-response for phytotoxic observations with oilseed rape despite plant condition scores less than 100 in the control on day 14. Chlorosis, leaf curl, and lodging were noted in the control plants for sorghum on day 14, however, in the 15.2, 45.5, and 136 g product/ha treatment groups on day 14 only necrosis was noted in most replicates. Lodging was not noted in the controls on day 21 for sorghum.

The results from DuPont 49942 are representative of the effects to be expected in non-target terrestrial plants exposed to DPX-V4B07 24 WG. Visual injury effects noted in the control plants at test termination were infrequent, not consistent with symptoms noted in treated plants, and represent normal plant growth patterns for plants grown under greenhouse conditions. The more pronounced visual injury scores noted on day 14 for sorghum and oilseed rape were also not aligned with the symptoms noted in the treated plants at this observation interval.

A 2.6.2.4 Position paper comparing toxicity of GF-3969 with adjuvants DPX-KG691 and Codacide

Comments of zRMS:	<p>The studies on effects of GF-3969 to non-target terrestrial plants were performed only with addition of adjuvant DPX-KG691 (Vivolt) and in none of the studies adjuvant Codacide was included. For this reason the zRMS had some concerns if mixture of GF-3969 with Codacide would not result with more pronounced effects and in consequence – lower endpoints, which could have significant impact on the outcome of the risk assessment. This concern was raised since the study on toxicity of GF-3969+Codacide gave slightly lower results comparing to study performed with GF-3969+DPX-KG691 (Vivolt).</p> <p>In order to address concerns of the zRMS the Applicant provided comparison of results of efficacy trials performed with GF-3969 and both adjuvants. The trials included efficacy against various monocotyledonous (100 datapoints) and dicotyledonous (204 datapoints) weeds. Full details of the efficacy trials may be found in the Core Assessment, Part B, Section 3 and below only overall summary of results is provided. Obtained results indicate that effects of GF-3969 on target weeds were similar, regardless of the adjuvant used. Some fluctuations were observed, but these were negligible (differences up to ~5%). Furthermore, in majority studies mixture of GF-3969 with DPX-</p>
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	<p>KG691 (Vivolt) had more pronounced effects on investigated weeds, which is also reflected in the overall mean efficacy calculated separately for monocot and dicot weeds.</p> <p>Based on the obtained results it is not expected that addition of adjuvant Codacide would result with more pronounced effects in non-target terrestrial plants studies and endpoints derived from studies performed with addition of DPX-KG691 (Vivolt) cover also effects from the mixture with Codacide.</p> <p>The zRMS agrees that slightly lower endpoints obtained for Lemna gibba in the study performed with GF-3969+Codacide comparing to the study performed with addition of DPX-KG691 (Vivolt) were due to intra-laboratory variation (endpoints differed by factor 1.4, i.e. considerable below factor of 3, considered to be a benchmark used to determine significant difference in toxicity values).</p>
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Reference:	KCP 10.6.2/03
Report:	Ellis, S. (2022); Position paper to address zRMS comments on the risk to non-target plants from GF-3969
DuPont Report No.:	-
Testing Facility Report No.:	Not relevant, position paper
Guidelines	Not relevant, position paper
Deviations:	Not relevant, position paper
GLP:	Not relevant, position paper
Acceptability:	Not relevant, position paper

In the review the zRMS commented *'it is noted that the studies on effects of GF-3969 on NTTPs were performed only with DPX-KG691 used as a surfactant. However, based on results of studies performed with Lemna gibba it seems that addition of Codacide leads to more pronounced toxic effects. Taking into account that GF-3969 may be used also with Codacide, studies on effects of GF-3969 with this surfactant on non-target terrestrial plants or other sufficient information demonstrating phytotoxic effects of GF-3969+Codacide should be also provided.'*

With regard to the comment on the difference in toxicity of the formulation with the adjuvants DPX-KG691 and Codacide the applicant would like to highlight that Codacide is generic 3rd party vegetable oil whereas DPX-KG691 is a specific adjuvant owned by the notifier. As such this adjuvant is included in studies as the default adjuvant.

With the *Lemna gibba* studies conducted with both adjuvants, the difference in toxicity between the formulation studies containing DPX-KG691 and Codacide is shown to be within a factor of 3 which is used in several guidance documents as the benchmark to determine if products/batches vary in toxicity.

This is highlighted in the EFSA recurring issues document 2019 (EFSA supporting publication 2019: EN-1673):

"In relation to 'when a formulation should be considered more toxic than the active substance', the proposal was to account for a difference of a factor of three, as recommended in the guidance from the Directorate-General for Health and Food Safety (SANCO/10597/2003rev.10.1) (European Commission, 2012) on the equivalence of batches and in the aquatic guidance (EFSA PPR Panel, 2013). This means that when the endpoint of the PPP (expressed in terms of the active substance) is at least three times lower than the equivalent endpoint for the active substance, it should be considered to be more toxic. This factor was agreed by the majority of the experts, to be applied consistently to Tier 1 studies for all groups of non-target organisms"

The E_rC_{50} values derived for *Lemna gibba* with the formulation + DPX-KG691 and formulation + Codacide differ by a factor of 1.4 and so based on the above recommendation this variation is considered to be within the typical level of variation which will occur when conducting studies, and not necessarily a result of a difference in toxicity. Further studies with the formulation plus both adjuvants have been conducted with bees, soil organisms and non-target arthropods and no difference in toxicity was shown.

Efficacy data is also available which indicates there is no difference in the toxicity of the formulation with either adjuvants. Please refer to Appendix 2 for full details of the reported efficacy with both adjuvants. Notably no difference in phytotoxic effects between the adjuvants were reported.

Overall, data from 37 efficacy trials were summarised to demonstrate equal efficacy between applications of GF- 3969 + Vivolt and GF-3969 + Codacide as surfactant for grass and dicot control across different climatic conditions of the central regulatory zone.

Moreover, data from 28 selectivity trials across various climatic conditions are included herein to demonstrate equal crop safety between Vivolt and Codacide in terms of phytotoxicity assessments and yield.

Overall it is concluded that the data presented hereafter clearly demonstrate equal efficacy between adjuvants as well as equal crop safety if application was done in accordance to label recommendations.

APPENDIX 1 to the position paper by Ellie, 2022 (detailed comparison of the adjuvant efficacy data)

GF-3969 is recommended to be used with non-ionic adjuvant (DPX-KG691 – Vivolt) and vegetable oil – Codacide. The study on effects non-target terrestrial plants was performed with GF-3969 mixed with DPX-KG691. Studies are not available on GF-3969 mixed with Codacide.

The following paragraph was prepared to compare DPX-KG691 (Vivolt, a non-ionic surfactant) with Codacide (vegetable oil) in terms of efficacy as well as to compare crop safety of both adjuvants if applied to corn. The data presented herein is an extract of the zonal Biological Assessment Dossier that was submitted to the zRMS Poland to evaluate the biological claims of GF-3969.

Overall, data from 37 efficacy trials is summarized hereafter to demonstrate equal efficacy between applications of GF-3969 + Vivolt and GF-3969 + Codacide as surfactant for grass (Table 1) and dicot control (Table 2) across different climatic conditions of the central regulatory zone. WEEDS with less than 3 trials were removed.

Moreover, data from 28 selectivity trials across various climatic conditions are included herein to demonstrate equal crop safety between Vivolt and Codacide in terms of phytotoxicity assessments (Table 3) and yield (Table 4).

Overall it is concluded that the data presented hereafter clearly demonstrate equal efficacy between adjuvants as well as equal crop safety if application was done in accordance to label recommendations.

Table A 44: Summary efficacy of GF-3969 at 135 g fp/ha using different (non-ionic and vegetable oil) surfactants – grasses across climatic regions

Target grasses	EPO and administrative Zone	N° of trials	Infestation in the untreated control (pl/m ²)		% Control			
					GF-3969 (135 g fp/ha)		GF-3969 (+CODACI)	
					[E9636+M6316+X4145+KG691*] [20 gA/ha+12.5 gA/ha+15 gA/ha+0.2% v/v]		[E9636+M6316+X4145+CODACI] [20 gA/ha+12.5 gA/ha+15 gA/ha+1296 gA/ha]	
Mean	Min-Max	Mean	Min-Max	Mean	Min-Max			
AGRRE	Maritime	8	29.5	5-118	89.3	73.8-98.3	88.1	65-97
	Central Zone	8	29.5	5-118	89.3	73.8-98.3	88.1	65-97
DIGSA	Maritime	5	66.4	10-150	77.6	48.8-96.8	89.6	86.3-94.3
	South East	1	26	-	99.5	-	98.8	-
	Central Zone	6	59.7	10-150	81.3	48.8-99.5	91.5	86.3-98.8
ECHCG	Maritime	11	49.8	4-121	97.1	87.3-100	97.2	90.4-100
	North East	6	6.1	5-10	94.2	72.8-100	83.9	20-100
	South East	9	19.5	5-75	99.2	97.5-100	97.7	91.3-100
	Central Zone	26	29.2	4-121	97.2	72.8-100	94.2	20-100
POAAN	Maritime	3	7	5-10	97.5	92.5-100	98.8	97.5-100
	Central Zone	3	7	5-10	97.5	92.5-100	98.8	97.5-100
SETVI	Maritime	1	10	-	57.5	-	45	-
	South East	3	12.3	4-16.5	99.7	99-100	97	93.8-100
	Central Zone	4	11.8	4-16.5	89.1	57.5-100	84	45-100
SORHA	South East	3	12	6-24	92.8	86.8-97.5	92	84.5-98.8
	Central Zone	3	12	6-24	92.8	86.8-97.5	92	84.5-98.8
Average overall (n=100 datapoints)					90.7		89.8	

*Vivolt

The overall efficacy against grasses is comparable, independently if KG691 or Codacide was added to GF-3969.

Table A 45: Summary efficacy of GF-3969 at 135 g fp/ha using different (non-ionic and vegetable oil) surfactants – broad leaf weeds across climatic regions

Target BLW	EPPO and ad- ministrative Zone	N° of trials	Infestation in the untreated con- trol (pl/m ²)		% Control			
					GF-3969 (135 g fp/ha)		GF-3969 (+CODACI)	
					[E9636+M6316+X4145+KG691*] [20 gA/ha+12.5 gA/ha+15 gA/ha+0.2% v/v]		[E9636+M6316+X4145+CODACI] [20 gA/ha+12.5 gA/ha+15 gA/ha+1296 gA/ha]	
Mean	Min- Max	Mean	Min- Max	Mean	Min- Max			
ABUTH	Maritime	1	90	-	87.5	-	91	-
	South East	2	4.5	4-5	68.8	65-72.5	63.8	35-92.5
	Central Zone	3	33	4-90	75	65-87.5	72.8	35-92.5
AMARE	Maritime	3	92.3	9-230	99.8	99.3-100	99.2	97.5-100
	North East	6	5.7	4-7	97.8	95-99	98.4	96-99.8
	South East	1	12.5	-	100	-	100	-
	Central Zone	10	32.4	4-230	98.6	95-100	98.8	96-100
AMBEL	South East	6	26.8	10-48	89.4	80-100	83.9	66.3-98
	Central Zone	6	26.8	10-48	89.4	80-100	83.9	66.3-98
CHEAL	Maritime	14	31.4	9.5-83	96.8	77.5-100	92	40-100
	North East	8	9.5	5-18	90.9	77.5-98.5	86.1	47.5-97.5
	South East	5	12.4	5-19	99	96.5-100	95	87.5-100
	Central Zone	27	21.4	5-83	95.4	77.5-100	90.7	40-100
CHEPO	Maritime	4	19.2	5-56.3	93.9	80-100	92.4	71.3-100
	Central Zone	4	19.2	5-56.3	93.9	80-100	92.4	71.3-100
DATST	Maritime	2	71.5	33-110	68.1	60-76.3	74.4	70-78.8
	South East	4	19	6-48	67.7	26.3-88	54.4	17.5-89
	Central Zone	6	36.5	6-110	67.8	26.3-88	61.1	17.5-89
GASPA	Maritime	3	27	13-48	96.7	91.5-100	95	91.3-98.8
	Central Zone	3	27	13-48	96.7	91.5-100	95	91.3-98.8
HELAN	Maritime	1	5	-	90	-	80	-
	North East	1	6	-	30	-	30	-
	South East	3	5.3	4-7	94.5	83.5-100	95.3	86-100
	Central Zone	5	5.4	4-7	80.7	30-100	79.2	30-100
HIBTR	South East	3	17.7	13-26	96.3	93.8-100	93.8	87.5-100
	Central Zone	3	17.7	13-26	96.3	93.8-100	93.8	87.5-100
MATIN	Maritime	2	6.5	6-7	97.5	95-100	99.4	98.8-100
	North East	1	5	-	100	-	100	-
	Central Zone	3	6	5-7	98.3	95-100	99.6	98.8-100
	Maritime	5	23.8	5-63	91.5	75-96.8	79.2	35-97
	North East	4	5.8	4-7	80.9	37.5-100	85.1	60-98

Target BLW	EPPO and administrative Zone	N° of trials	Infestation in the untreated control (pl/m ²)		% Control			
					GF-3969 (135 g fp/ha)		GF-3969 (+CODACI)	
					[E9636+M6316+X4145+KG691*] [20 gA/ha+12.5 gA/ha+15 gA/ha+0.2% v/v]		[E9636+M6316+X4145+CODACI] [20 gA/ha+12.5 gA/ha+15 gA/ha+1296 gA/ha]	
Mean	Min-Max	Mean	Min-Max	Mean	Min-Max			
POLCO	South East	1	5	-	85.8	-	85.8	-
	Central Zone	10	14.7	4-63	86.7	37.5-100	82.2	35-98
POLLA	Maritime	3	7.7	5-9	99.7	99-100	98	95-100
	Central Zone	3	7.7	5-9	99.7	99-100	98	95-100
POLPE	Maritime	5	14.8	3-28.2	86.2	60-100	42.5	37.5-47.5
	North East	1	6	-	100	-	96.3	-
	Central Zone	6	13.4	3-28.2	88.5	60-100	60.4	37.5-96.3
SOLNI	Maritime	3	6.4	4.1-10	46.7	37.5-55.0	57.5	57.5-57.5
	North East	1	5	-	57.5	-	37.5	-
	Central Zone	4	6	4.1-10	49.4	37.5-57.5	47.5	37.5-57.5
STEME	Maritime	2	24.5	16-33	100	100-100	100	100-100
	North East	2	6.5	5-8	100	100-100	100	-
	Central Zone	4	15.5	5-33	100	100-100	100	100-100
VERPE	Maritime	2	5	5-5	98.3	97.5-99	95.8	92.5-99
	North East	3	6.4	5.3-8	67.9	40-88.8	61.3	35-86.3
	Central Zone	5	5.9	5-8	80.1	40-99	75.1	35-99
Average overall (n=204 datapoints)					86.7		82.8	

*Vivolt

The overall efficacy against broadleaf weeds is comparable, independently if KG691 or Codacide was added to GF-3969.

EPPO Zone	Trial number	ZEAMX variety	Appl. Date	BBCH at Appl	GF-3969 (+DPX-KG691)				GF-3969 (+Codacide)				
					135g + 0.2%		270g + 0.4%		135g + 1080g		270g + 2160g		
					N		2N		N		2N		
					Max	Final	Max	Final	Max	Final	Max	Final	
Maritime	DUT-18-015	KORYNT	15-May-2018	MAZ14	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
South east	HUM-17-123	DKC-4717	30-May-2017	MAZ18	3.0	0.0	3.5	0.0	2.0	0.0	2.0	0.0	
South east	HUS-17-123	DKC5542	17-May-2017	MAZ11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
South east	HUS-18-104	DKC 4795	17-May-2018	MAZ13	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
South east	HUS-18-105	DKC5542	29-May-2018	MAZ18	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
North East	PLR-17-123	SUBITO	22-Jun-2017	MAZ19	1.3	0.8	1.3	1.3	0.8	0.8	1.0	1.0	
North East	PLA-18-143	MALAWI CS	06-Jun-2018	MAZ18	10.0	10.0	12.0	12.0	0.0	0.0	0.0	0.0	
North East	PLJ-18-143	LG 30.254	30-May-2018	MAZ15	0.0	0.0	4.0	0.0	0.0	0.0	0.0	0.0	
South east	ROE-17-123	DKC 4590	30-May-2017	MAZ18	2.5	0.0	10.0	5.0	0.0	0.0	0.0	0.0	
South east	ROE-18-143	P9911	07-Jun-2018	MAZ14	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
South east	ROE-18-243	NK COBALT	02-Jun-2018	MAZ18	2.5	0.0	3.8	3.8	0.0	0.0	0.0	0.0	
PHYSTU % AREA/PLOT													
Maritime	DUC-17-019	DKC3623	09-Jun-2017	MAZ18	7.5	0.0	12.5	2.0	5.0	1.3	11.3	1.3	
Maritime	AST-18-100	PANDORAS	04-Jun-2018	MAZ18	17.5	0.0	25.0	0.0	7.5	0.0	7.5	0.0	
Maritime	DUC-18-143	PR38A75	09-May-2018	MAZ13	0.0	0.0	0.0	0.0	0.0	0.0	2.0	0.0	
Maritime	BNB-17-656	CODIBIRD	20-Jun-2017	MAZ18	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	
Maritime	BNB-17-657	MAS15.P	09-Jun-2017	MAZ18	16.3	0.0	41.5	16.3	3.0	0.0	7.8	2.0	
Maritime	BNB-18-656	Pan 36008	15-Jun-2018	MAZ18	7.5	0.0	30.0	3.8	8.3	0.0	13.3	0.0	
Maritime	BNB-18-657	Pan 36008	31-May-2018	MAZ15	8.8	2.5	7.5	2.5	6.3	4.0	8.8	4.0	
Maritime	CZL-17-123	JOKARI	15-Jun-2017	MAZ18	0.0	0.0	3.8	0.0	3.8	0.0	5.0	0.0	
Maritime	DUT-17-041	GL14205	08-Jun-2017	MAZ17	1.5	0.0	2.3	0.0	2.5	1.3	1.5	0.0	
Maritime	DUU-17-123	RICARDINIO	23-May-2017	MAZ13	25.0	0.0	20.0	0.0	30.0	0.0	32.5	0.0	
Maritime	DUI-18-722	SY KAIRO	08-Jun-2018	MAZ18	10.8	0.0	13.8	0.0	0.0	0.0	0.0	0.0	
Maritime	DUT-18-015	KORYNT	15-May-2018	MAZ14	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
South east	HUM-17-123	DKC-4717	30-May-2017	MAZ18	0.0	0.0	5.0	0.0	0.0	0.0	0.0	0.0	
South east	HUS-17-123	DKC5542	17-May-2017	MAZ11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
South east	HUS-18-104	DKC 4795	17-May-2018	MAZ13	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
South east	HUS-18-105	DKC5542	29-May-2018	MAZ18	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
North East	PLR-17-123	SUBITO	22-Jun-2017	MAZ19	1.5	0.0	1.5	0.0	0.5	0.0	0.8	0.0	
North East	PLA-18-143	MALAWI CS	30-May-2018	MAZ17	40.5	3.0	45.0	7.8	27.5	0.0	30.5	5.0	
North East	PLJ-18-143	LG 30.254	30-May-2018	MAZ15	0.0	0.0	3.0	0.0	0.0	0.0	0.0	0.0	
North East	PLR-18-142	SUBITO	12-Jun-2018	MAZ18	1.3	0.0	10.0	0.0	0.0	0.0	2.5	0.0	
South east	ROE-17-123	DKC 4590	30-May-2017	MAZ18	5.0	0.0	7.5	5.0	0.0	0.0	5.0	5.0	
South east	ROE-18-143	P9911	07-Jun-2018	MAZ14	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
South east	ROE-18-243	NK COBALT	02-Jun-2018	MAZ18	5.0	5.0	3.8	2.5	0.0	0.0	5.0	0.0	

Table A 47: Yield effect to seed corn and silage corn varieties if GF-3969 was applied using either Vivolt (KG691) or Codacide at target (N) and double (2N) rate across different climates of the central regulatory zone.

EPPO	Trial number	Variety	BBCH At appl	Yield in UTC (t/ha)	Yield at 1N as % of the untreated		Yield at 2N rate as % of the untreated	
					GF-3969	GF-3969 N rate	GF-3969 2N rate	GF-3969 2N rate
					N rate+ KG691	+CODACIDE	+KG691	+ CODACIDE
Maritime	AST-17-104	ES CUBUS	MAZ13	12.85	102	106	101	99
Maritime	AST-18-100	PANDORAS	MAZ18	13.2	102	96	100	100
Maritime	BNB-17-657	MAS15.P	MAZ18	11.94	84	91	84	93
Maritime	CZL-17-123	JOKARI	MAZ18	0.6	120	105	104	122
Maritime	CZM-18-143	LG 31.233	MAZ14	16.77	99	103	102	101
Maritime	DUC-17-019	DKC3623	MAZ18	8.49	100	102	99	95
Maritime	DUC-18-143	PR38A75	MAZ13	13.79	103	102	100	100
Maritime	DUT-17-041	GL14205	MAZ17	10.64	100	98	98	100
South east	HUM-17-123	DKC-4717	MAZ18	11.42	98	98	107	105
South east	HUS-18-104	DKC 4795	MAZ13	10.66	103	95	96	94
Maritime	BNB-18-656	Pan 36008	MAZ18	8.72	82	91	69	92
Maritime	BNB-18-657	Pan 36008	MAZ15	8.06	92	97	90	90
North east	PLA-18-143	MALAWI CS	MAZ18	11.28	93	103	83	90
North east	PLR-18-143	ES CONCORD	MAZ14	8.7	100	102	103	103
South east	ROE-18-143	P9911	MAZ14	10.03	96	98	97	98
AVG 15 trials (seed corn varieties)				10.5	98	99	96	99
Maritime	BNB-17-656	CODIBIRD	MAZ18	18.92	94	103	100	101
Maritime	CZF-17-123	SY WERENA	MAZ13	29.78	98	96	99	102
Maritime	CZL-18-143	SY KAIRO	MAZ18	10.89	103	99	115	116
Maritime	DUI-18-722	SY KAIRO	MAZ18	12.56	113	107	119	113
Maritime	DUT-18-015	KORYNT	MAZ14	20.69	94	93	95	89
Maritime	DUU-17-123	RICARDINIO	MAZ13	202.43	99	94	90	96
South east	HUS-17-123	DKC5542	MAZ18	23.99	99	98	98	93
South east	HUS-18-105	DKC5542	MAZ18	20.71	100	100	103	97
South east	ROE-17-123	DKC 4590	MAZ18	10.75	104	100	88	96
North east	PLJ-18-143	LG 30.254	MAZ15	19.82	103	105	100	103
North east	PLR-17-123	SUBITO	MAZ19	16.43	99	100	100	100
North east	PLR-18-142	SUBITO	MAZ18	41.37	99	102	100	101
South east	ROE-18-243	NK COBALT	MAZ18	19.81	100	100	100	99
AVG 13 trials (silage corn varieties)				34.5	100	100	101	100

A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants

No new or additional studies have been submitted.

A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

Comments of zRMS:	The study was evaluated in area of Efficacy section. Based on the results of the study it may be concluded that both tested thifensulfuron-methyl metabolites (IN-JZ789 and IN-U5F72) do not exhibit herbicidal activity.
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Reference:	KCP 10.7.1/01
Report:	Pur, A., Ochoa-Acuna, H., (2015); Herbicide non-relevance screen results for thifensulfuron methyl metabolites (IN-JZ789 and IN-U5F72)
DuPont Report No.:	DuPont-43667 EU
Testing Facility Report No.:	DuPont-43667 EU
Guidelines	Not applicable
Deviations:	None
GLP:	No
Acceptability:	Acceptable

MATERIALS AND METHODS

Seeds of two dicot (soybean and oilseed rape) and two monocot crops (wheat and corn) and 15 species of broadleaf and grass weeds were planted in 2 ½” square x 3 ½” deep pots held within a 32 cell carrying tray containing Redi-earth potting media. The plants are grown in a greenhouse for 9 to 14 days. Once the plants reach their appropriate stage of growth (seedling), the plants are then ready for herbicide application. Daytime and night-time temperatures in greenhouse were targeted at 25° - 30° C and 22° – 25° C, respectively. The test plants were supplemented with artificial lighting as needed. Day length was maintained for approximately 14 hours. Peter’s 20-20-20 (200 ppm) and chelated iron (10 ppm) was applied as the fertilizer during watering through an inline greenhouse fertilizer injection system.

The test chemicals were dissolved in a non-phytotoxic solvent (91.3% acetone, 4.2% glycerine, 4.2% water and 0.25% Tween 20) in concentrations required to obtain the desired rate of application. The solutions or suspensions were then applied as foliage sprays to the plants at the rate 100 g/ha of active substance and metabolite, respectively. Application was made using an automated spray machine at a spray volume of 457 liters per hectare. Immediately after treatment, the pots were transferred to a greenhouse and subsequently watered on a demand basis. Care was taken to not wet the foliage of the plants for at least the first 24 hours.

Visual plant response ratings were made 10 days after treatment. The ratings were made on a percentage scale (0 to 100, where 0 = no injury or control, and 100 = death of the plant) compared to untreated control treatment.

RESULTS AND DISCUSSION

The visual results of the injury evaluation screen are presented in Table 1. As can be seen, there was a consistent response (>80% injury) for all plant species treated with thifensulfuron methyl at 100 g/ha. On the other hand, testing using the thifensulfuron methyl metabolites IN-JZ789 and IN-U5F72 at the same high rate (100 g/ha) resulted in significantly lower visual responses than seen from application of the parent substance thifensulfuron methyl, to any of the plant species tested.

The results demonstrate that these metabolites have lost the biological activity ascribed to the parent compound and therefore they should be considered herbicidally inactive.

Table A 48: Visual plant response ratings for thifensulfuron methyl when applied post (foliar spray) to crop and weed species. The ratings were made on a percentage scale (0 to 100, where 0 = no injury or control, and 100 = death of the plant) compared to untreated control treatment

Plant species	Thifensulfuron methyl technical @ 100 g/ha	IN-JZ789 @ 100 g/ha	IN-U5F72 @ 100 g/ha
Crop species			
Corn	95	0	0
Oilseed Rape	90	0	0
Soybean	85	0	0
Wheat	40	0	0
Weed species			
Pigweed	98	0	0
Morning glory	100	0	20
Velvetleaf	100	0	0
Ragweed	85	0	20
Lambsquarters	100	0	0
Waterhemp	95	0	0
Galium	95	0	0
Kochia	98	0	0
Chickweed	100	0	0
Foxtail	80	0	0
Crabgrass	85	0	0
Barnyardgrass	95	0	0
Nutsedge	40	0	0
Wild Oat	0	0	0
Ryegrass	85	0	0
Blackgrass	80	0	0

A 2.8 KCP 10.8 Monitoring data

No new or additional studies have been submitted.

Appendix 3 Thifensulfuron methyl study summaries from FMC

Comments of zRMS:	Studies summarised in Appendix 3 were evaluated and agreed by the RMS (UK) in the course of the evaluation of the confirmatory data. For details, please refer to EFSA Supporting publication 2020:EN-1627. The summaries below were struck through as being not validated by the zRMS.
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Fish toxicity

Chronic toxicity to fish

Thifensulfuron methyl

~~Report: xxxxxxxxxxxxxxxxxxxxxxxxThifensulfuron Methyl (DPX-M6316) Technical: Early Life Stage Toxicity Test with the Rainbow Trout, *Oncorhynchus mykiss*, Under Flow Through Conditions~~

~~Report No.: DuPont 28722~~

~~Guidelines: OECD 210, OPPTS 850.1400~~

~~Deviations: None~~

~~Testing Facility: ABC Laboratories, USA~~

~~Testing Facility Report No.: 64908~~

~~GLP: Yes~~

~~Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA.~~

Executive summary:

~~The early life stage toxicity of thifensulfuron methyl to fed rainbow trout was determined in a 96-day flow through test. The test was conducted in accordance with the U.S. EPA, Office of Prevention, Pesticides and Toxic Substance (OPPTS), Ecological Effects Test Guideline 850.1400 and the Organization for Economic Cooperation and Development (OECD), Guideline 210. Treatments consisted of a dilution water control and five nominal concentrations of 0.63, 1.3, 2.5, 5.0, and 10 mg a.s./L. Based on mean measured concentrations of thifensulfuron methyl, the NOEC values for egg hatchability, first day of hatch, fry survival, standard length, and blotted wet weight was 10.6 mg a.s./L, the highest concentration tested. There was a statistically significant difference in the last day of hatch in the 10.6 mg a.s./L treatment level when compared to the control. Due to very small difference (4% difference versus the control) in the last day of hatch, the lack of any adverse biological effect in any other test parameter (hatching, growth, and survival), and historical control hatch data from two previous studies that bracket the last day of hatch at the highest test concentration, this difference was not considered to be biologically meaningful relative to the control performance. Therefore, the NOEC for last day of hatch was 10.6 mg a.s./L, the highest mean measured concentration tested.~~

~~I. MATERIALS AND METHODS~~

~~A. MATERIALS~~

- | | |
|--|--|
| 1. Test material: | Thifensulfuron methyl |
| Lot/Batch #: | M6316-259 |
| Purity: | 99.0% |
| Description: | Solid |
| CAS#: | 79277-27-3 |
| Stability of test compound: | Stable in the test system |
| 2. Control: | Dilution (laboratory blended water) water |
| Solvent control: | None |

Test vehicle:	Dilution (laboratory blended water) water
Toxic reference:	None
3. Test organism:	Rainbow trout
Species:	<i>Oncorhynchus mykiss</i>
Age at dosing:	<24 hours
Initial population:	25 embryos per test chamber
Source:	Trout Lodge (Sumner, Washington)
Diet:	Brine shrimp nauplii and/or salmon starter at least twice daily except 24 hours prior to termination
Test chamber:	Glass aquaria measuring approximately 16 cm wide by 31 cm long by 32 cm high with a test solution depth of 25 cm
4. Environmental conditions (in life period)	
Temperature:	9.7 to 10.5°C for embryos 11.8 to 12.6°C for fry
Photoperiod:	16 hr photoperiod (454 lux) and 8 hr darkness which included 30 min transitional light preceding and following the 16 hr light interval

B. STUDY DESIGN AND METHODS

1. In life initiated/completed

06 October 2009 to 11 January 2010

2. Experimental treatments

The early life stage toxicity of thifensulfuron methyl to fed *Oncorhynchus mykiss* was determined in an un-aerated, flow through, 62 day post hatch test. Treatments consisted of a dilution water control, and five nominal concentrations of 0 (control), 0.63, 1.3, 2.5, 5.0, and 10 mg a.s./L. Twenty five embryos were used per replicate with four replicates per test concentration and control.

3. Observations

Mortality and behavioral observations were made daily throughout the exposure.

4. Statistics

The no observed effect concentration (NOEC) and lowest observed effect concentration for egg hatchability and fish survival (62 day post hatch) data were determined by using a Fisher's exact test. A Hochberg adjustment was used to control the experiment wise error rate for the Fisher's test at the same alpha level. The NOEC and LOEC, based on first day of hatch, last day of hatch, swim up, standard length and blotted wet weight, were estimated using a one way analysis of variance (ANOVA) procedure and a one tailed Dunnett's test (with the exception of the first day of hatch and last day of hatch data where two tailed Dunnett's tests were used), with the alternate hypothesis being the mean for the length or weight was reduced or day was increased in comparison to the control mean. Prior to the Dunnett's test, a Shapiro-Wilk's test and a Levene's test were conducted to test for normality and homogeneity of variance, respectively, over treatments at each time point. The results from the Shapiro-Wilk's and Levene's tests indicated non-normality and heterogeneity of variance for the first day of hatch, last day of hatch, and swim up data. Therefore, these parameters were analyzed with non-parametric analyses on the ranks of the values. The results from the Shapiro-Wilk's and Levene's tests indicated normality and homogeneity of variance for standard length and blotted wet weight. Therefore, these parameters were analyzed with a parametric ANOVA and Dunnett's test on the non-transformed data. Where possible, the point estimates of the maximum acceptable toxicant concentration (MATC) were calculated as the geometric mean of the NOEC and LOEC values of the sensitive endpoints.

H. RESULTS AND DISCUSSION

A. FINDINGS

The mean measured concentrations of thifensulfuron methyl in the control and test substance treatments during the study were <LOD (control), 0.650, 1.38, 2.54, 5.25, and 10.6 mg a.s./L and ranged from 102 to 106% of the nominal concentrations. No residues of thifensulfuron methyl were detected in the control above the LOD of 0.00773 mg a.s./L. All test acceptability criteria were met. A summary of hatching and survival is presented the following table.

Table 1
~~Summary of observed mortality of *Oncorhynchus mykiss* exposed to thifensulfuron methyl in a flow-through test~~

Mean Measured Thifensulfuron Methyl Concentration (mg a.s./L)	Hatch (No. of Hatched Fry/Initial No. of Embryos)				Survival (No. of Surviving Fry/Total No. of Hatched Fry)			
	A	B	C	D	A	B	C	D
Control	15/15	15/15	14/14	15/15	15/15	15/15	14/14	15/15
0.650	14/14	15/15	15/15	13/13	12/14	15/15	14/15	12/13
1.38	14/15	15/15	15/15	15/15	14/14	14/15	15/15	14/15
2.54	15/15	15/15	15/15	15/15	15/15	13/15	14/15	14/15
5.25	14/15	15/15	15/15	13/15	11/14	14/15	13/15	13/13
10.6	14/15	15/15	14/15	15/15	14/14	15/15	13/14	14/15

III. CONCLUSION

Based on mean measured concentrations of thifensulfuron methyl, the NOEC values for egg hatchability, first day of hatch, last day of hatch, fry survival, standard length, and blotted wet weight was 10.6 mg a.s./L, the highest mean measured concentration tested.

Gerke (AG), 2010

Toxicity to aquatic species other than fish and aquatic species field testing

Acute toxicity to aquatic invertebrates

Acute toxicity (24 and 48-hour) for *Daphnia*

Thifensulfuron methyl

Report: Brougher, D.S., Lockard, L., Gallagher, S.P. (2017); Thifensulfuron methyl (DPX M6316) technical: A 48-hour static acute toxicity test with the cladoceran (*Daphnia magna*)

Report No.: DuPont 46007, Revision No. 1

Guidelines: OECD 202 (2004), OPPTS 850.1010 (1996)

Deviations: None

Testing Facility: Wildlife International Ltd (USA), Easton, Maryland, USA

Testing Facility Report No.: 112A 649

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA.

Executive summary:

The acute toxicity of thifensulfuron methyl to unfed <24-hour-old *Daphnia magna* neonates was determined in an unacrated, static, 48-hour test. The test was conducted in accordance with the OECD Guideline for Testing of Chemicals, Guideline 202, *Daphnia sp.*, *Acute Immobilisation Test* and U.S. Environmental Protection Agency Series 850—Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.1010, *Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids*. Treatments consisted of a dilution water control, and five nominal test concentrations of 7.5, 15, 30, 60 and 120 mg a.s./L. Mean, measured concentrations of thifensulfuron methyl were 7.5, 15, 30, 60 and 120 mg a.s./L. The 48-hour EC₅₀ in *Daphnia magna* was >120 mg a.s./L, based on mean, measured thifensulfuron methyl test concentrations and immobility data.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Thifensulfuron-methyl technical
Lot/Batch #: M6316-293
Purity: 99.3%
Description: Solid
CAS#: 79277-27-3
Stability of test compound: Shown to be stable under the conditions of the test.
2. Controls: Dilution water (laboratory well water) control
Test vehicle: Dilution water (laboratory well water)
Toxic reference: Not applicable
3. Test organism: Cladoceran
Species: *Daphnia magna*
Age/life stage at dosing: <24 hours
Initial population: Four replicate test chambers with 5 daphnids per test chamber
Source: Wildlife International in-house culture
Diet: Unfed during test
Test chamber: 250 mL glass beaker containing approximately 210 mL of test solution (5.9 cm test solution depth)
4. Environmental conditions: Dissolved oxygen: ≥ 8.1 mg/L ($\geq 90\%$ of saturation)
pH: 7.7 to 8.4
Temperature: 19.8 to 20.9°C in test chambers; 19.22 to 19.78°C measured continuously in an adjacent container of water.
Photoperiod: 16 hr light (446 lux at initiation) and 8 hr dark including 30 min transitional period preceding and following the 16 hr light interval.

B. STUDY DESIGN AND METHODS

1. In life initiated/completed
03 May 2016 to 05 May 2016
2. Experimental treatments
The acute toxicity of thifensulfuron-methyl to unfed *Daphnia magna* (<24 hour old neonates) was determined in an unaerated, static, 48 hour test. Treatments consisted of a dilution water control, and five mean, measured test concentrations of 7.5 to 120 mg a.s./L. Five daphnids were used per replicate with four replicates per test concentration and control.
3. Observations
Immobility and behavioural observations were made at approximately 3.5 hours, and at 24 and 48 hours (± 1 hour) following initiation of exposure.
4. Statistics
The absence of immobile daphnids in any of the thifensulfuron-methyl treatment groups during the test precluded the statistical calculation of EC₅₀ values at 24 and 48 hours. Therefore, the EC₅₀ values were estimated to be greater than the highest concentration tested. The highest test concentration causing no immobility at test end and the lowest test concentration causing 100% immobility at test end were assessed by visual observation of the immobility and observation data.

II. RESULTS AND DISCUSSION

A. FINDINGS

Nominal test concentrations of thifensulfuron-methyl were 7.5, 15, 30, 60 and 120 mg a.s./L. Mean, measured concentrations of thifensulfuron-methyl were 7.5, 15, 30, 60 and 120 mg a.s./L, each with mean percent recoveries equal to 100% of nominal concentrations. All validation

criteria were met for the study. Summaries of cumulative immobility and sub-lethal effects are presented in Table 7 and Table 8, respectively.

Table 7

Summary of observed immobility of unfed *Daphnia magna* exposed to thifensulfuron methyl for 48 hours in an unaerated, static, acute test

Mean, Measured Test Concentration (mg a.s./L)	Immobility (No. immobile/No. at test start) ^a							
	24 Hours ^b				48 Hours			
	A	B	C	D	A	B	C	D
Dilution water control (0.0)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
7.5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
15	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
30	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
60	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
120	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

^a— A-D represent replicate test chambers containing 5 daphnids each at test start.

^b— There were no immobile daphnids noted at the 3.5-hour observation interval.

Table 8

Summary of sub-lethal effects of unfed *Daphnia magna* exposed to thifensulfuron methyl for 48 hours in an unaerated, static, acute test

Mean, Measured Test Concentration (mg a.s./L)	Sub-lethal Effects (Number affected / Number alive ^a)							
	24 Hours ^b				48 Hours			
	A	B	C	D	A	B	C	D
Dilution Water Control (0.0)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
7.5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
15	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
30	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
60	0/5	0/5	0/5	0/5	0/5	1 Q, AN ^c /5	0/5	0/5
120	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

^a— A-D represent replicate test chambers containing 5 daphnids each at test start.

^b— All organisms appeared normal at the 3.5-hour observation interval.

^c— Observations: Q, AN = daphnid trapped at water surface but appeared normal after gentle submersion.

III. CONCLUSION

The 48-hour EC₅₀ value, based on the mean, measured test concentrations of thifensulfuron methyl and immobility, was >120 mg a.s./L, the highest concentration tested. The highest mean, measured test concentration causing no immobility at test end was 120 mg a.s./L. The lowest mean, measured test concentration causing 100% immobility at test end was >120 mg a.s./L, the highest concentration tested.

(Brougher, D.S., Lockard, L., Gallagher, S.P., 2017)

Chronic toxicity in *Daphnia magna* (21 day)

Thifensulfuron methyl

HA 8.3.2.1/04

Report: Hutton, D.G. (1989); Chronic toxicity of IN-M6316-25 to *Daphnia magna*

Report No.: HLR 70-89

Guidelines: OECD 202 (1984), U.S. EPA 72-4 (1988)

Deviations: None

Testing Facility: DuPont Haskell Laboratory, Newark, Delaware, USA

Testing Facility Report No.: HLR 70-89

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections.

Executive summary:

The effects of thifensulfuron methyl on the growth and reproduction of *Daphnia magna* (<24 hour old) were assessed in an un aerated, static renewal, 21-day test. The test was conducted in accordance with the appropriate Good Laboratory Practice standards and test guidelines OECD Guideline for Testing of Chemicals: 202 and U.S. EPA Pesticide Assessment Guidelines Subdivision E, 72-4. Treatments consisted of a dilution water control and six nominal concentrations of 42, 64, 99, 152, 235, and 350 mg thifensulfuron methyl/L. The corresponding mean, measured concentrations of thifensulfuron methyl were 40, 66, 100, 150, 240, and 340 mg a.s./L. The 21-day NOEC for *Daphnia magna* based on mean, measured concentrations and adult survival was >340 mg thifensulfuron methyl/L, the highest concentration tested. The EC_{50s} for *Daphnia magna* based on mean, measured concentrations and growth and reproductive parameters, respectively, were both >340 mg thifensulfuron methyl/L, the highest concentration tested. The 21-day LOEC for *Daphnia magna* based on mean, measured concentrations and reproductive parameters (total offspring and offspring per surviving adult) was 340 mg thifensulfuron methyl/L, the highest concentration tested, and the 21-day NOEC for reproduction (offspring per surviving adult) was 240 mg a.s./L. The 21-day NOEC for reproduction (first day of reproduction) was 150 mg a.s./L and the LOEC was 240 mg a.s./L. For growth, the most sensitive parameter, the 21-day LOEC, was 100 mg/L and the NOEC was 150 mg a.s./L. The maximum acceptable toxicant concentration (MATC) for survival was >340 mg a.s./L, the MATC for reproduction (total offspring and offspring per surviving adult) was between 240 and 340 mg a.s./L, and the MATC for growth was between 100 and 150 mg a.s./L based on mean measured concentration.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Thifensulfuron methyl technical
Lot/Batch #: M6316-25
Purity: 97.0%
Description: Powder
CAS#: 79277-27-3
Stability of test compound: A separate test for chemical stability of the test substance in the vehicle (well water) was not performed; concentrations were maintained by renewal.
2. Untreated control: Dilution (laboratory well water) water
Test vehicle: Dilution (laboratory well water) water used for fathead minnow culturing in a flow through system, then filtered through a 0.8 µm filter.
Toxic reference: None
3. Test organism
Species: *Daphnia magna*
Age at dosing: <24 hours old
Initial population: Four daphnids per test chamber
Source: Haskell Laboratory, in house culture
Diet: Trout chow (Glencoe) and yeast (Fleischmann's)
Test chamber: 250 mL glass beaker containing approximately 200 mL of test solution (approximately 6.8 cm test solution depth), covered with a glass plate
4. Environmental conditions (in life period)
Dissolved oxygen: 6.2 to 8.8 mg/L
pH: 7.0 to 8.0
Temperature: 19.3 to 20.4°C
Photoperiod: 16 hour photoperiod (approximately 550 lux)

B. STUDY DESIGN AND METHODS

1. In life initiated/completed

07-December-1988 to 28-December-1988

2. Experimental treatments

The effects of thifensulfuron methyl on the growth and reproduction of *Daphnia magna* (<24 hour old) were assessed in an unacrated, static renewal, 21 day test. Treatments consisted of a dilution water control and six nominal concentrations of 42, 64, 99, 152, 235, and 350 mg thifensulfuron methyl/L. A total of 10 replicates, each containing four <24-hour old neonates, were tested per concentration (40 neonates/concentration) and control. Test concentrations were renewed three times per week.

3. Observations

Observations were made three times per week of the number of surviving adult daphnids and production of live young. Length of surviving adult daphnids was determined at test end (21 days).

4. Statistics

Survival data were analysed by Fisher's Exact test. Probit analysis was used to determine the EC₅₀ for survival and reproductive parameters. Reproduction and growth data were analysed by one way analysis of variance technique. Multiple comparisons of treatment and control groups were then carried out using Dunnett's method (p <0.05).

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical verification of thifensulfuron methyl concentrations was made on test solutions sampled on Days 0, 7, 14, and 21. Both "fresh" and "old" samples were analysed. Mean, measured concentrations were 40, 66, 100, 150, 240, and 340 mg thifensulfuron methyl/L and ranged from 95 to 103% of nominal concentrations. All chemical and physical parameters for the 21 day study were within acceptable ranges. All validation criteria were met for the study.

First day of reproduction varied considerably from one test concentration to the next, with no discernible trend until the 340 mg/L test concentration was reached (see Table 9). However, from a statistical standpoint, the 66, 240, and 340 mg a.s./L test concentrations were significantly later than the control. Because the 100 and 150 mg a.s./L test concentrations were not statistically different from the control, the 66 mg a.s./L test results are considered biologically insignificant. A summary of percent adult survival, total live young produced per surviving female, total immobile adults, and length of surviving adults is shown in Table 9.

Table 9

Summary of effects following exposure of *Daphnia magna* to thifensulfuron methyl for 21 days

Mean, measured thifensulfuron methyl concentration (mg/L)	Mean % adult survival ^{a,b}	Mean first day of reproduction	Mean total live young ^c	Mean total immobile adults	Mean adult length (mm)
Water Control (0.0)	100	9.0	70	0.000	3.8
40	98	9.9	54	0.025	3.7
66	100	10.5*	71	0.000	3.6
100	98	8.8	87	0.025	3.7
150	100	9.3	63	0.000	3.4*
240	98	10.2*	69	0.025	3.4*
340	100	12.0*	38*	0.000	3.1*

^a— Percent of adult daphnids alive at the end of the test (immobility was synonymous with death)

^b— There were no significant differences from the control (p <0.05)

^c— Mean of live young produced per surviving female

*— Significantly different from the control (Dunnett's test, p <0.05)

III. CONCLUSION

The 21 day NOEC for *Daphnia magna* based on mean, measured concentrations and adult survival was >340 mg thifensulfuron methyl/L, the highest concentration tested. The EC_{50s} for *Daphnia magna* based on mean, measured concentrations and growth and reproductive parameters, respectively, were both >340 mg thifensulfuron methyl/L, the highest concentration tested. The 21-day LOEC for *Daphnia magna* based on mean, measured concentrations and reproductive parameters (total offspring and offspring per surviving adult) was 340 mg thifensulfuron methyl/L, the highest concentration tested, and the 21 day NOEC was 240 mg a.s./L. The 21 day NOEC for reproduction (first day of reproduction) was 150 mg a.s./L and the LOEC was 240 mg a.s./L. For growth, the most sensitive parameter, the 21 day LOEC, was 100 mg a.s./L and the NOEC was 150 mg a.s./L.

(Hutton, D.G., 1989)

Effects on algal growth and growth rate

Thifensulfuron methyl

Report: Arnie, J.R., Lockard, L., Martin, K.H., Porch, J.R. (2016); Thifensulfuron methyl (DPX-M6316) technical: A 72-hour toxicity test with the freshwater alga (*Pseudokirchneriella subcapitata*)

Report No.: DuPont 46004, Revision No. 1

Guidelines: OCSPP 850.4500 (2012), OECD 201 (2006) **Deviations:** None

Testing Facility: Wildlife International Ltd (USA), Easton, Maryland, USA

Testing Facility Report No.: 112P-268

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA.

Executive summary:

The effect of thifensulfuron methyl on the area under the growth curve, growth rate, and yield of the freshwater alga, *Pseudokirchneriella subcapitata* was determined in a 72 hour test without test medium renewal. The test was conducted according to OECD Guidelines for the Testing of Chemicals: 201 (2006). Treatments consisted of six nominal concentrations of 0.10, 0.26, 0.64, 1.6, 4.0, and 10 mg thifensulfuron methyl/L, an untreated blank control, and an abiotic (stability) control. The EC₅₀ and NOEC values for *P. subcapitata* were based on mean, measured concentrations of thifensulfuron methyl for area under the growth curve (biomass), growth rate, and yield. The 72-hour E_bC₅₀, E_rC₅₀ and E_yC₅₀ values based on biomass, growth rate, and yield, respectively, were 0.27, 1.4, and 0.30 mg a.s./L, based on mean, measured concentrations of thifensulfuron methyl. The NOEC and LOEC for biomass were <0.10 and 0.10 mg a.s./L, respectively. The NOEC and LOEC for growth rate and yield were 0.10 and 0.25 mg a.s./L, respectively. The 72-hour E_bC₀₅ value was determined to be 0.038 mg a.s./L.

I. MATERIALS AND METHODS

A. MATERIALS

1	Test material:	Thifensulfuron methyl technical
	Lot/Batch #:	M6316-293
	Purity:	99.3%
	Description:	Solid
	CAS#:	79277-27-3
	Stability of test compound:	Thifensulfuron methyl was stable under test conditions.
2	Control:	Freshwater algal (AAP) medium
	Test vehicle:	Freshwater algal (AAP) medium
	Toxic reference:	None

- | | | |
|---|---|--|
| 3 | Test organism:
Species:
Initial population:
Source:
Growth medium:
Test chamber: | Freshwater alga
<i>Pseudokirchneriella subcapitata</i>
approximately 10000 cells/mL
Wildlife International, Easton, MD in-house culture
Freshwater algal medium
250 mL Erlenmeyer flask containing 100 mL of test solution and plugged with foam stoppers |
| 4 | Environmental conditions
(in life period):
Temperature:

Photoperiod:
pH |
24.34 to 24.78°C (measured in a container of water located adjacent to the test)

24-hour photoperiod (5020 to 7030 lux)
7.2 to 9.8 throughout the exposure period |

B. STUDY DESIGN AND METHODS

1. In life initiated/completed

26 April 2016 to 29 April 2016

2. Experimental treatments

A study was conducted to determine the effect of thifensulfuron methyl on the area under the growth curve, growth rate, and yield of the freshwater alga, *P. subcapitata*. The algae were exposed to an untreated blank control and six nominal, concentrations of 0.10, 0.26, 0.64, 1.6, 4.0, and 10 mg a.s./L, in freshwater AAP medium for 72 hours, without test medium renewal. Each test concentration was tested as four replicates and eight replicates were maintained in the blank control. An abiotic (stability) control was also included and was tested as a single replicate in the 10 mg a.s./L treatment group.

3. Observations

Test concentrations were measured on Day 0 and Day 3 (72 hours) to verify stability of the test item. Cell counts were recorded for samples collected approximately 24, 48, and 72 hours after test initiation. Area under the growth curve (biomass), growth rate, and yield were recorded and expressed as percent inhibition relative to the control replicates following exposure to thifensulfuron methyl for 72 hours.

4. Statistics

Area under the growth curve, growth rate and yield data were evaluated for normality and homogeneity of variance ($\alpha = 0.01$) using the Shapiro Wilk's and Levene's tests, respectively. Area under the growth curve and growth rate data met assumptions of normality and homogeneity of variance. Yield data violated assumptions of normality; however, log transformation of the data resolved this issue. The treatment groups were compared to the control using ANOVA and Dunnett's test ($\alpha = 0.05$). The results of the statistical analyses, as well as an evaluation of the concentration response pattern, were used to determine the NOEC relative to each parameter at 72 hours. In instances where an experimental NOEC could not be determined, EC_{05} values and their corresponding 95% confidence intervals were calculated and reported.

II. RESULTS AND DISCUSSION

A. FINDINGS

Measured concentrations of thifensulfuron methyl in the test solutions on Day 0 ranged from 98 to 102% of nominal, and recoveries on Day 3 ranged from 91 to 97% of nominal concentrations. The measured concentration of thifensulfuron methyl in the abiotic control solution at test termination was 99%, of nominal. Thifensulfuron methyl was determined to be stable over the course of the test. The untreated control solutions contained no quantifiable concentrations of thifensulfuron methyl. Mean, measured concentrations of thifensulfuron methyl in the biotic treatment groups were determined to be 0.10, 0.25, 0.62, 1.6, 3.9, and 9.9 mg a.s./L, equivalent to 100, 96, 97, 100, 98, and 99% of nominal,

respectively. The results of the study are based on mean, measured concentrations. All validity criteria were met.

A summary of algal growth inhibition following exposure of *P. subcapitata* to thifensulfuron methyl for 72 hours is presented in the following table.

Table 10

Summary of algal growth inhibition following exposure of *Pseudokirchneriella subcapitata* to thifensulfuron methyl for 72 hours

Nominal Thifensulfuron Methyl Concentration (mg a.s./L)	% Inhibition Relative to Blank Control		
	Area Under Curve	Growth rate	Yield
Blank Control (0.0)	—	—	—
0.10	13 ^a	2	11
0.26	48 ^a	11 ^a	44 ^a
0.64	74 ^a	24 ^a	73 ^a
1.6	95 ^a	55 ^a	95 ^a
4.0	99 ^a	78 ^a	99
10	99 ^a	87 ^a	100 ^a

* — Treatment group mean was significantly different from the blank control mean (Dunnett's test, $p < 0.05$).

III. CONCLUSIONS

The effects of thifensulfuron methyl on area under the growth curve, growth rate, and yield of *Pseudokirchneriella subcapitata* as calculated using mean, measured concentrations were as follows:

	Mean, Measured Thifensulfuron Methyl Concentration
Area Under Growth Curve (biomass):	72 hr $E_b C_{50}$ = 0.27 mg a.s./L (95% confidence interval: 0.23 to 0.33 mg a.s./L) 72 hr $E_b C_{20}$ = 0.10 mg a.s./L (95% confidence interval: 0.076^a to 0.13 mg a.s./L) 72 hr $E_b C_{10}$ = 0.059 mg a.s./L^a (95% confidence interval: 0.043^a to 0.081^a mg a.s./L) 72 hr $E_b C_{05}$ = 0.038 mg a.s./L^a (95% confidence interval: 0.026^a to 0.055^a mg a.s./L) 72 hr LOEC = 0.10 mg a.s./L 72 hr NOEC < 0.10 mg a.s./L^b
Growth Rate:	72 hr $E_r C_{50}$ = 1.4 mg a.s./L (95% confidence interval: 1.2 to 1.6 mg a.s./L) 72 hr $E_r C_{20}$ = 0.38 mg a.s./L (0.30 to 0.47 mg a.s./L) 72 hr $E_r C_{10}$ = 0.19 mg a.s./L (95% confidence interval: 0.15 to 0.25 mg a.s./L) 72 hr LOEC = 0.25 mg a.s./L 72 hr NOEC = 0.10 mg a.s./L
Yield:	72 hr $E_y C_{50}$ = 0.30 mg a.s./L (95% confidence interval: 0.25 to 0.36 mg a.s./L) 72 hr $E_y C_{20}$ = 0.12 mg a.s./L (0.093^a to 0.15 mg a.s./L) 72 hr $E_y C_{10}$ = 0.074 mg a.s./L^a (95% confidence interval: 0.054^a to 0.10 mg a.s./L) 72 hr LOEC = 0.25 mg a.s./L 72 hr NOEC = 0.10 mg a.s./L

^a—Estimated value is extrapolated.

^b— EC_{05} value provided where NOEC value could not be determined.

(Arnie, J.R., Lockard, L., Martin, K.H., Porch, J.R., 2017)

IN-D8858

Report: Arnie, J.R., Zhang, L., Porch, J.R., Martin, K.H. (2016); IN-D8858: A 72-hour toxicity test with the freshwater alga (*Pseudokirchneriella subcapitata*)

Report No.: DuPont 42163, Revision No. 1

Guidelines: OECD 201 (2006), OCSPP 850.4500 (2012)

Deviations: None

Testing Facility: Wildlife International Ltd (USA), Easton, Maryland, USA

Testing Facility Report No.: 112P-236

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA.

Executive summary:

The effect of IN-D8858 on the area under the growth curve, growth rate and yield of the freshwater alga *Pseudokirchneriella subcapitata* was determined in a 72-hour test without test medium renewal. The test was conducted according to OECD Guidelines for the Testing of Chemicals: 201 (2006) and U.S. EPA Series 850—Ecological Effects Test Guidelines OCSPP Number 850.4500 (2012). Treatments consisted of five IN-D8858 concentrations of 3.1, 6.3, 13, 25 and 50 µg/L, an untreated blank control and an abiotic (stability) control. Geometric mean, measured concentrations of IN-

D8858 were 2.3, 5.1, 10, 21 and 45 µg/L. The EC_x, NOEC and LOEC values for *P. subcapitata* were based on geometric mean, measured concentrations of IN-D8858 for area under the growth curve (biomass), growth rate and yield. The 72-hour E_bC₅₀, E_rC₅₀ and E_yC₅₀ values based on biomass, growth rate and yield, respectively, were all >45 µg/L. The NOEC and LOEC for biomass, growth rate and yield were 45 and >45 µg/L, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1	Test material:	IN-D8858 technical metabolite
	Lot/Batch #:	D8858-002
	Purity:	95.0%
	Description:	Solid
	CAS#:	None
	Stability of test compound:	Stable under test conditions
2	Control:	Freshwater AAP algal medium
	Test vehicle:	Freshwater AAP algal medium
	Toxic reference:	None
3	Test organism:	Freshwater alga
	Species:	<i>Pseudokirchneriella subcapitata</i>
	Initial population:	approximately 10000 cells/mL
	Source:	Wildlife International, Easton, MD in house culture
	Growth medium:	Freshwater AAP algal medium
	Test chamber:	250 mL Erlenmeyer flask containing 100 mL of test solution and plugged with foam stoppers
4	Environmental conditions (in life period):	
	Temperature:	23.55 to 24.17°C (measured in a container of water located adjacent to the test)
	Photoperiod:	24-hour photoperiod (5280 to 7010 lux)
	pH	7.4 to 8.5 throughout the exposure period

B. STUDY DESIGN AND METHODS

1. In life initiated/completed
31 March 2015 to 03 April 2015
2. Experimental treatments
A study was conducted to determine the effect of IN-D8858 on the area under the growth curve, growth rate and yield of the freshwater alga *Pseudokirchneriella subcapitata*. The algae were exposed to an untreated blank control and five geometric mean, measured concentrations of 2.3, 5.1, 10, 21 and 45 µg/L, in freshwater algal medium for 72 hours, without test medium renewal. Each test concentration was tested as four replicates, and the blank control group was tested as eight replicates. An abiotic (stability) control was also included and was tested as a single replicate.
3. Observations
Test concentrations were measured on Day 0 and Day 3 (72 hours) to verify stability of the test item. Cell counts were recorded for samples collected approximately 24, 48, and 72 hours after test initiation. Area under the growth curve (biomass), growth rate and yield were recorded and expressed as percent inhibition relative to the control replicates following exposure to IN-D8858 for 72 hours.
4. Statistics
Area under the growth curve, growth rate and yield data were evaluated for normality and homogeneity of variance ($\alpha = 0.01$) using the Shapiro Wilk's and Levene's tests, respectively. The data met all assumptions, therefore the treatment groups were compared to the control using ANOVA and Dunnett's test ($\alpha = 0.05$). The results of the statistical

analyses, as well as an evaluation of the concentration response pattern, were used to determine the NOEC and LOEC relative to each parameter at 72 hours. Due to the lack of dose response, the EC_x values were empirically determined to be greater than the highest concentration tested.

II. RESULTS AND DISCUSSION

A. FINDINGS

Geometric mean, measured concentrations of IN D8858 in the biotic test solutions ranged from 74 to 90% of nominal concentrations. The measured concentration of IN D8858 in the abiotic control solution at test termination was 99% of the nominal concentration. IN D8858 was determined to be stable under test conditions. The decline of measured concentrations in the biological replicates indicates that the test substance may have either adhered to or been metabolised by the test organism over the 72-hour exposure. The results of the study are based on geometric mean, measured test concentrations. The untreated control solutions contained no quantifiable concentrations of the test substance. All validity criteria were met.

A summary of algal growth inhibition following exposure of *P. subcapitata* to IN D8858 for 72-hours is presented in the table that follows.

Table 11

Summary of algal growth inhibition following exposure of *Pseudokirchneriella subcapitata* to IN D8858 for 72 hours

Geometric Mean, Measured IN-D8858 Concentration (µg/L)	% Inhibition Relative to Blank Control		
	Area Under Growth Curve	Growth rate	Yield
Blank Control (0.0)	—	—	—
2.3	-25	-4	-21
5.1	-13	-2	-10
10	-9	-1	-4
21	-32	-5	-26
45	-29	-3	-19

Note: No treatment group mean was significantly different from the blank control mean (Dunnett's test, p > 0.05).

III. CONCLUSIONS

The effects of IN D8858 on area under the growth curve, growth rate and yield of *Pseudokirchneriella subcapitata* as calculated using geometric mean, measured IN D8858 concentrations were as follows:

Area Under Growth Curve (biomass):	Geometric Mean, Measured IN-D8858 Concentration 72-hr $E_b C_{50} > 45 \mu\text{g/L}$ (Confidence interval not applicable) 72-hr NOEC = $45 \mu\text{g/L}$ 72-hr LOEC = $> 45 \mu\text{g/L}$
Growth Rate:	72-hr $E_f C_{50} > 45 \mu\text{g/L}$ (Confidence interval not applicable) 72-hr $E_f C_{20} > 45 \mu\text{g/L}$ (Confidence interval not applicable) 72-hr $E_f C_{10} > 45 \mu\text{g/L}$ (Confidence interval not applicable) 72-hr NOEC = $45 \mu\text{g/L}$ 72-hr LOEC = $> 45 \mu\text{g/L}$
Yield:	72-hr $E_y C_{50} > 45 \mu\text{g/L}$ (Confidence interval not applicable) 72-hr $E_y C_{20} > 45 \mu\text{g/L}$ (Confidence interval not applicable) 72-hr $E_y C_{10} > 45 \mu\text{g/L}$ (Confidence interval not applicable) 72-hr NOEC = $45 \mu\text{g/L}$ 72-hr LOEC = $> 45 \mu\text{g/L}$

(Arnie, J.R., Zhang, L., Porch, J.R., Martin, K.H., 2016)

Effects on aquatic plants

Lemna gibba

IN-D8858

HA 8.6/21

Report: Arnie, J.R., Zhang, L., Porch, J.R., Martin, K.H. (2016); IN-D8858: A 7-day static-renewal toxicity test with duckweed (*Lemna gibba*-G3)

Report No.: DuPont 42164, Revision No. 1

Guidelines: OCSPP Guideline 850.4400 (2012), OECD 221 (2006)

Deviations: None

Testing Facility: Wildlife International Ltd (USA), Easton, Maryland, USA

Testing Facility Report No.: 112P-237

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA.

Executive summary:

Toxicity of IN-D8858 to the floating, freshwater vascular plant *Lemna gibba*-G3 was determined in a static-renewal, 7-day test. The test was conducted in accordance with U.S. Environmental Protection Agency (EPA) Series 850—Ecological Effects Test Guidelines, OCSPP Number 850.4400 and OECD Guideline 221. Treatments consisted of five nominal concentrations of IN-D8858 of 3.1, 6.3, 13, 25 and 50 $\mu\text{g/L}$, an untreated control, and an abiotic (stability) control at 50 $\mu\text{g/L}$.

The 7-day EC_{50} values for each tested parameter based on geometric mean, measured concentrations of IN-D8858 were all determined to be $> 44 \mu\text{g/L}$, the highest concentration tested. The 7-day NOEC

and LOEC for all endpoints based on geometric mean, measured concentrations of IN D8858 were 44 and >44 µ/L, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN D8858 technical metabolite
Lot/Batch #: D8858-002
Purity: 95.0%
Description: Solid
CAS#: Not available
Stability of test compound: Stable under test conditions
2. Control: 20X AAP nutrient medium
Test vehicle: 20X AAP nutrient medium
Toxic reference: None
3. Test organism: Duckweed
Species: *Lemna gibba* G3
Initial population: 4 plants, totalling 12 fronds
Source: Wildlife International in house culture
Growth medium: 20X AAP nutrient medium
Test chamber: 250 mL beaker containing approximately 100 mL of test solution and covered with a disposable petri dish lid to permit gas exchange
4. Environmental conditions (in life period):
Temperature: 24.72 to 25.47°C (Surrogate vessel)
Photoperiod: 24 hr photoperiod (5870 to 6860 lux)
pH: 8.1 to 9.3 throughout the exposure period

B. STUDY DESIGN AND METHODS

1. Experimental initiated/completed
03 April 2015 to 13 April 2015
2. Experimental treatments
Toxicity of IN D8858 to the floating, freshwater vascular plant *Lemna gibba* G3 was determined in a static renewal, 7 day test. The effect of IN D8858 on *Lemna gibba* G3 was determined in 20X AAP nutrient medium. Treatments consisted of five IN D8858 nominal concentrations of 3.1, 6.3, 13, 25 and 50 µg/L, a blank control and an abiotic (stability) control. Each test concentration was tested as four replicate test vessels and the blank control included eight replicate test vessels. The abiotic control was tested as a single unit. Four plants totalling 12 fronds were used per biotic replicate. Plants were incubated in an environmental chamber for 7 days, with renewal of test solutions occurring on Days 3 and 5.
3. Observations
Test concentrations were measured at test initiation, from new and old solutions at each renewal period and test termination to verify stability and concentrations of IN D8858. Frond counts were made on Days 0, 3, 5, and 7. Biomass was determined at the completion of the 7 day test. Growth rates were determined on Day 7 and were based on frond count and on biomass. Healthy frond count yield and biomass yield were determined by subtracting the initial frond count or biomass from the end test values. Healthy frond count, frond count yield, biomass, biomass yield, growth rate based on frond count, and growth rate based on biomass were expressed as percent inhibition relative to the blank control.
4. Statistics
Day 7 EC₅₀, EC₂₀ and EC₁₀ values and their corresponding 95% confidence intervals were calculated, when possible, using non linear regression with treatment response (frond number, frond number yield, biomass, biomass yield and respective growth rates) and

geometric mean, measured test concentrations. Dead, chlorotic, and necrotic fronds were counted and combined in order to calculate the percentage of abnormal fronds relative to the total number of fronds present in each test chamber.

The data were evaluated for normality and homogeneity of variances ($\alpha = 0.01$) using the Shapiro-Wilk's and Levene's tests, respectively. Treatment group means were compared to the means of the blank control group ($\alpha = 0.05$) using analysis of variance (ANOVA) and Dunnett's t test. Results of the statistical analyses, as well as an evaluation of the concentration response pattern and other observations of effects, were used to determine the NOEC and LOEC. All calculations and statistical analyses were conducted using "Microsoft Excel 2010" or "The SAS System for Windows Version 9.4."

H. RESULTS AND DISCUSSION

A. FINDINGS

The geometric mean, measured concentrations of IN-D8858 in the biological replicates were 2.9, 5.7, 11, 22 and 44 $\mu\text{g/L}$, which were 94, 90, 85, 88 and 88% of nominal, respectively. Measured concentrations of IN-D8858 in the abiotic replicate included in the nominal 50 $\mu\text{g/L}$ treatment group on Days 3, 5 and at test termination were 78, 80 and 87% of nominal. Blank control solution showed no detectable concentrations of the active substance. The test item was determined to be stable over the course of the test. The validation criterion was met for the study. Data on healthy frond count and biomass, frond count yield and biomass yield, and growth rate based on frond count and on biomass are summarized in the tables that follow.

Table 12
 Summary of growth inhibition (frond count and biomass) following exposure of *Lemna gibba* G3 to IN-D8858 for 7 days

Geometric mean, measured IN-D8858 concentration ($\mu\text{g/L}$)	Frond count		Biomass	
	7-Day mean frond count ^a	% Inhibition relative to blank control	7-Day mean biomass (mg) ^a	% Inhibition relative to blank control
Blank Control	234	—	30.3	—
2.9	245	-5	31.6	-4
5.7	232	+1	31.5	-4
11	253	-8	32.9	-9
22	232	+1	31.3	-3
44	229	+2	29.9	+1

^a—None of the treatment responses were significantly reduced from the blank control response (Dunnett's test, $p > 0.05$).

Table 13
 Summary of growth inhibition (frond count yield and biomass yield) following exposure of *Lemna gibba* G3 to IN-D8858 for 7 days

Geometric mean, measured IN-D8858 concentration ($\mu\text{g/L}$)	Frond count yield		Biomass yield	
	7-Day mean frond count yield ^a	% Inhibition relative to blank control	7-Day mean biomass yield (mg) ^a	% Inhibition relative to blank control
Blank Control	222	—	29.0	—
2.9	233	-5	30.3	-4
5.7	220	+1	30.2	-4
11	241	-8	31.6	-9
22	220	+1	30.0	-3
44	217	+2	28.6	+2

^a—None of the treatment responses were significantly reduced from the blank control response (Dunnett's test, $p > 0.05$).

Table 14
 Summary of growth inhibition (growth rate) following exposure of *Lemna gibba* G3 to IN-D8858 for 7 days

Geometric mean, measured IN-D8858 concentration (µg/L)	0-7 day growth rate based on frond count		0-7 day growth rate based on biomass	
	0-7 day mean growth rate ^a	% Inhibition relative to blank control	0-7 day mean growth rate ^a	% Inhibition relative to blank control
Blank Control	0.424	—	0.450	—
2.9	0.431	-2	0.455	-1
5.7	0.422	+1	0.453	-1
11	0.435	-3	0.461	-2
22	0.423	0	0.454	-1
44	0.421	+1	0.447	+1

^a—None of the treatment responses were significantly reduced from the blank control response (Dunnett's test, $p > 0.05$).

III. CONCLUSIONS

Lemna gibba G3 was exposed to five geometric mean, measured concentrations of IN-D8858 ranging from 2.9 to 44 µg/L and evaluated for effects on frond count, frond yield, frond count growth rate, biomass, biomass yield, and biomass growth rate. Dunnett's test indicated that for all six endpoints, treatment group means were not significantly reduced ($p > 0.05$) in any of the IN-D8858 treatment groups when compared to the blank control group means. Based on geometric mean measured concentrations, the NOEC and LOEC for all endpoints were determined to be 44 and >44 µg/L, respectively.

EC₅₀ (95% confidence interval), NOEC and LOEC values based on geometric mean, measured concentrations of IN-D8858 on *Lemna gibba* G3 were as follows:

	Geometric mean, measured concentration of IN-D8858
7-Day Frond Count:	EC₅₀ > 44 µg/L (not applicable) NOEC = 44 µg/L LOEC > 44 µg/L
7-Day Frond Count Yield:	EC₅₀ > 44 µg/L (not applicable) NOEC = 44 µg/L LOEC > 44 µg/L
0-7 Day Frond Count Growth Rate:	EC₅₀ > 44 µg/L (not applicable) NOEC = 44 µg/L LOEC > 44 µg/L
7-Day Biomass:	EC₅₀ > 44 µg/L (not applicable) NOEC = 44 µg/L LOEC = 44 µg/L
7-Day Biomass Yield:	EC₅₀ > 44 µg/L (not applicable) NOEC = 44 µg/L LOEC > 44 µg/L
0-7 Day Biomass Growth Rate:	EC₅₀ > 44 µg/L (not applicable) NOEC = 44 µg/L LOEC > 44 µg/L

(Arnie, J.R., Zhang, L., Porch, J.R., Martin, K.H., 2016)

~~Effects on non-target terrestrial arthropods~~

~~Effects on non-target terrestrial arthropods using artificial substrates~~

~~Effects on non-target terrestrial arthropods in extended laboratory/semi-field tests~~

~~Other terrestrial invertebrates~~

~~*Folsomia candida*~~

~~Study submitted for the first time at EU level in the June 2017 confirmatory data submission. Listed under Reference List 1 “Documents Submitted, List by Annex Point” and under Reference List 2, “Documents Submitted, List by Author.”~~

~~Report: Lührs, U. (2015a); IN JZ789: Effects on the Collembola *Folsomia candida* in artificial soil with 5% peat~~

~~DuPont Report No.: DuPont 42165~~

~~Guidelines: OECD 232 (2009), ISO 11267 (1999)~~

~~Deviations: None~~

~~Testing Facility: IBACON, Rossdorf, Germany~~

~~Testing Facility Report No.: 96321016~~

~~GLP: Yes~~

~~Certifying Authority: Hessisches Ministerium für Umwelt, Energie, Landwirtschaft und Verbraucherschutz (Weisbaden, Germany)~~

Executive summary:

The effects of IN JZ789 on the mortality and reproduction of Collembola (*Folsomia candida*) were determined in a 28 day soil exposure laboratory study according to OECD 232, 2009 and ISO 11267, 1999. Ten to twelve day old Collembola were exposed for 28 days to artificial soil (prepared according to OECD 232) treated with five nominal concentrations of IN JZ789 of 6.42, 12.83, 25.67, 51.33 and 102.7 mg test item/kg dry artificial soil (corresponding to 5.662, 11.32, 22.65, 45.30 and 90.58 mg IN JZ789/kg dry artificial soil, adjusted for purity) and an untreated control. Mortality and reproduction (number of juveniles produced) were assessed after 28 days. The overall 28 day NOEC (No Observed Effect Concentration) based on mortality and reproduction was determined to be 90.58 mg IN JZ789/kg soil dry weight.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN JZ789 technical metabolite
Lot/Batch #: JZ789-001
Purity: 88.2%
Description: Solid
CAS #: 171628-02-7
Stability of test compound: Not analysed in the test system
2. Control: Untreated (and moistened with deionised water)
Test vehicle: Deionised water
3. Test System: Collembola
Species: *Folsomia candida*, Willem (Collembola: Isotomidae)
Age at dosing: 10 to 12 days
Weight at dosing: Not determined
Source: In-house laboratory culture
Acclimation period: 10 to 12 days
Test chamber: Glass containers (volume: 100 mL; diameter: 5.0 cm),
closed, filled with 30 ± 1.0 g artificial soil fresh weight
Test medium: Artificial soil prepared according to OECD 232, maximum
water holding capacity of the artificial soil, as measured:
42%
Diet: Granulated dry yeast
Water content of soil: Initiation: 21.6 to 22.0% equivalent to 51.4 to 52.3% of
the maximum water holding capacity
Termination: 18.5 to 21.3% equivalent to 44.1 to 50.8% of
the maximum water holding capacity
Soil pH: 6.0 to 6.1 at test start; 5.8 to 6.0 at test termination
4. Environmental conditions
Temperature: Within the range of 18 to 22°C
Photoperiod: 16 h light, 8 h dark, photoperiod within the range of 400 to
800 lux

B. STUDY DESIGN AND METHODS

1. In life initiated/completed
02 February 2015 to 03 March 2015

2. Experimental treatments

A study was conducted to determine the effects of IN JZ789 on the mortality and reproduction of Collembola (*Folsomia candida*). Eight replicates for the control and four replicates per test item group, containing ten Collembola each (total 80 per control and 40 individuals per test item group) were each exposed for 28 days to the nominal concentrations of IN JZ789 of 6.42, 12.83, 25.67, 51.33 and 102.7 mg test item/kg dry artificial soil (corresponding to 5.662, 11.32, 22.65, 45.30 and 90.58 mg IN JZ789/kg dry artificial soil, adjusted for purity) and an untreated control (deionised water only). A reference item (boric acid, at a concentration range of 30.5 to 200 mg/kg artificial soil dry weight) is tested at least once per year to ensure sensitivity of the test system. The most recent test to this study was conducted in November/December 2014.

3. Observations

After the 28 day exposure period, adult Collembola were counted and the mean number of adults in each treatment group was determined. The number of juveniles produced in each treatment group over 28 days exposure period was also determined and the percent reduction in juveniles produced relative to the untreated control group was calculated.

4. Statistics

Mortality data were analysed for significance by using Fisher's Exact Test (one-sided greater, alpha = 0.05).

Reproduction data were tested for normal distribution and homoscedasticity using Shapiro-Wilk's test and Levene's test (alpha = 0.05). Further statistical evaluation of the NOEC for reproduction was performed using Williams t test (multiple comparison, alpha = 0.05, one-sided smaller). EC₅₀ was not determined by statistical analysis as reduction of reproduction was less than 50%.

II. RESULTS AND DISCUSSION

A. FINDINGS

All validity criteria were met. The EC₅₀ for reproduction of the reference item (boric acid) in the most recent test was 145.1 mg boric acid/kg dry artificial soil.

A summary of the results is provided in the table below.

Table 15

The effects on mortality and reproduction of Collembola, *Folsomia candida*, exposed to IN JZ789 in artificial soil for 28 days

Nominal IN-JZ789 concentration, adjusted for purity (mg/kg soil)	Mean % mortality ^a	Reproduction	
		Mean juveniles per replicate ^a	% of control
Untreated control (0.0)	6	575	-
5.662	3	578	101
11.32	5	491	85.4
22.65	5	561	97.6
45.30	8	544	94.6
90.58	3	533	92.7

^a—There were no significant differences from the control (mortality: Fisher's Exact Test, alpha = 0.05, one-sided greater; number of juveniles: Williams t test, alpha = 0.05, one-sided smaller)

III. CONCLUSIONS

The 28 day EC₅₀ and the Lowest Observed Effect Concentration (LOEC) for IN JZ789 were estimated to be greater than 90.58 mg IN JZ789/kg dry artificial soil (adjusted for purity), the highest concentration tested. The overall 28 day No Observed Effect Concentration (NOEC) based on mortality and reproduction was determined to be 90.58 mg IN JZ789/kg dry artificial soil (adjusted for purity).

(Lührs, U., 2015a)

~~Study submitted for the first time at EU level in the June 2017 confirmatory data submission. Listed under Reference List 1 “Documents Submitted, List by Annex Point” and under Reference List 2, “Documents Submitted, List by Author.”~~

~~**Report:** Lührs, U. (2015b); IN U5F72: Effects on the Collembola *Folsomia candida* in artificial soil with 5% peat~~

~~**DuPont Report No.:** DuPont 42481~~

~~**Guidelines:** OECD 232 (2009), ISO 11267 (1999)~~

~~**Deviations:** None~~

~~**Testing Facility:** IBACON, Rossdorf, Germany~~

~~**Testing Facility Report No.:** 97821016~~

~~**GLP:** Yes~~

~~**Certifying Authority:** Hessisches Ministerium für Umwelt, Energie, Landwirtschaft und Verbraucherschutz (Wiesbaden, Germany)~~

Executive summary:

The effects of IN U5F72 on the mortality and reproduction of Collembola (*Folsomia candida*) were determined in a 28-day soil exposure laboratory study according to OECD 232, 2009 and ISO 11267, 1999. Eleven to twelve day old Collembola were exposed for 28 days to artificial soil (prepared according to OECD 232) treated with five nominal concentrations of IN U5F72 of 6.339, 12.68, 25.35, 50.71 and 101.4 mg IN U5F72/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0 and 100 mg IN U5F72/kg dry artificial soil, adjusted for purity) and to an untreated control (using the same amount of acetone and fine quartz sand per g substrate as in the test item groups). Mortality and reproduction (number of juveniles produced) were assessed after 28 days. The overall 28-day NOEC (No Observed Effect Concentration) based on mortality and reproduction was determined to be 100 mg IN U5F72/kg soil dry weight.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-U5F72 technical metabolite
Lot/Batch #: U5F72-000
Purity: 98.6%
Description: Solid
CAS #: 171628-03-8
Stability of test compound: Not analysed in the test system
2. Control: Untreated (using the same amount of acetone and sand per g substrate as in the test item groups and moistened with deionised water)

Test vehicle: Acetone
3. Test System: Collembola
Species: *Folsomia candida*, Willem (Collembola: Isotomidae)
Age at dosing: 11 to 12 days
Weight at dosing: Not determined
Source: In-house laboratory culture
Acclimation period: 11 to 12 days
Test chamber: Glass containers (volume: 100 mL; diameter: 5.0 cm), closed, filled with 30 ± 1.0 g artificial soil fresh weight
Test medium: Artificial soil prepared according to OECD 232, maximum water holding capacity of the artificial soil, as measured: 45%

Diet: Granulated dry yeast
Water content of soil: Initiation: 23.2 to 23.7% equivalent to 51.7 to 52.6% of the maximum water holding capacity
Termination: 19.9 to 21.0% equivalent to 44.1 to 46.6% of the maximum water holding capacity

Soil pH: 5.6 to 6.1 at test start; 5.8 to 6.3 at test termination
4. Environmental conditions
Temperature: Within the range of 18 to 22°C
Photoperiod: 16 h light, 8 h dark, photoperiod within the range of 400 to 800 lux

B. STUDY DESIGN AND METHODS

1. In life initiated/completed
11 May 2015 to 09 June 2015
2. Experimental treatments
A study was conducted to determine the effects of IN-U5F72 on the mortality and reproduction of Collembola (*Folsomia candida*). Eight replicates for the control and four replicates per test item group, containing ten Collembola each (total 80 per control and 40 individuals per test item group) were each exposed for 28 days to the nominal concentrations of IN-U5F72 of 6.339, 12.68, 25.35, 50.71 and 101.4 mg IN-U5F72/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0 and 100 mg IN-U5F72/kg dry artificial soil, adjusted for purity) and to an untreated control (using the same amount of acetone and fine quartz sand per g substrate as in the test item groups). A reference item (boric acid, at a concentration range of 30.5 to 200 mg/kg artificial soil dry weight) is tested at least once per year to ensure sensitivity of the test system. The most recent test to this study was conducted in November/December 2014.
3. Observations
After the 28 day exposure period, adult Collembola were counted and the mean number of adults in each treatment group was determined. The number of juveniles produced in each

treatment group over 28 days exposure period was also determined and the percent reduction in juveniles produced relative to the untreated control group was calculated.

4. Statistics

Mortality data were analysed for significance by using Fisher's Exact Test (one-sided greater, alpha = 0.05).

Reproduction data were tested for normal distribution and homoscedasticity using Shapiro-Wilk's test and Levene's test (alpha = 0.05). Further statistical evaluation of the NOEC for reproduction was performed using Williams t test (multiple comparison, alpha = 0.05, one-sided smaller). EC₅₀ was not determined by statistical analysis as reduction of reproduction was less than 50%.

II. RESULTS AND DISCUSSION

A. FINDINGS

All validity criteria were met. The EC₅₀ for reproduction of the reference item (boric acid) in the most recent test was 145.1 mg boric acid/kg dry artificial soil.

A summary of the results is provided in the table below.

Table 16

The effects on mortality and reproduction of Collembola, *Folsomia candida*, exposed to IN U5F72 in artificial soil for 28 days

Nominal IN-U5F72 concentration, adjusted for purity (mg/kg soil)	Mean % mortality ^a	Reproduction	
		Mean juveniles per replicate ^a	% of control
Untreated control (0)	13	704	-
6.25	5	614	87.2
12.5	13	686	97.5
25.0	8	751	107
50.0	13	619	88.0
100	15	623	88.6

^a—There were no significant differences from the control (mortality: Fisher's Exact Test, alpha = 0.05, one-sided greater; number of juveniles: Williams t test, alpha = 0.05, one-sided smaller)

III. CONCLUSIONS

The 28 day EC₅₀ and the Lowest Observed Effect Concentration (LOEC) for IN U5F72 were estimated to be greater than 100 mg IN U5F72/kg dry artificial soil, the highest concentration tested. The overall 28 day No Observed Effect Concentration (NOEC) based on mortality and reproduction was determined to be 100 mg IN U5F72/kg dry artificial soil.

(Lührs, U., 2015b)