REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: GLOB182F

Product name(s): SURRENDER

Chemical active substance:

Fludioxonil, 100 g/L

Interzonal

Zonal Rapporteur Member State: PL

CORE ASSESSMENT

(Authorisation)

Applicant: Globachem NV

Submission date: January 2021

MS Finalisation date: October 2021 (initial Core Assessment)

March 2022 (final Core Assessment)

Version history

When	What
January 2021	Initial dRR – Globachem NV
October 2021	Initial izRMS assessment The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the izRMS 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~~.
March 2022	Final report (Core Assessment updated following the commenting period) Additional information/assessments included by the izRMS 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)

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 I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			PHI (days)	Remarks: e.g. g safener/ synergist per ha	**Conclusion**						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	L/ton seeds a) max. rate per appl. b) max. total rate per crop/season	Kg a.s./ton seeds a) max. rate per appl. b) max. total rate per crop/season	g a.s./ha a) max. rate per appl. b) max. total rate per crop/season	Sowing rate (kg seeds/ha) min/ max	Water L/ton seeds min / max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
Interzonal uses (use as seed treatment, in greenhouses (or other closed places of plant production), as post-harvest treatment or for treatment of empty storage rooms)																				
1 [1)]	izRMS + all cMS*	Maize (forage) ZEAMX	I (treatment seeds) F (sowing)	*Fusarium* sp. FUSASP	Seed treatment	BBCH 00	a) 1 b) 1	/	a) 0.5 b) 0.5	a) 0.050 b) 0.050	a) 1.2-2.375 b) 1.2-2.375	24-47.5	4-8 L (incl. product)	N/A	TGW: 240-380 Sowing density: 100,000-125,000 12-23.75 mL product/ha	A	A	A	A	A	A	A
4, 5 (at higher rate), 6 [2)]	izRMS + cMS*	Sunflower HELAN	I (treatment seeds) F (sowing)	*Botrytis cinerea* (BOTRCI) Downy mildew (PLASHA) *Fusarium* sp. (FUSASP)	Seed treatment	BBCH 00	a) 1 b) 1	/	a) 1.5 b) 1.5	a) 0.150 b) 0.150	a) 0.525-1.6875 b) 0.525-1.6875	3.5-11.3	4-8 L (incl. product)	N/A	TGW: 20-50 Sowing density: 175,000-225,000 5.25-16.95 ~~16.88~~ mL product/ha	C	A	A	A	A	A	A
5 (lower rate)	HU, RO, SI	Sunflower (HELAN)	I (treatment seeds) F (sowing)	*Botrytis cinerea* (BOTRCI) Downy mildew (PLASHA) *Fusarium* sp. (FUSASP)	Seed treatment	BBCH 00	a) 1 b) 1	/	a) 1.25~~-1.5~~ b) 1.25~~-1.5~~	a) 0.125~~-0.150~~ b) 0.125~~-0.150~~	a) 0.4375~~-1.6875~~ b) 0.4375~~-1.6875~~	3.5-11.3	4-8L (incl. product)	N/A	TGW: 20-50 Sowing density: 175,000-225,000 4.375~~-16.88~~ mL product/ha	A	A	A	A	A	A	A

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

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

and non-professional greenhouse use, I: indoor application

[1)] Protective also for uses No 2 (maize at 0.96-1.71 g a.s./ha) and No 3 (sweet corn at 0.2925-0.825 g a.s./ha); see Core Assessment, Part B, Section 0 for more details

[2)] Protective also for use No 6 (sunflower at 1.15 g a.s./ha); see Core Assessment, Part B, Section 0 for more details

Explanation for column 15 – 21 "Conclusion"

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

Remarks table:

(1) Numeration necessary to allow references
(2) Use official codes/nomenclatures of EU
(3) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure)
(4) F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application
(5) Scientific names and EPPO-Codes of target pests/diseases/ weeds or when relevant the common names of the pest groups (e.g. biting and sucking insects, soil born insects, foliar fungi, weeds) and the developmental stages of the pests and pest groups at the moment of application must be named
(6) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
(7) Growth stage at first and last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(8) The maximum number of application possible under practical conditions of use must be provided
(9) Minimum interval (in days) between applications of the same product.
(10) For specific uses other specifications might be possible, e.g.: g/m³ in case of fumigation of empty rooms. See also EPPO-Guideline PP 1/239 Dose expression for plant protection products
(11) The dimension (g, kg) must be clearly specified. (Maximum) dose of a.s. per treatment (usually g, kg or L product / ha).
(12) If water volume range depends on application equipments (e.g. ULVA or LVA) it should be mentioned under "application: method/kind".
(13) PHI - minimum pre-harvest interval
(14) Remarks may include: Extent of use/economic importance/restrictions

9.1.1 Overall conclusions

izRMS comments:

Conclusions provided in points below were corrected by the izRMS accordingly depending on the outcome of the performed risk assessment.

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

Performed risk assessment demonstrated acceptable acute and long-term risk to birds from seeds and seedlings scenario following uses of GLOB182F as a seed treatment in maize and sunflower at application rates equivalent to 50 and 125 mg a.s./kg seeds, respectively.
For intended uses in sunflower at rate equivalent to 150 mg a.s./kg seeds acceptable acute risk could be concluded for seeds and seedling scenario and acceptable long-term risk for seedling scenario. However, the long-term TER for granivorous birds feeding on seeds was below the trigger of 5 indicating potentially unacceptable risk from this use. No risk refinement options were available, since application to sunflower was not considered in the Applicants' calculations and further data needs to be provided in order to resolve the risk from the intended uses in sunflower at the higher rate.

During the commenting period the Applicant provided a Letter of Access to the additional reproductive toxicity study performed with the Northern bobwhite quail at higher doses comparing to the study available during the first EU review of fludioxonil (study already evaluated and agreed during the ongoing renewal process). When results of this study are taken into account, acceptable long-term risk may be concluded for uses in sunflower at the higher rate. However, according to the restrictions indicated in the LoA by the owner of the study (Syngenta), its results may be considered only in support of authorisation of GLOB182F in Poland, while use of these data in other Member States and countries is not possible without the prior consent of Syngenta. Taking this into account, the additional risk assessment performed with consideration of endpoint derived from this new study has been presented only in Part A (relevant for Poland). Results of these calculations and derived conclusions may be taken into account by the concerned Member States once the Applicant for GLOB182F presents respective Syngenta consent at the national level.

~~The TER_a and TER_{lt} values exceed the triggers of 10 and 5 for the acute and long-term assessment respectively, indicating that Fludioxonil does not pose an acute and a long-term risk to wild birds and mammals after the use of GLOB182F according to the intended GAP.~~

The ratio of the effective application rate to the acute and long term toxicity endpoint is less than 3000 for fludioxonil. Therefore it is considered that there is low risk of acute/long term toxicity to birds and mammals from the uptake of contaminated drinking water and no further assessment is required.

Fludioxonil has a log P_{OW} value of 4.12. It was therefore necessary to consider the risk from secondary poisoning further. The risk assessments for earthworm and fish-eating birds and mammals show that there is no unacceptable long-term risk for these birds and mammals after exposure to GLOB182F when used according to the proposed GAP. Evaluation of the risk of secondary poisoning was not required for fludioxonil soil metabolites since in the regulatory studies they were formed only in presence of light while due to the type of application (seed treatment) photolysis will not play a major role in degradation of fludioxonil in soil. For this reason metabolites formed via photolysis in soil may not be taken into account in the risk assessment for the intended uses of GLOB182F. Nevertheless, due to potential migration of fludioxonil to surface water bodies and subsequent degradation, aquatic photolytic metabolites ((CGA339833, CGA344623 and A5) are considered relevant for the risk assessment together with metabolite CGA192155 formed in water/sediment studies. In the course of the EU review photolytic metabolites CGA344623 and A5 were considered to be minor metabolites since they were detected only

in the sterile photolysis study and not in the water/sediment study performed under light conditions. Therefore, only photolytic aquatic metabolite CGA339833 should be taken into account in the evaluation. However, according to information available in the DAR (Vol. 3, B.8 of January 2005), log Pow values for CGA192155 and CGA339833 are <3, hence the evaluation of the risk of secondary poisoning was not triggered for these compounds.

Mammals

The TERa and TERlt values exceed the triggers of 10 and 5 for the acute and long-term risk assessment respectively, indicating that Fludioxonil does not pose an acute and a long-term risk to wild birds and mammals after the use of GLOB182F according to the intended GAP.

The ratio of the effective application rate to the acute and long-term toxicity endpoint is less than 3000 for Fludioxonil. Therefore, it is considered that there is low risk of acute/long term toxicity to mammals from the uptake of contaminated drinking water and no further assessment is required.

Fludioxonil has a log POW value of 4.12. It was therefore necessary to consider the risk from secondary poisoning further. The risk assessments for earthworm and fish-eating mammals show that there is no unacceptable long-term risk for these mammals after exposure to GLOB182F when used according to the proposed GAP. As in case of birds, evaluation of the risk of secondary poisoning was either not triggered or not required for fludioxonil metabolites.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

An acceptable acute and long-term risk to aquatic organisms is identified for the intended use of GLOB182F in sunflower and maize seeds. No risk mitigation measures are required.

9.1.1.3 Effects on bees (KCP 10.3.1)

The hazard quotients after oral and contact exposures are below the trigger value of 50. According to SANCO/10329/2002 (final, 2002), it can therefore be concluded that the intended use of GLOB182F gives a low acute oral and contact risk to honey bees.

The chronic TER's for honey bee adults and larvae are higher than the respective trigger values defined by the EFSA guidance on bees (2013) indicating that the proposed use of GLOB182F as seed treatment on sunflower and maize poses an acceptable chronic risk to honey bee adults and larvae.

The acute TER's for bumblebees at screening step are higher than the respective trigger values defined by the EFSA guidance on bees (2013) indicating that the proposed use of GLOB182F as seed treatment poses an acceptable acute risk to bumblebees.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

The in-field and off-field HQ values for *A. rhopalosiphi*, *T. pyri*, *Aleochara bilineata* and *Poecilus cupreus* fall below the trigger values indicating that GLOB182F does not pose an unacceptable risk to non-target arthropods in in-field and off-field areas following application according to the proposed use patterns. No risk mitigation measures are deemed necessary for the off-crop habitats.

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

The ~~acute and~~ long-term TER values all exceed the Annex VI ~~acute and~~ long-term trigger value of ~~10 and~~ 5 ~~respectively~~, indicating that GLOB182F poses low ~~acute and~~ long-term risk to earthworms when applied according to the proposed use rates.

As the PEC_{soil} is much lower than the concentration at which no significant effects are detected, it can be

concluded that the risk of GLOB182F to soil micro-organisms is acceptable in accordance with the intended use.

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

Since GLOB182F is applied as a seed treatment, it is of no risk to non-target plants and therefore no assessment is required.

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

Not required.

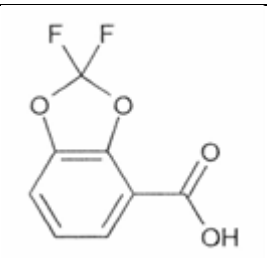
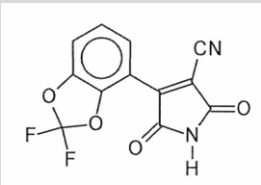
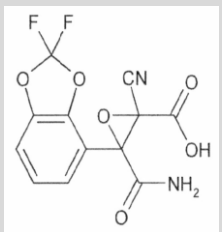
9.1.2 Grouping of intended uses for risk assessment

As the application for GLOB182F is used as a seed treatment for maize and sunflower, the highest dose rate occurring from the highest sowing rate and dose is proposed.

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 GLOB182F is indicated in the table.

Table 9.1-2 Metabolites of Fludioxonil

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
CGA192155		202.1	Soil: Max. 11.7% AR in lab soil photolysis study. Max. 13% AR in the field. Water/sediment: 17.3% ~~13.7%~~	Not required for soil organisms since route of formation of this metabolite (in presence of light) is not relevant for seed treatment Yes, for aquatic organisms
CGA265378		278.2	Soil: Max. 12.3% AR in lab soil photolysis study. Not detected in the field. Water/sediment: 3.8%	Not required for soil organisms since route of formation of this metabolite (in presence of light) is not relevant for seed treatment Yes, for aquatic organisms
CGA339833		312.2	Soil: Max. 9.1% AR in lab soil photolysis study. Max. 8% AR in the field. Water/sediment: not detected Max. 30.5% in aqueous photolysis study	Not required for soil organisms since route of formation of this metabolite (in presence of light) is not relevant for seed treatment Yes, for aquatic organisms

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
CGA344623	Not available	not given in the LoEP	Soil: - Water/sediment: 12.4% in aqueous photolysis study	In the course of the EU review this compound was considered to be minor metabolite since it was detected only in the sterile photolysis study and not in the water/sediment study performed under light conditions
A5	Not available	not given in the LoEP	Soil: - Water/sediment: 11.3% in aqueous photolysis study	In the course of the EU review this compound was considered to be minor metabolite since it was detected only in the sterile photolysis study and not in the water/sediment study performed under light conditions

izRMS comments:

Information regarding fludioxonil metabolite CGA192155 is in general in line with EU agreed data reported in EFSA Scientific Report (2007) 110 with correction regarding the maximum formation in water/sediment study.
It is noted that two other metabolites were formed via photodegradation in soil (CGA265378 and CGA339833). However, due to the type of application (seed treatment) photolysis will not play a major role in degradation of fludioxonil in soil and for this reason metabolites formed exclusively via photolysis in soil may not be taken into account in exposure assessment for the intended uses of GLOB182F. Nevertheless, metabolites formed via photolysis in water (CGA339833, CGA344623 and A5) may be relevant, since fludioxonil applied as a seed treatment may migrate to surface water bodies where it will undergo photodegradation. It is noted that in the course of the EU review photolytic metabolites CGA344623 and A5 were considered to be minor metabolites since they were detected only in the sterile photolysis study and not in the water/sediment study performed under light conditions. Therefore, in addition to CGA192155, also photolytic aquatic metabolite CGA339833 is considered to be relevant for the surface water exposure assessment.

Respective information has been provided by the izRMS in Table 9.1-3 for completeness.

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with Fludioxonil. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on birds of GLOB182F were not evaluated as part of the EU assessment of Fludioxonil. However further data on GLOB182F are not relevant as active substance data on toxicity to birds is used and additional formulation data are not considered essential. Birds are typically exposed to dry residues on their food items following the dilution and spraying of the formulated product. During these processes, much of the formulation constituents are likely to be lost by volatilisation. Since oral exposure is the main route of exposure, toxicity data for the active substances are therefore used in preference to data from tests with the formulated material. On this basis, the risk to birds from the proposed use of GLOB182F will be assessed using data on Fludioxonil. Therefore all relevant data were assessed in the EU review. Risk assessments for GLOB182F with the proposed use pattern are provided here and are considered adequate.

The EU agreed endpoints for the avian toxicity studies are summarized in table 9.2-1 below.

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

Species	Substance	Exposure System	Results	Reference
Bobwhite quail	Fludioxonil	Oral 1 d Acute	LD_{50} > 2000 mg/kg bw/d	EFSA, 2007 DK, 2006
		Dietary 8 d Short-term	LDD_{50} > 833 mg/kg bw/d (LC_{50} >5200 ppm)	EFSA, 2007 DK, 2006
		Dietary Reproductive toxicity	NOAEL = 62.8 mg/kg bw/d	EFSA, 2007 DK, 2006
Mallard duck	Fludioxonil	Oral 1 d Acute	LD_{50} > 2000 mg/kg bw/d	EFSA, 2007 DK, 2006

izRMS comments:

Avian toxicity data provided in Table 9.2-1 are in line with EU agreed endpoints reported in EFSA Scientific Report (2007) 110.

It is noted that in the LoEP two reproductive toxicity endpoints are reported: NOAEL of 62.8 mg a.s./kg bw/d (provided in Table 9.2-1) and NOEL of 11.1 mg a.s./kg bw/d. However, during the experts' meeting in area of ecotoxicology (PRAPeR 08) held in November 2006 it was decided that NOAEL of 62.8 mg a.s./kg bw/d should be used for the risk assessment purposes. Taking this into account, the lower endpoint is not provided in table above.

Comments concerning the use of dietary toxicity endpoints for acute risk assessment

Under point 2.2 of the EFSA Guidance Document (EFSA Journal 2009; 7(12): 1438) it is recommended that short-term avian toxicity studies should only be conducted for those active substances which have a mode of action (e.g. organochlorines or anticoagulants) or have results from mammalian testing that indicate a potential for the dietary LD_{50} measured by short -term exposure to be lower than the LD_{50} based upon an acute oral study. In such instances where a study exists and the dietary LD_{50} is lower than the acute LD_{50}, then the dietary LD_{50} should be used in the acute risk assessment. The Guidance Document recognises that although a requirement for data on dietary LD_{50} is no longer triggered for the majority of active substances, pre-existing data may be available and although its routine use is not required it may be used in higher tier assessments. For fludioxonil, a short term toxicity has been conducted with the Bobwhite quail resulting in a dietary LD_{50} of >833 mg/kg bw/day (the highest rate

tested). Although the dietary LD_{50} is potentially lower than the acute LD_{50} of >2000 mg/kg bw (also obtained with the Bobwhite), since there were no mortalities in the dietary study there is no indication of increased toxicity due to dietary exposure and therefore the acute risk assessment will be conducted using the acute endpoint as recommended.

izRMS comments:

izRMS confirms that no mortality was observed in the short-term dietary toxicity study with the bobwhite quail up to the maximum dose tested. Slightly reduced bodyweight in three highest test concentrations was correlated with the reduced food consumption, but from the study summary in the DAR (June 2005) it seems that this reduction was statistically not significant and not accompanied by any symptoms of intoxication. Possibly, active substance present in the diet at higher concentrations reduced food palatability which in consequence resulted with slightly lower bodyweight comparing to controls.
Overall, performed short-term toxicity study have not indicated that fludioxonil is more toxic to birds via the diet and for this reason it is justified to use the acute LD_{50} for purposes of the risk assessment.

9.2.1.1 Justification for new endpoints

EU agreed endpoints were used in the risk assessment. No deviations were made.

9.2.2 Risk assessment for spray applications

Not relevant.

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

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).

The potential exposure of birds to Fludioxonil was estimated following 1 application of GLOB182F at 0.5 L/ton seeds which corresponds to 50 mg as/kg seeds.

Exposure of birds will be predominantly dietary, through the consumption of treated seeds or by eating the shoots of germinated maize or sunflower seedlings.

Uptake and translocation of a chemical in plants are largely determined by its physicochemical properties (i.e. pKa and Log P_{OW}). Bromilov and Chamberlain (1989)[1] related these two properties to predict movement within the plant. Based on a pKa of 14.1 and a Log P_{OW} of 4.12, Fludioxonil would be classified as non-systemic. Indeed, Fludioxonil is not designed as a systemic seed treatment but is rather used to control soil borne fungi in the seed and root zones. Therefore it is expected that potential exposure of birds to Fludioxonil in GLOB182F will be trough the consumption of treated seeds and that the exposure via consumption of fresh green plant parts will be negligible. Nevertheless, and as a worst-case scenario, a risk assessment for fludioxonil for birds consuming seedlings from treated seeds will also be presented.

Exposure via other routes such as dermal, consumption of insects and inhalation is considered to be negligible and therefore exposure via these routes will not be considered further.

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

Exposure to standard generic indicator species was estimated according to the EFSA Guidance Document - Risk Assessment for Birds and Mammals (2009). According to this guidance, there is no screening step

[1] Bromilov, R.H. and Chamberlain, K. (1989) In mechanisms and Regulation of Transport Processes, Monograph 18, British Plant Growth Regulator Group. Ed. Atkin, R.K. and Clifford, D.R.

for seed treatments. Therefore, the assessment starts at Tier I. The recommended scenario for the seed treatment application of GLOB182F in maize/sunflower is the following:

- A large granivorous bird with a food intake rate over body weight (FIR/bw) of 0.1 and feeding on large seeds (like maize and sunflower) only;

The Tier I evaluation is carried out for a dose rate of 0.5 L/ton seeds, equivalent to 50 mg as/kg. At Tier I, it is assumed that birds do not avoid contaminated food items, that they feed exclusively in the treated area, and that they feed on a single food type. Factors AV, PT and PD are therefore equal to 1.

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

Table 9.2-2: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of GLOB182F in maize/~~sunflower~~ seeds

Intended use	Maize ~~and sunflower~~ (large seeds)				
Active substance	Fludioxonil				
Nominal application rate (mg a.s./kg seed)	1 × 50 mg as/kg				
Residues in seedlings (mg a.s./kg)	10 (based on NAR/5)				
Acute toxicity (mg/kg bw)	> 2000				
TER criterion	10				
Crop scenario Growth stage	**Indicator species**	**NAR[1] (mg a.s./kg)**	**FIR/bw**	**DDD (mg/kg bw/d)**	**TER_a**
Large seeds (maize/peas/beans)	Large granivorous bird	50	0.1	5	> 400
Seedlings	Small omnivorous bird	10	0.5	5	> 400
	Large herbivorous bird	10	0.3	3	> 666
Reprod. toxicity (mg/kg bw/d)	62.8				
TER criterion	5				
Crop scenario Growth stage	**Indicator species**	**NAR[1] (mg a.s./kg)**	**FIR/bw**	**TWA**	**Short-cut value[2] = DDD (mg/kg bw/d)**	**TER_{lt}**
Large seeds (maize/peas/beans)	Large granivorous bird	50	0.1	1	5	12.56
Seedlings	Small omnivorous bird	10	0.5	0.53	2.65	23.70
	Large herbivorous bird	10	0.3	0.53	1.59	39.5

[1] NAR=nominal loading/application rate of active substance in mg/kg seed
[2] Short-cut value equals to 0.5 x NAR/5 according to the EFSA guidance document
DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Since the long term tier I exposure of Fludioxonil leads to an acceptable risk for granivorous birds, no refinement of these exposure estimates was done.

izRMS comments:

The risk assessment presented in Table 9.2-2 above was performed correctly and is accepted by the izRMS for uses of GLOB182F as a seed treatment in maize, for which acceptable acute and long-term risk for seeds and seedlings scenario may be concluded. Additional indicator species for seedling scenario (large herbivorous bird) has been added in Table 9.2-2 above, since it is missing in EFSA (2009). However, it was already covered by calculations for small omnivorous bird, which has higher FIR/bw and represents thus worst case.

For uses of GLOB182F as a seed treatment in sunflower significantly higher application rates are proposed (125 and 150 mg a.s./kg seeds) and for this reason separate risk assessment for this crop is necessary and is presented below, separately for each intended rate.

Intended use	Sunflower (large seeds)
Active substance	Fludioxonil
Nominal application rate (mg a.s./kg seed)	1 × 150 mg as/kg
Residues in seedlings (mg a.s./kg)	30 (based on NAR/5)
Acute toxicity (mg/kg bw)	> 2000
TER criterion	10

Crop scenario Growth stage	**Indicator species**	**NAR[1] (mg a.s./kg)**	**FIR/bw**	**DDD (mg/kg bw/d)**	**TER_a**
Large seeds	Large granivorous bird	150	0.1	15	> 133
Seedlings	Small omnivorous bird	30	0.5	15	> 133
	Large herbivorous bird	30	0.3	9	> 222

Reprod. toxicity (mg/kg bw/d)	62.8
TER criterion	5

Crop scenario Growth stage	**Indicator species**	**NAR[1] (mg a.s./kg)**	**FIR/bw**	**TWA**	**Short-cut value[2] = DDD (mg/kg bw/d)**	**TER_{lt}**
Large seeds	Large granivorous bird	150	0.1	1	15	**4.2**
Seedlings	Small omnivorous bird	30	0.5	0.53	7.95	7.9
	Large herbivorous bird	30	0.3	0.53	4.77	13.2

[1] NAR=nominal loading/application rate of active substance in mg/kg seed
[2] Short-cut value equals to 0.5 x NAR/5 according to the EFSA guidance document
Values in **bold** indicate unacceptable risk

Intended use	Sunflower (large seeds)
Active substance	Fludioxonil
Nominal application rate (mg a.s./kg seed)	1 × 125 mg as/kg
Residues in seedlings (mg a.s./kg)	25 (based on NAR/5)
Acute toxicity (mg/kg bw)	> 2000
TER criterion	10

Crop scenario Growth stage	**Indicator species**	**NAR[1] (mg a.s./kg)**	**FIR/bw**	**DDD (mg/kg bw/d)**	**TER_a**
Large seeds	Large granivorous bird	125	0.1	12.5	> 160
Seedlings	Small omnivorous bird	25	0.5	12.5	> 160
	Large herbivorous bird	25	0.3	7.5	> 266

Reprod. toxicity (mg/kg bw/d)	62.8
TER criterion	5

Crop scenario Growth stage	**Indicator species**	**NAR[1] (mg a.s./kg)**	**FIR/bw**	**TWA**	**Short-cut value[2] = DDD (mg/kg bw/d)**	**TER_{lt}**
Large seeds	Large granivorous bird	125	0.1	1	12.5	5.02
Seedlings	Small omnivorous bird	25	0.5	0.53	6.63	9.5
	Large herbivorous bird	25	0.3	0.53	4.0	15.7

[1] NAR=nominal loading/application rate of active substance in mg/kg seed
[2] Short-cut value equals to 0.5 x NAR/5 according to the EFSA guidance document

Based on performed above calculations, acceptable acute and long-term risk for seeds and seedlings scenario may be concluded for uses of GLOB182F as a seed treatment in sunflower at lower application rate equivalent to 125 mg a.s./kg seeds.
For higher rate equivalent to 150 mg a.s./kg seeds acceptable acute risk may be concluded for both considered scenarios while acceptable long-term risk may be concluded for seedlings scenario. However, long-term TER for birds exposed via seeds is below the trigger of 5 indicating potentially unacceptable risk.
The indicator species for seedling scenario missing in EFSA, 2009 (large herbivorous bird) has been added in tables

above. However, it was already covered by calculations for small omnivorous bird, which has higher FIR/bw and represents thus worst case.

It is acknowledged by the izRMS that calculations performed in area of the long-term risk are conservative as being based on nominal initial loading of the active substance on treated seeds with dissipation/degradation of the active compound not taken into account in the respective time-window of 21 days (relevant for the long-term risk assessments). In addition to that, PD and PT of 1 have been assumed while it is highly unlikely that the diet of granivorous birds would be represented in 100% by treated sunflower seeds obtained exclusively in the area where seeds treated with GLOB182F were sown. Taking this into account the potential exposure would be likely considerably lower. Nevertheless, all these assumptions must be supported by respective ecological data which were not provided by the Applicant since intended use in sunflower was not considered in Applicants' calculations.
It is also noted that due to the TER of 4.2 being relatively close to the trigger the risk would be potentially addressed using the "foraging area approach", provided that the area to be foraged would be sufficiently large. It is noted that no criteria for this approach has been developed in the Central Zone, but in the Northern Zone B&M GD version 2.0, 2020 it is indicated that area to be foraged by large granivores would need to be at least 70 m^2 in case of the long-term risk. Nevertheless, in order to calculate the area to be foraged to obtain NOAEL/5, respective focal species needs to be determined and data on the number of seeds remaining on the soil surface in the mid-field and field margins after drilling has to be provided.

In absence of these data long-term risk to birds exposed to fludioxonil after application of GLOB182F as a seed treatment in sunflower at rate equivalent to 150 mg a.s./kg seeds remains unresolved.

During the commenting period the Applicant provided a Letter of Access to the additional reproductive toxicity study performed with the Northern bobwhite quail at higher doses comparing to the study available during the first EU review of fludioxonil (study already evaluated and agreed during the ongoing renewal process). When results of this study are taken into account, acceptable long-term risk may be concluded for uses in sunflower at the higher rate. However, according to the restrictions indicated in the LoA by the owner of the study (Syngenta), its results may be considered only in support of authorisation of GLOB182F in Poland, while use of these data in other Member States and countries is not possible without the prior consent of Syngenta. Taking this into account, the additional risk assessment performed with consideration of endpoint derived from this new study has been presented only in Part A (relevant for Poland). Results of these calculations and derived conclusions may be taken into account by the concerned Member States once the Applicant for GLOB 182F presents respective Syngenta consent at the national level.

9.2.3.2 Higher-tier risk assessment

Not relevant.

izRMS comments:

As demonstrated by the additional risk assessment performed by the izRMS, higher-tier risk assessment is deemed necessary for uses of GLOB182F as a seed treatment in sunflower at application rate equivalent to 150 mg a.s./kg seeds.

See also additional information presented in the commenting box in point 9.2.3 above.

9.2.3.3 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 GLOB182F is not a product for spray applications / 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 (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 500 L/kg).

With a K(f)oc of 145600 ~~145000~~ L/kg, Fludioxonil belongs to the group of more sorptive substances. The effective application rate is calculated by multiplying the application rate with the MAF_m. As only one application is intended, the MAF_m is equal to 1 and can be omitted. The application rate of 0.5 L/ton seeds based on a worst case sowing rate of 47.5 kg seeds/ha, gives an effective application rate of 2.375 g as/ha.

Effective application rate (g/ha)	=	2.375		
Acute toxicity (mg/kg bw)	=	2000	quotient =	0.0012
Reprod. toxicity (mg/kg bw/d)	=	62.8	quotient =	0.0378

Since the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) doesn't exceed the critical value of 3000, no quantitative risk assessment (calculation of TER values) is necessary.

izRMS comments:

The drinking water scenario is not required for seed treatment uses. However, since it was performed by the Applicant, it was evaluated by the izRMS for precautionary reasons. The drinking water risk assessment provided by the Applicant in table above is agreed by the izRMS. Since the maximum intended application rate was considered, performed calculations are protective for all intended uses of GLOB182F in maize and sunflower.
The Kfoc mentioned in the text above has been corrected by the izRMS to comply with endpoints reported in EFSA Scientific Report (2007) 110.

It is noted that the drinking water risk assessment should be also performed for relevant metabolites of the active substance. However, soil metabolites of fludioxonil (CGA192155, CGA265378 and CGA339833) were formed only in presence of light while due to the type of application (seed treatment) photolysis will not play a major role in degradation of fludioxonil in soil. For this reason metabolites formed via photolysis in soil may not be taken into account in the risk assessment for the intended uses of GLOB182F. No metabolites were formed in studies performed in the dark.

9.2.3.4 Effects of secondary poisoning

The log P_{ow} of Fludioxonil amounts to 4.12 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is therefore required.

Risk assessment for earthworm-eating birds via secondary poisoning

According to EFSA/2009/1438, the risk for vermivorous birds is assessed for a bird of 100 g body weight with a daily food consumption of 104.6 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil.

Table 9.2-3: Assessment of the risk for earthworm-eating birds due to exposure to Fludioxonil via bioaccumulation in earthworms (secondary poisoning) for the intended use in maize/sunflower seeds

Parameter	Fludioxonil	comments
PEC_{soil} (accumulation) (mg/kg soil) ~~PEC_{soil} (twa = 21 d) (mg/kg soil)~~	0.00882	dRR Part B8 Annex point 8.7.2.1
log P_{ow} / P_{ow}	4.12 / 13183	EFSA, 2007
Koc	145600 ~~145000~~	Mean (n = 5)
foc	0.02	Default
BCF_{worm}	~~0.544~~ 0.0546	$BCF_{worm/soil}$ = ($PEC_{worm,ww}/PEC_{soil,dw}$) = (0.84 + 0.012 × P_{ow}) / foc × Koc
PEC_{worm}	0.0005 ~~0.004794~~ ~~0.004813895~~	PEC_{worm} = PEC_{soil} × $BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.00053 ~~0.00503~~ ~~0.005054590~~	DDD = PEC_{worm} × 1.05
NOEL (mg/kg bw/d)	62.8	EFSA, 2007
TER_{lt}	118491 ~~12485~~ ~~12424.35~~	TER criterion = 5

TER values shown in bold fall below the relevant trigger.

The TER_{LT} value is greater than the Annex VI trigger of 5 for the earthworm-eating birds, indicating that Fludioxonil poses low long-term risk to these birds following application of GLOB182F at the proposed rate in maize/sunflower seeds.

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 9.2-4: Assessment of the risk for fish-eating birds due to exposure to Fludioxonil via bioaccumulation in fish (secondary poisoning) for the intended use in maize/sunflower seeds

Parameter	Fludioxonil	comments
PEC_{sw} (twa = 21 d) (mg/L)	0.0000046	dRR Part B8 Annex point 8.9.2 (highest 21 d- $PEC_{sw,twa}$ from the STEP 1)
BCF_{fish}	366	EFSA, 2007
BMF	/	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	0.00168	PEC_{fish} = PEC_{water} × BCF_{fish}
Daily dietary dose (mg/kg bw/d)	0.000268	DDD = PEC_{fish} × 0.159
NOEL (mg/kg bw/d)	62.8	EFSA, 2007
TER_{lt}	234597.62	TER criterion = 5

TER values shown in bold fall below the relevant trigger.

The TER_{LT} value is greater than the Annex VI trigger of 5 for the fish-eating birds, indicating that Fludioxonil poses low long-term risk to these birds following application of GLOB182F at the proposed rate in maize/sunflower seeds.

izRMS comments:

The approach of the Applicant in the evaluation of the risk of secondary poisoning for earthworm-eating birds presented above is correct, however Kfoc considered in performed calculations was not in line with value reported in EFSA Scientific Report (2007) 110. ~~In addition to that, a typing error was noted in BCF_{worm} value reported in Table 9.2-4 above (it should have been 0.546, while 0.0546 was indicated).~~ Respective corrections were thus made by the izRMS for consistency, but with no impact on the outcome of the performed evaluation. As the worst case soil exposure accounting for accumulation potential has been considered, calculations provided above are protective for all intended uses of GLOB182F.

The evaluation of the risk of secondary poisoning for fish-eating birds is agreed by the izRMS with no corrections.

It is noted that the risk of secondary poisoning should be also evaluated for relevant metabolites of the active

substance. Soil metabolites of fludioxonil (CGA192155, CGA265378 and CGA339833) were formed only in presence of light while due to the type of application (seed treatment) photolysis will not play a major role in degradation of fludioxonil in soil. For this reason metabolites formed via photolysis in soil may not be taken into account in the risk assessment for the intended uses of GLOB182F.
Nevertheless, metabolites formed via photolysis in water (CGA339833, CGA344623 and A5) may be relevant, since fludioxonil applied as a seed treatment may migrate to surface water bodies where it will undergo photodegradation. It is noted that in the course of the EU review photolytic metabolites CGA344623 and A5 were considered to be minor metabolites since they were detected only in the sterile photolysis study and not in the water/sediment study performed under light conditions. Therefore, photolytic aquatic metabolite CGA339833 should be taken into account in the evaluation. Furthermore, also metabolite CGA192155 is considered relevant, as it was formed in the water/sediment studies.
No log Pow values are reported in EFSA Scientific Report (2007) 110 for aquatic metabolites relevant for seed treatment uses. However, according to the DAR (Vol. 3, B.8 of January 2005), log Pow values for CGA192155 and CGA339833 are <3, hence the evaluation of the risk of secondary poisoning was not triggered for these compounds.

Overall, the risk of secondary poisoning is concluded to be low.

9.2.3.5 Biomagnification in terrestrial food chains

Not relevant.

The results of the ADME studies indicate that Fludioxonil has a low bioaccumulation potential. Fludioxonil is extensively metabolised and almost completely eliminated within 24 hours. Thus there will be a low secondary exposure and bioaccumulation of Fludioxonil, and a low risk to predatory birds is expected following the proposed use of GLOB182F.

9.2.4 Overall conclusions

izRMS comments:

Performed risk assessment demonstrated acceptable acute and long-term risk to birds from seeds and seedlings scenario following uses of GLOB182F as a seed treatment in maize and sunflower at application rates equivalent to 50 and 125 mg a.s./kg seeds, respectively.

For intended uses in sunflower at rate equivalent to 150 mg a.s./kg seeds acceptable acute risk could be concluded for seeds and seedling scenario and acceptable long-term risk for seedling scenario. However, the long-term TER for granivorous birds feeding on seeds was below the trigger of 5 indicating potentially unacceptable risk from this use. No risk refinement options were available, since application to sunflower was not considered in the Applicants' calculations and further data needs to be provided in order to resolve the risk from the intended uses in sunflower at the higher rate.

During the commenting period the Applicant provided a Letter of Access to the additional reproductive toxicity study performed with the Northern bobwhite quail at higher doses comparing to the study available during the first EU review of fludioxonil (study already evaluated and agreed during the ongoing renewal process). When results of this study are taken into account, acceptable long-term risk may be concluded for uses in sunflower at the higher rate. However, according to the restrictions indicated in the LoA by the owner of the study (Syngenta), its results may be considered only in support of authorisation of GLOB182F in Poland, while use of these data in other Member States and countries is not possible without the prior consent of Syngenta. Taking this into account, the additional risk assessment performed with consideration of endpoint derived from this new study has been presented only in Part A (relevant for Poland). Results of these calculations and derived conclusions may be taken into account by the concerned Member States once the Applicant for GLOB182F presents respective Syngenta consent at the national level.

~~The risk assessments for birds indicated that the TER_a and TER_{lt} values are greater than the Annex VI trigger of 10 or 5 respectively, indicating that the use of GLOB182F in maize and sunflower seeds according to the proposed GAP poses a low acute and long-term risk to birds.~~

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 Fludioxonil. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on mammals of GLOB182F were not evaluated as part of the EU assessment of Fludioxonil. However further data on GLOB182F is not relevant as active substance data on toxicity to terrestrial vertebrates other than birds is used and additional formulation data are not considered essential. Therefore all relevant data were assessed in the EU review. Mammals are typically exposed to dry residues on their food items following the dilution and spraying of the formulated product. During these processes, much of the formulation constituents are likely to be lost by volatilisation. Therefore, where oral exposure is the main route of exposure, toxicity data for the active substance are used in preference to data from tests with the formulated material. Exposure to GLOB182F *via* dermal and inhalation routes is considered unlikely, since at the time of application and for a short period thereafter, most wild mammals will leave the immediate vicinity of spray operations in response to the human disturbance.

Therefore, all relevant data were assessed in the EU review. Risk assessments for GLOB182F with the proposed use pattern are provided here and are considered adequate.

The EU agreed endpoints for the mammalian toxicity studies are summarized in tables 9.3-1 below. Further details can be found in the corresponding *EU review* for Fludioxonil.

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

Species	Substance	Exposure System	Results	Reference
Rat	Fludioxonil	Oral 1 d Acute	LD_{50} > 5000 mg/kg bw	EFSA, 2007 DK, 2006
		Dietary Reproductive toxicity Two-generation study	NOAEL = 200 mg/kg bw/d	EFSA, 2007 DK, 2006

izRMS comments:

Mammalian toxicity data provided in Table 9.3-1 are in line with EU agreed endpoints reported in EFSA Scientific Report (2007) 110.

9.3.1.1 Justification for new endpoints

EU agreed endpoints were used in the risk assessment. No deviations were made.

9.3.2 Risk assessment for spray applications

Not relevant.

9.3.3 Risk assessment for baits, pellets, granules, pills or treated seed

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

The potential exposure of birds to Fludioxonil was estimated following 1 application of GLOB182F at 0.5 L/ton seeds which corresponds to 50 mg as/kg seeds.

Exposure of mammals will be predominantly dietary, through the consumption of treated seeds or by eating the shoots of germinated maize/sunflower seedlings.

Uptake and translocation of a chemical in plants are largely determined by its physicochemical properties (i.e. pKa and Log P_{OW}). Bromilov and Chamberlain (1989)[2] related these two properties to predict movement within the plant. Based on a pKa of 14.1 and a Log P_{OW} of 4.12, Fludioxonil would be classified as non-systemic. Indeed, Fludioxonil is not designed as a systemic seed treatment but is rather used to control soil borne fungi in the seed and root zones. Therefore it is expected that potential exposure of mammals ~~birds~~ to Fludioxonil in GLOB182F will be trough the consumption of treated seeds and that the exposure via consumption of fresh green plant parts will be negligible.

Exposure via other routes such as dermal, consumption of insects and inhalation is considered to be negligible and therefore exposure via these routes will not be considered further.

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

Exposure to standard generic indicator species was estimated according to the EFSA Guidance Document - Risk Assessment for Birds and Mammals (2009). According to this guidance, there is no screening step for seed treatments. Therefore, the assessment starts at Tier I. The recommended scenario for the seed treatment application of GLOB182F in maize and sunflower is the following:

- A small omnivorous mammal with a food intake rate over body weight (FIR/bw) of 0.24 and feeding on maize/sunflower grains only;

The Tier I evaluation is carried out for a dose rate of 0.5 L/ton seeds, equivalent to 50 mg as/kg. At Tier I, it is assumed that mammals do not avoid contaminated food items, that they feed exclusively in the treated area, and that they feed on a single food type. Factors AV, PT and PD are therefore equal to 1.
The results of the acute and reproductive first-tier risk assessments are summarised in the following table.

Table 9.3-2: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of GLOB182F in maize/sunflower seeds

Intended use	Maize ~~and sunflower~~ (large seeds)					
Active substance	Fludioxonil					
Nominal application rate (mg a.s./kg seed)	1 × 50 mg as/kg					
Residues in seedlings (mg a.s./kg)	10 (based on NAR/5)					
Acute toxicity (mg/kg bw)	> 5000					
TER criterion	10					
Crop scenario Growth stage	**Indicator species**	**NAR[1] (mg a.s./kg)**	**FIR/bw**		**DDD (mg/kg bw/d)**	**TER_a**
Large seeds (maize/peas/beans)	Small omnivorous mammal	50	0.24		12	> 416.67
Seedlings	Small omnivorous mammal	10	0.24		2.4	> 2083.33
	Large herbivorous mammal	10	0.4		4.0	> 1250
Reprod. toxicity (mg/kg bw/d)	200					
TER criterion	5					
Crop scenario Growth stage	**Indicator species**	**NAR[1] (mg a.s./kg)**	**FIR/bw**	**TWA**	**Short-cut value[2] = DDD (mg/kg bw/d)**	**TER_{lt}**
Large seeds (maize/peas/beans)	Small omnivorous mammal	50	0.24	1	12	16.67
Seedlings	Small omnivorous mammal	10	0.24	0.53	1.272	157.23
	Large herbivorous mammal	10	0.4	0.53	2.12	94.3

[1] NAR=nominal loading/application rate of active substance in mg/kg seed
[2] Short-cut value equals to 0.24 x NAR/5 according to the EFSA guidance document
DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

[2] Bromilov, R.H. and Chamberlain, K. (1989) In mechanisms and Regulation of Transport Processes, Monograph 18, British Plant Growth Regulator Group. Ed. Atkin, R.K. and Clifford, D.R.

The acute and reproductive risks to small omnivorous mammals feeding on maize and sunflower seeds or shoots is acceptable since the TER respectively exceed the Annex VI triggers of 10 and 5.

izRMS comments:

The risk assessment presented in Table 9.3-2 above was performed correctly and is accepted by the izRMS for uses of GLOB182F as a seed treatment in maize, for which acceptable acute and long-term risk for seeds and seedlings scenario may be concluded. Additional indicator species for seedling scenario (large herbivorous bird) has been added in Table 9.2-2 above, since it is missing in EFSA (2009). Acceptable risk could be concluded also for this species.

For uses of GLOB182F as a seed treatment in sunflower significantly higher application rates are proposed (125 and 150 mg a.s./kg seeds) and for this reason separate risk assessment for this crop is necessary and is presented below, considering maximum application rate protective also for lower rate.

Intended use	Sunflower (large seeds)
Active substance	Fludioxonil
Nominal application rate (mg a.s./kg seed)	1 × 150 mg as/kg
Residues in seedlings (mg a.s./kg)	30 (based on NAR/5)
Acute toxicity (mg/kg bw)	> 5000
TER criterion	10

Crop scenario Growth stage	**Indicator species**	**NAR[1] (mg a.s./kg)**	**FIR/bw**	**DDD (mg/kg bw/d)**	**TER_a**
Large seeds	Small omnivorous mammal	150	0.24	36	> 138
Seedlings	Small omnivorous mammal	30	0.24	7.2	> 694
	Large herbivorous mammal	30	0.4	12.0	> 416

Reprod. toxicity (mg/kg bw/d)	200
TER criterion	5

Crop scenario Growth stage	**Indicator species**	**NAR[1] (mg a.s./kg)**	**FIR/bw**	**TWA**	**Short-cut value[2] = DDD (mg/kg bw/d)**	**TER_{lt}**
Large seeds (maize/peas/beans)	Small omnivorous mammal	150	0.24	1	36	5.6
Seedlings	Small omnivorous mammal	30	0.24	0.53	3.82	52.4
	Large herbivorous mammal	30	0.4	0.53	6.36	31.4

[1] NAR=nominal loading/application rate of active substance in mg/kg seed
[2] Short-cut value equals to 0.5 x NAR/5 according to the EFSA guidance document

Based on performed above calculations, acceptable acute and long-term risk for seeds and seedlings scenario may be concluded for uses of GLOB182F as a seed treatment in sunflower at both application rates equivalent to 150 and 125 mg a.s./kg seeds.
The indicator species for seedling scenario missing in EFSA, 2009 (large herbivorous bird) has been added in tables above. Acceptable risk could be concluded also for this species.

9.3.3.2 Higher-tier risk assessment

Not relevant.

9.3.3.3 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).

Leaf scenario

Since GLOB182F 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 (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 500 L/kg).

With a K(f)oc of 145600 ~~145000~~ L/kg, Fludioxonil belongs to the group of more sorptive substances. The effective application rate is calculated by multiplying the application rate with the MAF_m. As only one application is intended, the MAF_m is equal to 1 and can be omitted. The application rate of 0.5 L/ton seeds based on a worst case sowing rate of 47.5 kg seeds/ha, gives an effective application rate of 2.375 g as/ha.

Effective application rate (g/ha)	=	2.375		
Acute toxicity (mg/kg bw)	=	> 5000	quotient =	0.000475
Reprod. toxicity (mg/kg bw/d)	=	200	quotient =	0.011875

Since the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) doesn't exceed the critical value of 3000, no quantitative risk assessment (calculation of TER values) is necessary.

izRMS comments:

The drinking water scenario is not required for seed treatment uses. However, since it was performed by the Applicant, it was evaluated by the izRMS for precautionary reasons. The drinking water risk assessment provided by the Applicant in table above is agreed by the izRMS. Since the maximum intended application rate was considered, performed calculations are protective for all intended uses of GLOB182F in maize and sunflower.
The Kfoc mentioned in the text above has been corrected by the izRMS to comply with endpoints reported in EFSA Scientific Report (2007) 110.

It is noted that the drinking water risk assessment should be also performed for relevant metabolites of the active substance. However, soil metabolites of fludioxonil (CGA192155, CGA265378 and CGA339833) were formed only in presence of light while due to the type of application (seed treatment) photolysis will not play a major role in degradation of fludioxonil in soil. For this reason metabolites formed via photolysis in soil may not be taken into account in the risk assessment for the intended uses of GLOB182F. No metabolites were formed in studies performed in the dark.

9.3.3.4 Effects of secondary poisoning

The log P_{ow} of Fludioxonil amounts to 4.12 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is therefore required.

Risk assessment for earthworm-eating mammals via secondary poisoning

According to EFSA/2009/1438, the risk for vermivorous mammals is assessed for a small mammal of 10 g body weight with a daily food consumption of 12.8 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil.

Table 9.3-3: Assessment of the risk for earthworm-eating mammals due to exposure to Fludioxonil via bioaccumulation in earthworms (secondary poisoning) for the intended use in maize/sunflower seeds

Parameter	Fludioxonil	comments
PEC_{soil} (accumulation) (mg/kg soil) ~~PEC_{soil} (twa = 21 d) (mg/kg soil)~~	0.00882	dRR Part B8 Annex point 8.7.2.1
log P_{ow} / P_{ow}	4.12 / 13183	EFSA, 2007
Koc	145600 ~~145000~~	Mean (n = 5)
foc	0.02	Default
BCF_{worm}	~~0.544~~ 0.0546	$BCF_{worm/soil}$ = ($PEC_{worm,ww}/PEC_{soil,dw}$) = (0.84 + 0.012 × P_{ow}) / foc × Koc
PEC_{worm}	0.0005 ~~0.004794~~ ~~0.004813895~~	PEC_{worm} = PEC_{soil} × $BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.00064 ~~0.00614~~ ~~0.000616412~~	DDD = PEC_{worm} × 1.28
NOEL (mg/kg bw/d)	200	EFSA, 2007
TER_{lt}	312500 ~~32573~~ ~~324458.23~~	TER criterion = 5

TER values shown in bold fall below the relevant trigger.

The TER_{LT} value is greater than the Annex VI trigger of 5 for the earthworm-eating birds, indicating that Fludioxonil poses low long-term risk to these birds following application of GLOB182F at the proposed rate in maize/sunflower seeds.

Risk assessment for fish-eating mammals via secondary poisoning

According to EFSA/2009/1438, the risk for piscivorous mammals is assessed for a mammal of 3000 g body weight with a daily food consumption of 425 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water.

Table 9.3-4: Assessment of the risk for fish-eating mammals due to exposure to Fludioxonil via bioaccumulation in fish (secondary poisoning) for the intended use in maize/sunflower seeds

Parameter	Fludioxonil	comments
PEC_{sw} (twa = 21 d) (mg/L)	0.0000046	dRR Part B8 Annex point 8.9.2 (highest 21 d- $PEC_{sw,twa}$ from the STEP 1)
BCF_{fish}	366	EFSA, 2007
BMF	/	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	0.0016836	PEC_{fish} = PEC_{water} × BCF_{fish}
Daily dietary dose (mg/kg bw/d)	0.0002391	DDD = PEC_{fish} × 0.142
NOEL (mg/kg bw/d)	200 ~~62.8~~	EFSA, 2007
TER_{lt}	836470 ~~262683.25~~	TER criterion = 5

TER values shown in bold fall below the relevant trigger.

The TER_{LT} value is greater than the Annex VI trigger of 5 for the earthworm-eating mammals, indicating that Fludioxonil poses low long-term risk to these birds following application of GLOB182F at the proposed rate in maize/sunflower seeds.

izRMS comments:

The approach of the Applicant in the evaluation of the risk of secondary poisoning for earthworm-eating mammals presented above is correct, however Kfoc considered in performed calculations was not in line with value reported in EFSA Scientific Report (2007) 110. ~~In addition to that, a typing error was noted in BCF_{worm} value reported in Table 9.3-3 above (it should have been 0.546, while 0.0546 was indicated).~~ Respective corrections were thus made by the izRMS for consistency, but with no impact on the outcome of the performed evaluation. As the worst case soil exposure accounting for accumulation potential has been considered, calculations provided above are protective for all intended uses of GLOB182F.

The evaluation of the risk of secondary poisoning for fish-eating mammals is agreed by the izRMS, it is however noted that the avian long-term endpoint of 62.8 mg a.s./kg bw/d was used instead of 200 mg a.s./g bw/d, relevant for the mammalian risk assessment. Respective corrections were made by the izRMS in Table 9.3-4 above.

It is noted that the risk of secondary poisoning should be also evaluated for relevant metabolites of the active substance. Soil metabolites of fludioxonil (CGA192155, CGA265378 and CGA339833) were formed only in presence of light while due to the type of application (seed treatment) photolysis will not play a major role in degradation of fludioxonil in soil. For this reason metabolites formed via photolysis in soil may not be taken into account in the risk assessment for the intended uses of GLOB182F.
Nevertheless, metabolites formed via photolysis in water (CGA339833, CGA344623 and A5) may be relevant, since fludioxonil applied as a seed treatment may migrate to surface water bodies where it will undergo photodegradation. It is noted that in the course of the EU review photolytic metabolites CGA344623 and A5 were considered to be minor metabolites since they were detected only in the sterile photolysis study and not in the water/sediment study performed under light conditions. Therefore, photolytic aquatic metabolite CGA339833 should be taken into account in the evaluation. Furthermore, also metabolite CGA192155 is considered relevant, as it was formed in the water/sediment studies.
No log Pow values are reported in EFSA Scientific Report (2007) 110 for aquatic metabolites relevant for seed treatment uses. However, according to the DAR (Vol. 3, B.8 of January 2005), log Pow values for CGA192155 and CGA339833 are <3, hence the evaluation of the risk of secondary poisoning was not triggered for these compounds.

Overall, the risk of secondary poisoning is concluded to be low.

9.3.3.5 Biomagnification in terrestrial food chains

Not relevant.

The results of the ADME studies indicate that Fludioxonil has a low bioaccumulation potential. Fludioxonil is extensively metabolised and almost completely eliminated within 24 hours. Thus there will be a low secondary exposure and bioaccumulation of Fludioxonil, and a low risk to predatory mammals is expected following the proposed use of GLOB182F.

9.3.4 Overall conclusions

The risk assessments for mammals indicated that the TER_a and TER_{lt} values are greater than the Annex VI trigger of 10 or 5 respectively, indicating that the use of GLOB182F in maizes/sunflower seeds according to the proposed GAP poses a low acute and long-term risk to mammals.

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

Not required.

izRMS comments:

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

9.5 Effects on aquatic organisms (KCP 10.2)

9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with Fludioxonil and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on aquatic organisms of GLOB182F were not evaluated as part of the EU assessment of Fludioxonil. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment deviates from the results of the EU review process. Justifications are provided below.

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

Species	Substance	Exposure System	Results	Reference
Acute toxicity to fish				
Oncorhynchus mykiss	Fludioxonil	96 h, f	LC_{50} = 0.23 mg a.s./L	EFSA, 2007
	CGA339833	96 h, s	LC_{50} > 100 mg as/L	EFSA, 2007
	CGA192155	96 h, s	LC_{50} > 100 mg/L	EFSA, 2007
Chronic toxicity to fish				
Oncorhynchus mykiss	Fludioxonil	28d, f	NOEC = 0.040 mg as/L	EFSA, 2007
Pimephales promelas	Fludioxonil	28d, f	NOEC = 0.039 mg as/L	EFSA, 2007
Acute toxicity to aquatic invertebrates				
Daphnia magna	Fludioxonil	48 h, f	EC_{50} = 0.4 mg/L	EFSA, 2007
Mysidiopsis bahia	Fludioxonil	96 h, f	LC_{50} = 0.27 mg as/L	EFSA, 2007
Daphnia magna	CGA339833	48 h, f	EC_{50} > 100 mg/L	EFSA, 2007
	CGA192155	48 h, s	EC_{50} > 100 mg/L	EFSA, 2007
	CGA344623	48 h, s	EC_{50} > 100 mg/L	EFSA, 2007
Chronic toxicity to aquatic invertebrates				
Daphnia magna	Fludioxonil	21 d, ss	NOEC = 0.005 mg as/L	EFSA, 2007
Chronic toxicity to aquatic insects				
Chironomus riparius	Fludioxonil	28 d, spiked sediment	NOEC = 0.2 mg a.s./L NOEC = 40 mg a.s./kg sed. (dw)	EFSA, 2007
Toxicity to green algae				
Pseudokirchneriella subcapitata (formerly Selenastrum capricornutum)	Fludioxonil	120 h	E_rC_{50} = 0.33 mg a.s./L E_bC_{50} = 0.024 mg a.s./L	EFSA, 2007
	CGA339833	72 h	E_rC_{50} = 104.7 mg a.s./L E_bC_{50} = 95.8 mg a.s./L	EFSA, 2007
Scenedesmus subspicatus	CGA192155	96 h	E_rC_{50} > 100 mg a.s./L E_bC_{50} > 100 mg a.s./L	EFSA, 2007
Toxicity to aquatic plants				
No data available, not necessary for a fungicide				
Higher-tier studies (micro- or mesocosm studies)				
Outdoor aquatic microcosm	Fludioxonil	112d	NOAEC = 0.0164 mg a.s./L	EFSA, 2007

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

Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – GLOB182F

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	Not available	Not available	Not available	Not available
Daphnia magna	GLOB182F	48 h, s	EC_{50} = 5.38 mg product/L $_{nom}$ (0.51 mg a.s./L)	Renner P., 2021a
Pseudokirchneriella subcapitata	GLOB182F	72 h, s	E_rC_{50} = 7.7 mg product /L $_{nom}$ (0.73 mg a.s./L) E_yC_{50} = 4.46 mg product /L $_{nom}$ (0.43 mg a.s./L)	Renner P., 2021b

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

No specific acute toxicity study to fish was carried out on the formulation in order to avoid studies conducted on vertebrates. As the formulation consists of only one active substance, the toxicity of the formulation is expected to be mainly based on the toxicity of Fludioxonil for the relevant ecotoxicological data of fish. Therefore, it can be agreed to refer to the risk assessment to fish performed on the active substance Fludioxonil.

izRMS comments:

Data on toxicity of the active substance and its metabolites to aquatic organisms provided in Table 9.5-1 are in line with EU agreed endpoints reported in EFSA Scientific Report (2007) 110. Results of the study on toxicity of metabolite CGA344623 to *Daphnia magna* were added by the izRMS for completeness, however in the course of the EU review this metabolite was considered to be minor since it was detected only in the sterile photolysis study and not in the water/sediment study performed under light conditions. Hence, it is not relevant for the risk assessment.

Studies on toxicity of GLOB182F to selected aquatic species were evaluated by the izRMS and considered acceptable. For details of evaluation, please refer to Appendix 2.
Testing of the acute toxicity of the formulated product to fish was deemed not necessary as it is possible to extrapolate from the active substance data. Furthermore, studies performed with *Daphnia magna* and algae demonstrated that GLOB182F is not more toxic comparing to the active substance and it is justified to base the risk assessment on the active substance endpoints which will cover also risk resulting from exposure to formulation.
In line with EFSA (2013), investigation of toxicity to higher aquatic plants is not required for fungicides.

9.5.1.1 Justification for new endpoints

As GLOB182F is not identical to the reference formulation Celeste 025 FS used during the EU Review of Fludioxonil, toxicity to the aquatic organisms *Daphnia magna* and *Pseudokirchneriella subcapitata* from the formulation was also tested and used in the risk assessment.

9.5.2 Risk assessment

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

The relevant global maximum FOCUS Step 1 and 2 for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the tables below.

Table 9.5-3: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Fludioxonil for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GLOB182F in maize and sunflower seeds

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**		**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**	~~**Higher-tier information**~~		**Sed. dwell. prolonged**
Test species		***Oncorhynchus mykiss***	***Pimephales promelas***	***Daphnia magna***	***Mysidiopsis bahia***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Chironomus riparius***	~~***Microcosm***~~		***Chironomus riparius***
Endpoint		LC_{50}	NOEC	EC_{50}	LC_{50}	NOEC	E_bC_{50}	NOEC	~~NOAEC~~		NOEC
(µg/L)		230	39	400	270	5	24	200	~~16.4~~		40000
AF		100	10	100	100	10	10	10	~~10~~		10
RAC (µg/L)		2.3	3.9	4	2.7	0.5	2.4	20	~~1.64~~		4000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**									**PEC $_{gl\text{-}max}$ (µg/kg)**	
Step 1											
	0.0073	0.003	0.002	0.002	0.003	0.015	0.003	<0.001	~~0.004~~	5.8826	0.001
Step 2											
N-Europe	0.0014	0.001	0.000	0.000	0.001	0.003	0.001	<0.001	~~0.001~~	1.1580	<0.001
S-Europe	0.0029	0.001	0.001	0.001	0.001	0.006	0.001	<0.001	~~0.002~~	2.3209	0.001

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

For the intended use in maize and sunflower seeds, calculated PEC/RAC ratios for the active substance Fludioxonil did indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for aquatic invertebrates as characterised by a NOEC for *Daphnia magna* of 5 µg/L in connection with an assessment factor of 10) in all FOCUS Steps 1 and 2 scenarios. Therefore, no further assessment is necessary.

Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the Fludioxonil metabolite CGA192155 for each organism group based on FOCUS Steps 1 and 2 calculations for the use in maize and sunflower seeds

Group		**Fish acute**	**Inverteb. acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Scenedesmus subspicatus***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}
(µg/L)		100000	100000	100000
AF		100	100	10
RAC (µg/L)		1000	1000	10000
FOCUS Scenario	**PEC $_{sw-max}$ (µg/L)**			
Step 1				
	0.1825	<0.001	<0.001	<0.001
Step 2				
N-Europe	0.0331	<0.001	<0.001	<0.001
S-Europe	0.0662	<0.001	<0.001	<0.001

For the intended use in maize and sunflower seeds, calculated PEC/RAC ratios for the metabolite CGA192155 did indicate an acceptable risk for the most sensitive groups of aquatic organisms (risk for fish/aquatic invertebrates as characterised by a LC_{50}/EC_{50} for *Oncorhynchus mykiss/Daphnia magna* of 100000 µg/L in connection with an assessment factor of 100) in all FOCUS Steps 1 and 2 scenarios. Therefore, no further assessment is necessary.

PECsw calculations for the formulation are not necessary as GLOB182F is used exclusively as a seed treatment, therefore there will be no direct exposure to spray drift of the formulated product.

izRMS comments:

The risk assessment provided by the Applicant in tables above is agreed by the izRMS. In performed calculations the overall maximum $PC_{SW/SED}$ values were used covering all intended uses in both crops (maize and sunflower).
Since acceptable risk from exposure to fludioxonil could be concluded using standard laboratory toxicity tests and Step 1 exposure estimates, calculation of PEC/RAC values based on results of the higher-tier studies (mesocosm) was not necessary and is struck through in Table 9.5-3 above. It is also noted that in the risk assessment for algae from fludioxonil the E_bC_{50} value was used instead of E_rC_{50} recommended by EFSA aquatic guidance (2013). Nevertheless, since considered E_bC_{50} (24 µg a.s./L) is more than 10 times lower comparing to E_rC_{50} (330 µg a.s./L) and acceptable risk could be concluded with this assumption, the risk assessment in Table 9.5-3 represents worst case and was thus not corrected.

Overall, acceptable risk could be concluded for fludioxonil and metabolite CGA192155 following application of GLOB182F as a seed treatment in maize and sunflower with no need for risk mitigation measures.

It should be, however, noted that in line with information provided in area of environmental fate and behaviour (and reproduced in point 9.1.3 of this document), apart of metabolite CGA192155 also metabolite CGA339833 is considered relevant for the intended uses of fludioxonil in GLOB182F. Respective risk assessment was thus performed by the izRMS below. Maximum PEC_{SW} from both uses were considered covering all intended uses.

Risk assessment for metabolite CGA339833

Group		**Fish acute**	**Inverteb. acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Pseudokirchneriella subcapitata***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}
(µg/L)		100000	100000	104700
AF		100	100	10
RAC (µg/L)		1000	1000	10470
FOCUS Scenario	**PEC $_{sw-max}$ (µg/L)**			
Step 1	0.3027	<0.001	<0.001	<0.001

Based on performed above calculations, acceptable risk from metabolite CGA339833 may be concluded already at Step 1.

9.5.3 Overall conclusions

An acceptable acute and long-term risk to aquatic organisms is identified for the intended use of GLOB182F in maize and sunflower seeds. No risk mitigation measures are required.

9.6 Effects on bees (KCP 10.3.1)

9.6.1 Toxicity data

Studies on the toxicity to bees have been carried out with Fludioxonil. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on bees of GLOB182F were not evaluated as part of the EU assessment of Fludioxonil. New data submitted with this application are listed in Appendix 1and 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

Species	Substance	Exposure System	Results	Reference
Apis mellifera	Fludioxonil	Oral, 48h	LD_{50} > 100 µg/bee	EFSA, 2007
		Contact, 48h	LD_{50} > 100 µg/bee	
	GLOB182F	Oral, 48h	LD_{50} > 1100 µg/bee (= > 104.9 µg a.s./bee)	Franke M., 2020
		Contact, 48h	LD_{50} > 1100 µg/bee (= > 104.9 µg a.s./bee)	
		Adult, oral, 10d	LDD_{50} = 732 µg/bee/d (= 69.8 µg a.s./bee/d) NOEDD = 220 µg/bee/d (= 21.0 µg a.s./bee/d) NOEC = 6.777 g/kg food (= 0.646 g a.s./kg food)	Dreßler K., 2020
		Larval, oral, 22d	NOED = 40.3 µg/larva (= 3.8 µg a.s./larva) NOEC = 254.9 mg/kg food (= 24.3 mg a.s./kg food)	Schmidt K., 2021
Bombus terrestris	GLOB182F	Oral, 48h	LD_{50} >3688.3 µg/bumblebee (= > 351.6 µg a.s./bumblebee) ~~LD_{50} > 2129.6 µg/bumblebee (= > 203 µg a.s./bumblebee)~~	Amsel K., 2020
		Contact, 48h	LD_{50} > 2129.6 µg/bumblebee (= > 203 µg a.s./bumblebee)	
Higher-tier studies (tunnel test, field studies)				
Not necessary				

izRMS comments:

Data on toxicity of the active substance to bees provided in Table 9.6-1 are in line with EU agreed endpoints reported in EFSA Scientific Report (2007) 110.

Studies on toxicity of GLOB182F to bees and bumblebees ~~selected aquatic species~~ were evaluated by the izRMS and considered acceptable. For details of evaluation, please refer to Appendix 2.
The oral toxicity endpoint for bumblebees has been corrected in Table 9.6-1 in line with the outcome of the study. Overall, based on the obtained results it may be concluded that bumblebees are not more sensitive comparing to bees and for this reason acute risk assessment for bees is protective also for bumblebees.

9.6.1.1 Justification for new endpoints

As GLOB182F is not identical to the reference formulation Celeste 025 FS used during the EU Review of Fludioxonil, toxicity to bees from the formulation was also tested and used in the risk assessment.

9.6.2 Risk assessment

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

Applications of pesticides can potentially result in exposure of honeybees either through direct over-spray, or by contact with residues on plants whilst bees are foraging for food. However these sources of exposure are considered highly unlikely in the case of application of GLOB182F, as GLOB182F is used for seed treatment. The only way of exposure of bees will be through the consumption of nectar or pollen in off-field areas (flowering margins or neighbouring crops) contaminated with Fludioxonil via dust drift. In order to consider an extreme worst-case scenario, the maximum application rate for GLOB182F will be envisaged when it is applied at the maximum recommended rate of 0.5 L/ton seeds and seeds are sown at a rate of 47.5 kg/ha. This corresponds to 25.11 g GLOB182F/ha.

9.6.2.1 Hazard quotients for bees

Acute risk assessment

The acute risk to honeybees from use of GLOB182F was assessed using the maximum single application rate and the LD_{50} values to calculate hazard quotients ***(EPPO 2010)*** as follows:

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

Hazard quotients were calculated for oral exposure (Q_{HO}) and contact exposure (Q_{HC}) to Fludioxonil and GLOB182F. A hazard quotient of less than 50 indicates a low risk to bees in the field.

Table 9.6-2: First-tier assessment of the acute risk for bees due to the use of GLOB182F in maize seeds

Intended use	Maize seeds		
Active substance	Fludioxonil		
Application rate (g/ha)	1 × 2.375 g/ha		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 100	2.375	0.02375
Contact toxicity	> 100		0.02375
Product	GLOB182F		
Application rate (g/ha)	1 × 25.11 g/ha		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 1100	25.11	0.02283
Contact toxicity	> 1100		0.02283

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

All the hazard quotients are considerably less than 50, indicating that the active substance and formulation pose a low acute risk from dust drift to bees. Therefore a low acute risk to bees is expected from the application of GLOB182F.

Chronic risk assessment

The chronic risk to bees has been assessed according to the EFSA Guidance Document on bees (revision of 4 July 2014).

It should be noted that the EFSA guidance document for bees also states that a risk assessment for effects on the development of the hypopharyngeal glands (HPG) should be performed. However, as there is currently no validated methodology for the assessment of sublethal effects, this will not be considered in the risk assessment for the time being.

Larval assessment according to EFSA 2013

The chronic oral assessment for honey bee larvae was performed using the screening step for seed treatments.

At the screening step, two different ETR calculations have to be performed, one based on an application rates on kg a.s./ha have and one additional ETR calculation based on mg a.s./seed for the treated crop scenario. The different unit for the treated crop scenario for seed treatments (i.e. the mg/seed) is based on the assumption that the residue level in pollen and nectar of the individual crop plants are related to the seed loading rate of those individual plants rather than the total mass applied to a certain cropped area (i.e. application rate per hectare).

The first ETR calculation is carried out for a maximum single application rate of 2.375 g a.s./ha.

$$ETR_{larvae} = AR \times Ef \times SV / NOEL_{larvae} = 0.002375 \times 0.3 \times 4.4 / 3.8 = 0.000825$$

With: AR = application rate in kg as/ha = 0.002375 kg as/ha
Ef = Exposure factor
SV = shortcut value

An Additional ETR calculation was carried out for the treated crop scenario based on a maximum seed loading rate of 0.019 mg a.s./seed (maximum dose rate of 50 mg a.s./Kg seed and TWG of 380 ~~240~~ g/1000 seeds).

$$ETR_{larvae} = AR \times SV / NOEL_{larvae} = 0.019 \times 0.4 / 3.8 = 0.002$$

With: AR = application rate in kg a.s./ha = 0.019 mg as/seed
SV = shortcut value down-ward spraying

The resulting ETRs are lower than the trigger value of 0.2 defined by the EFSA guidance on bees (2013) so the protection goal is met, indicating that the proposed uses of GLOB182F pose an acceptable chronic risk to honey bee larvae.

Adult assessment according to EFSA 2013

The chronic oral assessment for adult honey bees was performed using the screening step for seed treatments.

At the screening step, two different ETR calculations have to be performed, one based on an application rates expressed on kg a.s./ha and one based on an application rate expressed on mg a.s./seed. The calculation based on an application rate expressed as mg a.s./seed is relevant only for the treated crop scenario for seed treatments. The calculation based on application rates expressed in kg a.s./ha are applicable for all other scenarios for seed treatments. The different unit for the treated crop scenario for seed treatments (i.e. the mg/seed) is based on the assumption that the residue level in pollen and nectar of the individual crop plants are related to the seed loading rate of those individual plants rather than the total mass applied to a certain cropped area (i.e. application rate per hectare).

The first ETR calculation is carried out for a maximum single application rate of 2.375 g a.s./ha.

$$ETR_{chronic\ adult\ oral} = AR \times Ef \times SV / 10\ d\ LDD_{50} = 0.002375 \times 0.3 \times 7.6 / 69.8 = 0.000078$$

With: AR = application rate in kg as/ha = 0.002375 kg as/ha
Ef = Exposure factor
SV = shortcut value

An Additional ETR calculation was carried out for the treated crop scenario based on a maximum seed loading rate of 0.019 mg a.s./seed (maximum dose rate of 50 mg a.s./Kg seed and TWG of 380 ~~240~~ g/1000 seeds).

$$ETR_{chronic\ adult\ oral} = AR \times SV / 10\ d\ LDD_{50} = 0.019 \times 0.7 / 155 = 0.00019$$

With: AR = application rate in mg as/seed = 0.019 mg as/seed
SV = shortcut value

The ETRs are lower than the respective trigger value of 0.03 which is defined by the EFSA guidance on bees (2013) so the protection goal is met, indicating that the proposed uses of GLOB182F as seed treatment pose an acceptable chronic risk to adult honey bees. A 1st Tier risk assessment is therefore not necessary.

izRMS comments:

Since no separate acute risk assessment scheme for bees is provided in SANCO/10329/2002 rev. 2 final, the evaluation has been carried out using assumptions for spray treatments. Although this scheme was not validated for seed treatments, it is considered acceptable by the izRMS since extremely worst case assumptions were made that bees will be exposed to 100% application rate. This approach covers also assumptions made for the acute risk assessment according to EFSA (2013) and no additional calculations were performed since acceptable risk could be concluded based on these extreme assumptions. Since maximum intended application rate was used in calculations, performed risk assessment covers all intended uses of GLOB182F in both crops (maize and sunflower).

With regard to the chronic and larvae risk assessment the izRMS agrees that in absence of the respective risk assessment scheme for seed treatments provided in SANCO/10329/2002 rev. 2 final it is most reasonable to perform respective evaluation based on indications of EFSA (2013). Acceptable chronic and larvae risk could be concluded based on the above calculations. It is noted that the maximum intended application rate and loading per seed (0.002375 kg a.s./ha and 0.019 mg a.s./seed) was considered by the Applicant (for sunflower it would be 1.6875 g a.s./ha and seed loading in range 0.003-0.008 mg a.s./seed) and for this reason performed calculations are protective for all intended uses of GLOB182F in both crops (maize and sunflower).

The izRMS agrees with the Applicant that currently no respective methods for evaluation of sub-lethal effects (e.g. effects on HPG) are available and for this reason no respective risk assessment may be performed.

Calculations performed by the Applicant were independently validated by the izRMS using EFSA Bee-Tool v.3 and the same results were obtained. In addition, acceptable risk from exposure via surface water and guttation water could be concluded.

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

Not relevant, risk is acceptable on lower tier.

9.6.3 Effects on bumble bees

A risk assessment scheme to assess the acute risk to bumblebees is not included in the SANCO/10329/2002 guidance document. However, such a risk assessment scheme is described in the EFSA guidance document for bees (EFSA, 2013; revised July 2014)12. Therefore, the acute risk assessment for bumblebees is performed as described in the EFSA guidance document for bees (revision

of 4 July 2014). According to the EFSA guidance document for bees, the same exposure routes as for honeybees are relevant for bumblebees. Regarding the accumulative toxicity, no methods/guidelines are currently available for testing potential accumulative effects. Therefore, only acute toxicity is considered in the risk assessment.

Acute contact assessment according to EFSA 2013

In the screening step hazard quotients were calculated for contact exposure ($HQ_{contact}$) to fludioxonil and GLOB182F. The specific protection goal is achieved if the calculated HQ value is smaller or equal to the trigger value of 2.3.

$HQ_{contact} = f_{dep}/100$ x AR / LD_{50} = 17/100 * 2.375 / 203 = 0.002

With: AR = application rate in g a.s./ha = 2.375 g fludioxonil/ha
f_{dep} = fraction of the dose deposited on foragers visiting plants in the field margin or an adjacent crop. The deposition value for maize without deflector is used as worst case.

The HQ value in the screening step is lower than the trigger value of 2.3 defined by the EFSA guidance on bees (2013) so the protection goal is met, indicating that the proposed uses of GLOB182F poses an acceptable acute contact risk to bumblebees.

Acute oral assessment according to EFSA 2013

The acute oral assessment for bumblebees for seed treatment applications was performed using the screening step for solid applications.

The first ETR calculation is carried out for a maximum single application rate of 2.375 g a.s./ha.

ETR_{acute}oral = AR x Ef x SV / LD_{50}oral = 0.002375 x 0.3 x 11.2 / 351.6 ~~203~~ = 0.00002 ~~0.00004~~

With: AR = application rate in kg a.s./ha = 0.002375 kg as/ha
Ef = exposure factor = 0.3
SV = shortcut value down-ward spraying

An Additional ETR calculation was carried out for the treated crop scenario based on a maximum seed loading rate of 0.019 mg a.s./seed (maximum dose rate of 50 mg a.s./Kg seed and TWG of 240 g/1000 seeds).

$ETR_{acute\ adult\ oral}$ = AR x SV / 10 d LD_{50} = 0.019 x 0.9 / 203 = 0.00008

With: AR = application rate in mg as/seed = 0.019 mg as/seed
SV = shortcut value

The resulting ETRs for the screening step is lower than the trigger value of 0.036 defined by the EFSA guidance on bees (2013) so the protection goal is met, indicating that the proposed uses of GLOB182F poses an acceptable acute oral risk to bumblebees.

izRMS comments:

Although the risk assessment for bumblebees is currently not mandatory and available toxicity data clearly indicated that bumblebees are less sensitive to GLOB182F comparing to bees, provided above calculations were validated by the izRMS and are considered acceptable.
Low acute risk to bumblebees from the intended uses of GLOB182F as a seed treatment in maize and sunflower may be concluded.

9.6.4 Effects on solitary bees

No information available.

9.6.5 Overall conclusions

The hazard quotients after oral and contact exposures are below the trigger value of 50. According to SANCO/10329/2002 (final, 2002), it can therefore be concluded that the intended use of GLOB182F gives a low acute oral and contact risk to honey bees.

The chronic TER's for honey bee adults and larvae are higher than the respective trigger values defined by the EFSA guidance on bees (2013) indicating that the proposed use of GLOB182F as seed treatment on sunflower and maize poses an acceptable chronic risk to honey bee adults and larvae.

The acute TER's for bumblebees at screening step are higher than the respective trigger values defined by the EFSA guidance on bees (2013) indicating that the proposed use of GLOB182F as seed treatment poses an acceptable acute risk to bumblebees.

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

Studies on the toxicity to non-target arthropods have been carried out with Fludioxonil. However, these studies were not conducted with the active ingredient alone. They were performed on the formulated product. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target arthropods of GLOB182F were not evaluated as part of the EU assessment of Fludioxonil. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2. Studies on *Poecilus cupreus* and *Aleochara bilineata* performed with the formulation Fludioxonil 480 FS (containing 480 g fludioxonil /L), similar to GLOB182F, are submitted.

The selection of studies and endpoints for the risk assessment deviates from the results of the EU review process. Justifications are provided below.

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

Species	Substance	Exposure System	Results	Reference
Aphidius rhopalosiphi (adults)	GLOB182F	Laboratory test glass plates (2D)	LR_{50} > 1.523 L/ha *(LR_{50} > 152.3 g as/ha)* ER_{50} > 1.523 L/ha *(ER_{50} > 152.3 g as/ha)*	Röhlig U., 2020a
Typhlodromus pyri (protonymphs)	GLOB182F	Laboratory test glass plates (2D)	LR_{50} > 1.523 L/ha *(LR_{50} > 152.3 g as/ha)* ER_{50} > 1.523 L/ha *(ER_{50} > 152.3 g as/ha)*	Röhlig U., 2020b
Aleochara bilineata (adults)	Fludioxonil 25 FS	Tier II extended laboratory test Fresh residues of formulation incorporated into soil	EC_{50} > 9.0234 mg a.s./kg soil d.w.	Röhlig U., 2020c
Aleochara bilineata (adults)	Fludioxonil 480 FS	Tier II extended laboratory test Fresh residues of formulation incorporated into soil	EC_{50} > 40 mg product/kg soil d.w. (*EC_{50} > 15.97 mg as/kg soil d.w.*)	Röhlig U., 2018a
Poecilus cupreus (adults)	Fludioxonil 25 FS	Tier II extended laboratory test Fresh residues of formulation incorporated into soil (2D)	EC_{50} > 9.0234 mg a.s./kg soil d.w.	Röhlig U., 2020d
Poecilus cupreus (adults)	Fludioxonil 480 FS	Tier II extended laboratory test Fresh residues of formulation incorporated into soil (2D)	LR_{50} > 40 mg product/kg soil d.w. (*LR_{50} > 15.97 mg as/kg soil d.w.*) NOER ≥ 40 mg product/kg soil d.w. (*NOER ≥ 15.97 mg as/kg soil d.w.*)	Röhlig U., 2018b

izRMS comments:

Studies on toxicity of GLOB182F and other fludioxonil seed treatment formulations to non-target arthropods were evaluated by the izRMS and considered acceptable. For details of evaluation, please refer to Appendix 2. Provided above endpoints are confirmed to be correct.

9.7.1.1 Justification for new endpoints

The applicant submitted studies on *Aleochara bilineata* (Röhlig U., 2020c and 2018a) and *Poecilus*

cupreus (Röhlig U., 2020d and 2018b) performed with the formulations Fludioxonil 25 FS and Fludioxonil 480 FS (containing 25 and 480 g fludioxonil/L, respectively). In these four studies the test organims are exposed to different concentrations of the test item (fludioxonil 25 FS or Fludioxonil 480 FS) mixed into the artificial soil substrate. Extrapolation has been made from these studies to the formulation GLOB182F and the resulted endpoints expressed in mg a.s./kg dw can be used in the risk assessment of GLOB182F. Extrapolation is justified since the Regulation 284/2013 allows to use studies on other formulations. Furthermore, the composition of both formulations are comparable since they contain the same solvent and similar co-formulants. This is certainly applicable for these type of studies where the test item is applied in or on the soil (not directly to the test organism) and thus the formulation has a minor role compared to the active substance.

The composition of Fludioxonil 25 FS and Fludioxonil 480 FS is also given in the Confidential Part C.

As can be seen in the risk assessment below, using the endpoints of the studies performed with both Fludioxonil 25 FS and Fludioxonil 480 FS gives large safety margins compared to the trigger value.

izRMS comments:

The izRMS agrees with the Applicant that toxicity data for formulations Fludioxonil 25 FS and Fludioxonil 480 FS may be used in the risk assessment performed for GLOB182F due to similar compositions. Majority of co-formulants is the same with some differences in their concentration, considered however to have no impact on the ecotoxicological profile of the formulated product. Single co-formulants are different, but they have the same role in the formulations. The only co-formulants classified in area of ecotoxicology (for aquatic hazard) are at the same level in all formulations. Different concentration of active compound in particular products is of no concern due to the dose-response test design of studies performed with *A. bilineata* and *P. cupreus*. It is also noted that contribution of co-formulants to the toxicity is reduced due to the exposure regime in these studies (test item incorporated in soil).

During the commenting period it was pointed out by FR that toxicity of GLOB182 F and Fludioxonil 480 FS to *Daphnia magna* and juvenile bees is comparable, while Fludioxonil 25 FS is slightly more toxic. Information on toxicity of particular formulations to selected non-target species was extracted by the izRMS from the respective Core Assessments and presented in table below. Please note that in this species for which endpoints were greater than the maximum dose/rate tested (i.e. acute bees, *T. pyri* and *A. rhopalosiphi*) were not included in the below comparison as being not informative.

Species	Endpoint	GLOB182F	Fludioxonil 25 FS [1)]	Fludioxonil 480 FS [2)]
Daphnia magna	EC_{50}	0.51 µg a.s./L	0.295 µg a.s./L	1.916 µg a.s./L
Pseudokirchneriella subcapitata	E_rC_{50}	0.73 µg a.s./L	0.154 µg a.s./L	1.064 µg a.s./L
Apis mellifera, adult, chronic	LDD_{50}	69.8 µg a.s./bee	2.78 µg a.s./bee	No study available, results for Fludioxonil 25 FS used
	NOEDD	21.0 µg a.s./bee	0.64 µg a.s./bee	
Apis mellifera, larvae	NOED, mortality, day 8	24 µg a.s./larva	10 µg a.s./larva	No study available, results for Fludioxonil 25 FS used
	NOED emergence, day 22	3.8 µg a.s./larva	Not investigated (study duration 8 days)	

[1)] Core Assessment for Fludioxonil 25 FS (Prepper), Part B, Section 9 (2019, izRMS: CZ)
[2)] Core Assessment for Fludioxonil 480 FS (Prepper 480 FS), Part B, Section 9 (2021, izRMS: NL)

Endpoints provided in the above table indicate that formulation Fludioxonil 25 FS is more toxic to selected non-target species comparing to GLOB182F and Fludioxonil 480 FS. This confirms that results for this product may be used in the risk assessment for GLOB182F.

9.7.2 Risk assessment

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

The risk assessment is carried out for the use as seed treatment in maize and sunflower (see 9.1.2).

The risk assessment is based on the standard species *Aphidius rhopalosiphi* and *Typhlodromus pyri* as well as on *Aleochara bilineata* and *Poecilus cupreus* which are relevant species for the intended use.

9.7.2.1 Risk assessment for in-field exposure

With regard to non-target arthropods, only soil-dwelling non-target arthropods have to be taken into account as they can be exposed in-field to residues from Fludioxonil through contact with residues on soil.

Tier II studies with *Poecilus cupreus* and *Aleochara bilineata* has been carried out with Fludioxonil 480 FS. The EC_{50} endpoints of these studies are presented in table 9.7-2. Since adult organisms were exposed to fresh residues of GLOB182F incorporated into sandy soil, risk assessment should be carried out with the predicted environmental concentration in soil (PECsoil).

Exposure
The initial value for PECsoil of GLOB182F after application is 0.04 mg product/kg soil d.w. based on worst-case GAP (30 g a.s./ha). See Part B, Section 8, Annex Point 8.7.2.1. As a worst case assumption, the initial PECsoil was multiplied by a factor 2 to account for two crop cycles.

Risk assessment
The risk assessment is conducted according to ESCORT 2 guidance. If the LC_{50} or EC_{50} value is greater than the PECsoil then no unacceptable effects would be predicted in-field following the use of GLOB182F in accordance with the proposed use pattern.

Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of GLOB182F as a seed treatment in maize and sunflower

Intended use **Product** **Application rate (g a.s./ha)**	Seed treatment GLOB182F 2.375 g a.s./ha		
Test species **Higher-tier**	**LR_{50}/ER_{50} (extended lab.) (mg product/kg soil d.w.)**	**PECsoil (mg a.s./kg)**	**Acceptable in-field risk?**
Aleochara bilineata (extrapolated from Fludioxonil 25 FS)	> 9.0234	0.00882	Yes
Aleochara bilineata (extrapolated from Fludioxonil 480 FS)	> 15.97	0.00882	Yes
Poecilus cupreus (extrapolated from Fludioxonil 25 FS)	> 9.0234	0.00882	Yes
Poecilus cupreus (extrapolated from Fludioxonil 480 FS)	> 15.97	0.00882	Yes

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient.
Criteria values shown in bold breach the relevant trigger.

Based on table 9.7-2 it can be concluded that there is low risk to in-field soil-dwelling non-target arthropods following application according to the proposed use pattern.

izRMS comments:

The risk assessment performed in Table 9.7-2 above is agreed by the izRMS. In evaluation the maximum PEC_{soil} values calculated with consideration of soil accumulation of fludioxonil were used, covering all intended uses of GLOB182F in maize and sunflower.

Due to the intended use pattern (seed treatment) in-field exposure of leaf-dwelling arthropods is not expected and the in-field risk assessment for soil-dwellers is deemed sufficient.

Acceptable in-field risk with large margin of safety could be concluded.

9.7.2.2 Risk assessment for off-field exposure

Risk assessment of areas immediately surrounding the crop is considered important since these areas represent a natural reservoir for immigration, emigration and reproduction of arthropod populations and provide increased species diversity. In case of GLOB182F, the formulation is applied as a seed dresser so non-target arthropods living in off-field areas will not be exposed to the formulation itself. Accordingly, risk to non-target arthropods living in the off-field is acceptable and no calculation of off-field exposure to the formulation is necessary.

However, foliar-dwelling non-target arthropods may be exposed off-field (in flowering margins or neighbouring crops) to dust drift derived from the sowing of seeds treated with plant protection products. Therefore, glass plate tests were conducted with the standard foliar-dwelling non-target arthropod species *A. rhopalosiphi* and *T. pyri* in which the glass plates were sprayed with GLOB182F. The standard endpoint, LR_{50}, from the species showing the highest sensitivity to the active substance will be used in the risk assessment.

~~The off-field exposure (predicted environmental residue, PER) is calculated according to ESCORT 2 using the following equation:~~

~~$PER_{off\text{-}field}$ = application rate (g as/ha or L/ha) * MAF *(drift factor / vegetation distribution factor)~~

~~The MAF is a generic multiple application factor, which is used to take into account the potential build-up of applied substances between applications based on the application interval, DT_{50} value and number of applications. Default foliar and soil MAF values are given in the ESCORT 2 Guidance Document. Since GLOB182F is applied as a seed treatment, the MAF can be omitted.~~

~~The basic drift value for a distance from the field border of 1m according to BBA 2000 (ESCORT 2 Guidance Document) is 2.77. The corresponding drift factor = % drift/100.~~

~~The drift value given above were determined over a non-vegetated area and only under windy conditions. However, the field boundary and the crop-relevant default drift distance is typically vegetated and serves as a filter strip trapping some drifted material. Therefore, the overestimated exposure given by the drift values should be corrected by a "vegetation distribution factor" to have a more realistic but still worst-case deposit estimation for off-field habitats. ESCORT 2 considers a default value of 10 as appropriate.~~

~~The potential risk of GLOB182F to off-field non-target arthropods was assessed by calculation of the hazard quotient (HQ = exposure/toxicity) with the predicted environmental rate (PER) and the lowest lethal rate (LR_{50}) values according to the following formula:~~

~~Off-field HQ = (off-field PER / LR_{50}) x correction factor~~

~~The correction factor is intended to cover uncertainty with regard to species sensitivity, the default value is 10.~~

~~The HQ trigger for Tier I laboratory studies is 2.~~

~~**Table 9.7-3: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of GLOB182F in maize and sunflower**~~

~~**Intended use**~~	~~Maize and sunflower seeds~~				
~~**Active substance/product**~~	~~Fludioxonil~~				
~~**Application rate (g/ha)**~~	~~2.375 g as/ha~~				
~~**MAF**~~	~~1~~				
~~**vdf**~~	~~10 (Tier 1)~~				
~~**Test species Tier I**~~	~~**LR_{50} (lab.) (g as/ha)**~~	~~**Drift factor**~~	~~**$PER_{off-field}$ (g as/ha)**~~	~~**CF**~~	~~**$HQ_{off-field}$ criterion: HQ ≤ 2**~~
~~*Typhlodromus pyri*~~	~~> 152.3 g as/ha~~	~~0.0277~~	~~0.00658~~	~~10~~	~~< 0.00043~~
~~*Aphidius rhopalosiphi*~~	~~> 152.3 g as/ha~~				~~< 0.00043~~

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

~~The off-field HQ values for exposure from drift for the representative species *A. rhopalosiphi* and *T. pyri* are less than the ESCORT 2 trigger value of 2 for the Tier I studies.~~

~~The off-field HQ values indicate that drift from GLOB182F poses low risk to off-field foliar-dwelling non-target arthropods following application according to the proposed use pattern.~~

izRMS comments:

Since currently no respective guidance document enabling off-field risk assessment for non-target arthropods from seed treatments is available, evaluation of the risk in off-crop habitat is not mandatory for GLOB182F.

Nevertheless, when performed, it should take into account that the drift rates determined for spray application will be not relevant for exposure resulting from the dust drift. EFSA bee guidance (2013) provides some information on the dust drift during sowing of certain crops, which was considered in determination of the extent of exposure of bees in the off-crop habitats. Although NTAs are not included in this guidance, provided dust drift rates are considered relevant also for this group of species. According to Appendix H of EFSA (2013), dust drift of 17% is relevant for maize sown without deflector ~~dust~~. No information on dust drift rates is available for sunflower, nevertheless the izRMS is of the opinion that in absence of respective data, the value for maize may be used as a surrogate.

The risk assessment performed with consideration of the dust drift indicated above is presented in table below. In absence of information on distribution of dust particles in vegetation, VDF of 1 is assumed as a worst case. Since studies on 4 species are available, CF of 5 is applicable. It has to be noted that HQ approach was validated for spray applications and not for seed treatments. Nevertheless, in absence of any other risk assessment scheme, calculation of HQ is considered sufficient for illustrative purposes.

Intended use	Maize and sunflower seeds				
Active substance/product	Fludioxonil				
Application rate (g/ha)	2.375 g as/ha				
MAF	1				
vdf	1 due to lack od data on distribution of dust particles in vegetation				
Test species Tier I	**LR_{50} (lab.) (g as/ha)**	**Drift factor**	**$PER_{off-field}$ (g as/ha)**	**CF**	**$HQ_{off-field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	> 152.3 g as/ha	0.17	2.02	5	< 0.013
Aphidius rhopalosiphi	> 152.3 g as/ha				< 0.013

Based on above calculations, acceptable off-field risk to non-target arthropods may be concluded with no need for risk mitigation measures. No separate evaluation was performed for *A. bilineata* and *P. cupreus*, since acceptable off-field risk may be concluded due to acceptable risk demonstrated for in-field situation.

It should be noted that provided above evaluation is only indicative since currently no risk assessment scheme exist to evaluate the off-crop risk to NTAs from seed treatments.

The Applicant calculations provided in Table 9.7-3 above were struck through since calculated exposure was relevant for the spraying formulation and not seed treatment.

9.7.2.3 Additional higher-tier risk assessment

Not relevant.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

The in-field and off-field HQ values for *A. rhopalosiphi*, ~~and~~ *T. pyri* and soil dwelling arthropods (*P. cupreus* and *A. bilineata*) fall below the trigger values indicating that GLOB182F does not pose an unacceptable risk to non-target arthropods in in-field and off-field areas following application according to the proposed use patterns. No risk mitigation measures are deemed necessary for the off-crop habitats.

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 Fludioxonil and its 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 GLOB182F were not evaluated as part of the EU assessment of Fludioxonil. Therefore new chronic toxicity studies on earthworms, *Folsomia candida* and *Hypoaspis aculeifer* performed with the formulations Fludioxonil 25 FS and Fludioxonil 480 FS (containing 25 and 480 g fludioxonil/L, respectively), similar to GLOB182F, are submitted.

New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment deviates from the results of the EU review process. Justifications are provided below.

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

Species	Substance	Exposure System	Results	Reference
~~*Eisenia fetida*~~	~~Fludioxonil~~	~~Mixed into substrate 14 d, acute 10 % peat content~~	~~$LC_{50} \geq$ 1000 mg/kg dw $LC_{50,corr} \geq$ 500 mg/kg dw*~~	~~EFSA, 2007~~
Eisenia fetida	Fludioxonil	Mixed into substrate 56 d, chronic 10 % peat content	NOEC $\geq$ 20 mg/kg dw $NOEC_{corr} \geq$ 10 mg/kg dw*	EFSA, 2007
Eisenia andrei	Fludioxonil 25 FS	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 425.48 mg/kg dw NOECcorr = 212.74 mg/kg dw* (NOEC = 9.979 mg a.s./kg dw $NOEC_{corr}$ = 4.990 mg a.s./kg dw*)	Friedrich S., 2017a
Eisenia andrei	Fludioxonil 480 FS	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 97.34 mg/kg dw $NOEC_{corr}$ = 48.67 mg/kg dw* (NOEC = 40.22 mg a.s./kg dw $NOEC_{corr}$ = 20.11 mg a.s./kg dw*)	Friedrich S., 2017b
Folsomia candida	Fludioxonil 25 FS	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 425.48 mg/kg dw $NOEC_{corr}$ = 212.74 mg/kg dw* (NOEC = 9.979 mg a.s./kg dw $NOEC_{corr}$ = 4.99 mg/kg dw*)	Friedrich S., 2017c
Folsomia candida	Fludioxonil 480 FS	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 97.34 mg/kg dw $NOEC_{corr}$ = 48.67 mg/kg dw* (NOEC = 40.22 mg a.s./kg dw $NOEC_{corr}$ = 20.11 mg/kg dw*)	Friedrich S., 2017d
Hypoaspis aculeifer	Fludioxonil 25 FS	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 425.48 mg/kg dw $NOEC_{corr}$ = 212.74 mg/kg dw* (NOEC = 9.979 mg a.s./kg dw $NOEC_{corr}$ = 4.99 mg/kg dw*)	Schulz L., 2017a
Hypoaspis aculeifer	Fludioxonil 480 FS	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 97.34 mg/kg dw $NOEC_{corr}$ = 48.67 mg/kg dw* (NOEC = 40.22 mg a.s./kg dw $NOEC_{corr}$ = 20.11 mg/kg dw*)	Schulz L., 2017b
Field studies				
Not necessary				
Litter bag test				
Not necessary				

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

izRMS comments:

The acute and chronic earthworms toxicity data for fludioxonil given in Table 9.8-1 are in line with the EU agreed endpoints reported in EFSA Scientific Report (2007) 110. Since acute toxicity to earthworms is no longer a data requirement, the acute endpoints were struck through as not considered in the risk assessment.

Studies on toxicity of various fludioxonil seed treatment formulations to soil macro and mesofauna were evaluated by the izRMS and considered acceptable. For details of evaluation, please refer to Appendix 2. Provided above endpoints are confirmed to be correct.

9.8.1.1 Justification for new endpoints

The applicant submitted chronic toxicity studies on earthworms (Friedrich S., 2017a and 2017b), *Folsomia candida* (Friedrich S., 2017c and 2017d) and *Hypoaspis aculeifer* (Schulz L., 2017a and 2017b) performed with the formulations Fludioxonil 25 FS and Fludioxonil 480 FS (containing 25 and 480 g fludioxonil/L, respectively). In these studies the test organims are exposed to different concentrations of the test item (fludioxonil 25 FS or Fludioxonil 480 FS) mixed into the artificial soil substrate. Extrapolation has been made from these studies to the formulation GLOB182F and the resulted endpoints expressed in mg a.s./kg dw can be used in the risk assessment of GLOB182F. Extrapolation is justified since the Regulation 284/2013 allows to use studies on other formulations. Furthermore, the composition of these formulations are comparable since they contain the same solvent and similar co-formulants. This is certainly applicable for these type of studies where the test item is applied in or on the soil (not directly to the test organism) and thus the formulation has a minor role compared to the active substance.

The composition of Fludioxonil 25 FS and Fludioxonil 480 FS is also given in the Confidential Part C.

As can be seen in the risk assessment below, using the endpoints of the studies performed with both Fludioxonil 25 FS and Fludioxonil 480 FS gives large safety margins compared to the trigger value of 5.

izRMS comments:

The izRMS agrees with the Applicant that toxicity data for formulations Fludioxonil 25 FS and Fludioxonil 480 FS may be used in the risk assessment performed for GLOB182F due to similar compositions. Majority of co-formulants is the same with some differences in their concentration, considered however to have no impact on the ecotoxicological profile of the formulated product. Single co-formulants are different, but they have the same role in the formulations. The only co-formulants classified in area of ecotoxicology (for aquatic hazard) are at the same level in all formulations. Different concentration of active compound in particular products is of no concern due to the dose-response test design of studies performed with earthworms, *F. candida* and *H. aculeifer*. It is also noted that contribution of co-formulants to the toxicity is reduced due to the exposure regime in these studies (test item incorporated in soil).

To further support consideration of results of studies performed with other fludioxonil FS formulations, the toxicity data for selected non-target species were compared in the izRMS commenting box in point 9.7.1.1 of this document. Available data indicate that Fludioxonil 25 FS is most toxic of all three formulated products.

9.8.2 Risk assessment

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

9.8.2.1 First-tier risk assessment

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3. According to the assessment of environmental-fate data, multi-annual accumulation in soil does not need to be considered for Fludioxonil.

Table 9.8-2: 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 GLOB182F in maize and sunflower seeds

Intended use	Maize and sunflower seeds		
~~**Acute effects on earthworms**~~			
~~**Product/active substance**~~	~~**LC_{50} (mg/kg dw)**~~	~~**PEC_{soil} (mg/kg dw)**~~	~~**TER_a (criterion TER ≥ 10)**~~
~~Fludioxonil~~	~~≥ 500~~	~~0.00882~~	~~56689.3~~
Chronic effects on earthworms			
Product/active substance	**NOEC (mg a.s./kg dw)**	**PEC_{soil} (mg a.s./kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Fludioxonil	≥ 10	0.00882	1133.8
Fludioxonil 25 FS	4.990	0.00882	565.76
Fludioxonil 480 FS	20.11	0.00882	2280.03
Chronic effects on *Folsomia candida*			
Product/active substance	**NOEC (mg a.s./kg dw)**	**PEC_{soil} (mg a.s./kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Fludioxonil 25 FS	4.99 ~~9.979~~	0.00882	565.76 ~~1131.41~~
Fludioxonil 480 FS	20.11 ~~40.22~~	0.00882	2280.03 ~~4560.06~~
Chronic effects on *Hypoaspis aculeifer*			
Product/active substance	**NOEC (mg a.s./kg dw)**	**PEC_{soil} (mg a.s./kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Fludioxonil 25 FS	4.99 ~~9.979~~	0.00882	565.76 ~~1131.41~~
Fludioxonil 480 FS	20.11 ~~40.22~~	0.00882	2280.03 ~~4560.06~~

TER values shown in bold fall below the relevant trigger.

All the acute TER values are much higher than the Annex VI acute trigger value of 10, indicating that GLOB182F poses low acute risk to earthworms when applied according to the proposed use rates.

The long-term TER values all exceed the Annex VI long-term trigger value of 5, indicating that GLOB182F poses low long-term risk to earthworms.

izRMS comments:

The risk assessment for earthworms provided in Table 9.8-2 above is agreed by the izRMS.

According to EFSA Supporting publication 2015:EN-924, corrected endpoints should be used in the risk assessment for soil macro- and mesofauna, irrespective of the peat content used in the study. Since in the risk assessment for *F. candida* and *H. aculeifer* not corrected endpoints were used by the Applicant, calculations provided in Table 9.8-2 were amended accordingly.

Overall, acceptable risk may be concluded for all relevant species.

Since in the risk assessment the maximum $PEC_{soil,accu}$ was considered, the performed evaluation is protective for all intended uses of GLOB182F as a seed treatment in maize and sunflower.

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

9.8.2.2 Higher-tier risk assessment

Not relevant as the risk is acceptable on lower tier.

9.8.3 Overall conclusions

The ~~acute and~~ long-term TER values all exceed the Annex VI ~~acute and~~ long-term trigger value of ~~10 and~~ 5 ~~respectively~~, indicating that GLOB182F poses low ~~acute and~~ long-term risk to earthworms and other soil macro- and meso-fauna when applied according to the proposed use rates.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

Studies on effects of soil microorganisms have been carried out with Fludioxonil. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on soil microorganisms of GLOB182F were not evaluated as part of the EU assessment of Fludioxonil. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment deviates from the results of the EU review process. Justifications are provided below.

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

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Fludioxonil	28 d, aerobic	3% effect at 1.3 mg as/kg (sand soil) 20% effect at 1.3 mg as/kg (sandy silt loam) No negative effect at 0.333 mg as/kg (sandy loam and loam soil)	EFSA, 2007
~~C-mineralisation~~	~~Fludioxonil~~	~~28 d, aerobic~~	~~No negative effect at 1.33 mg as/kg (sand and sandy silt loam) and 0.333 mg as/kg (sandy loam and loam)~~	~~EFSA, 2007~~
N-mineralisation	GLOB182F	28 d, aerobic Loamy sand/loam	No adverse effect at day 28 at 1 and 10 mg product/kg dry soil (0.952 mg a.s./kg dry soil)	Schulz L., 2020

izRMS comments:

Information regarding effects of fludioxonil on nitrogen mineralisation is in line with the EU agreed data reported in EFSA Scientific Report (2007) 110.

Study on effects of GLOB182F on soil nitrogen turnover was evaluated by the izRMS and considered acceptable. For details of evaluation, please refer to Appendix 2. Provided above endpoints are confirmed to be correct.

Information regarding effects on carbon mineralisation is no longer a data requirement and for this reason it was struck through in Table 9.9-1.

9.9.1.1 Justification for new endpoints

As GLOB182F is not identical to the reference formulation Celeste 025 FS used during the EU Review of Fludioxonil, toxicity to soil microorganisms from the formulation was also tested and used in the risk assessment.

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 is taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3 and was already used in the risk assessment for

earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.1).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of GLOB182F in maize and sunflower

Intended use	Maize and sunflower seed treatment		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
Fludioxonil	1.3 (at 28 d)	0.00882	yes
GLOB182F	10 (at 28 d)	0.03348	yes
~~**C-mineralisation**~~			
~~**Product/active substance**~~	~~**Max. conc. with effects ≤ 25 % (mg/kg dw)**~~	~~**PEC_{soil} (mg/kg dw)**~~	~~**Risk acceptable?**~~
~~Fludioxonil~~	~~1.33 (at 28 d)~~	~~0.00882~~	~~yes~~

izRMS comments:

The risk assessment for soil micro-organisms provided in Table 9.9-2 above is agreed by the izRMS.
No unacceptable effects on soil microbial activity are expected when GLOB182F is used according to the intended use pattern.

Since the risk assessment was based on the maximum $PEC_{soil,accu}$ for fludioxonil and maximum $PEC_{soil,ini}$ for the formulation, the performed evaluation is protective for all intended uses of GLOB182F as a seed treatment in maize and sunflower.

Evaluation based on results of study on soil carbon mineralisation has been struck through as being no longer a data requirement.

9.9.3 Overall conclusions

As the PEC_{soil} of Fludioxonil and the formulation are much lower than the concentration at which no significant effects are detected, it can be concluded that the risk of GLOB182F to soil micro-organisms is acceptable in accordance with the intended use.

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

Since GLOB182F is applied as a seed treatment, it is of no risk to non-target plants and therefore no assessment is required.

izRMS comments:

In line with the current guidance document (SANCO/10329/2002 rev 2 final), the risk assessment for non-target terrestrial plants is not required for seed treatments. Evaluation will be required once respective guidance document enabling determination of the off-field exposure from the dust drift is noted at the EU level.

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

No relevant information available.

9.12 Monitoring data (KCP 10.8)

No relevant information available.

9.13 Classification and Labelling

Based on the acute aquatic toxicity studies on *Daphnia magna* and *Pseudokirchneriella subcapitata*, GLOB182F must not be classified. For chronic classification, the summation method in accordance with EU Regulation 1272/2008 (CLP labelling) was applied. Only one ingredient of GLOB182F, the active substance Fludioxonil, is classified as chronic toxicity category 1 with M factor of 10. ~~As GLOB182F contains 9.53% of Fludioxonil, which has an E_bC_{50} of 0.024 mg/L for algae (M-factor = 10), the sum of components classified as Chronic Cat. 1 multiplied by the M-factor is below 25%. Therefore, GLOB182F is not classified as Chronic Cat. 1 (H410) but as Chronic Cat. 2 (H411).~~

~~The following classification is than proposed from an ecotoxicological point of view.~~

~~**Pictogram:** GHS09~~
~~**Signal word:** /~~

~~**H-statements**~~
~~**H411** Toxic to aquatic life with long lasting effects.~~

~~**P-statements**~~
~~**P391** Collect spillage.~~
~~**P501** Dispose of content/container to … in accordance with local/regional/national/international regulations (to be specified).~~

~~**Other safety/precautionary phrases:**~~
~~**SP1:** Do not contaminate water with the product or its container.~~
~~**EUH401** To avoid risks to human health and the environment, comply with the instructions for use.~~

izRMS comments:

The izRMS agrees with the classification and labelling provided by the Applicant above.

It should be, however, noted that full classification of GLOB182F for the acute aquatic hazard based on formulation toxicity data is not possible since no study on toxicity to fish is available. Therefore classification for acute aquatic hazard should be performed using summation method. Nevertheless, it will have the same outcome as indicated by the Applicant above (no classification for acute aquatic hazard) since concentration of the substance classified as Acute 1 (fludioxonil) multiplied by the respective M factor (1, as given in the Commission Delegated Regulation (EU) 2020/217) is <25%.
Information on the basis for classification as Chronic 1 (E_bC_{10} for algae of 0.024 mg a.s./L) has been struck through, since in the Commission Delegated Regulation (EU) 2020/217 only classification as Acute 1 (M=1) and Chronic 1

(M=10) is given with no justification.

During commenting period it was also noted that due to M factor of 10 for chronic aquatic hazard, the concentration of the active substance (9.53%) multiplied by the M factor (10) will result with concentration >25%, meaning that GLOB182F should be classified for chronic aquatic hazard in category 1. Therefore, the Applicants' classification proposal is not agreed and 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 content/container to … in accordance with local / regional / national / international regulations (to be specified).

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.1.1/01	xxxxxxxxxxxxxx	2015	Fludioxonil - A reproduction study with the Northern Bobwhite xxxxxxxxxxxxxxxxx xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx GLP Unpublished	Y	Syngenta (Globachem access via LoA, valid in PL only)
KCP 10.2.1	Renner, P.	2021a	Acute toxicity of GLOB182F to *Daphnia magna* in a 48-hour static test. 20 48 ADL 0012 BioChem agrar, Labor für biologische und chemische Analytik GmbH GLP Unpublished	N	Globachem NV
KCP 10.2.1	Renner, P.	2021b	Effects of GLOB182F on *Pseudokirchneriella subcapitata* in an algal growth inhibition test. 20 48 AAL 0015 BioChem agrar, Labor für biologische und chemische Analytik GmbH GLP Unpublished	N	Globachem NV
KCP 10.3.1.1	Franke, M.	2020	Acute toxicity of GLOB182F to the honeybee *Apis mellifera* L. under laboratory conditions. 20 48 BAA 0079 BioChem agrar, Labor für biologische und chemische Analytik GmbH GLP Unpublished	N	Globachem NV
KCP 10.3.1.1	Amsel, K.	2020	Acute toxicity of Fludioxonil 100 FS to the bumblebee *Bombus terrestris* L. under laboratory conditions, 20 48 BBA 0026. BioChem agrar, Labor für biologische und chemische Analytik GmbH GLP Unpublished	N	Globachem NV
KCP 10.3.1.2	Schmidt, K.	2021	GLOB182F – Repeated exposure of honey bee (*Apis mellifera*) larvae under laboratory conditions. 20 48 BLC 0050 BioChem agrar, Labor für biologische und chemische Analytik GmbH GLP Unpublished	N	Globachem NV

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	Vertebrate study Y/N	Owner
KCP 10.3.1.2	Deβler, K.	2020	Chronic toxicity of GLOB182F to the honey bee *Apis mellifera* L. under laboratory conditions. 20 48 BAC 0051 BioChem agrar, Labor für biologische und chemische Analytik GmbH GLP Unpublished	N	Globachem NV
KCP 10.3.2	Röhlig, U.	2020a	Effects of GLOB182F on the parasitic wasp *Aphidius rhopalosiphi* (DeStefani-Perez) in a laboratory test. 20 48 NAL 0009. BioChem agrar, Labor für biologische und chemische Analytik GmbH GLP Unpublished	N	Globachem NV
KCP 10.3.2	Röhlig, U.	2020b	Effects of GLOB182F on the predatory mite *Typhlodromus pyri* SCHEUTEN in a laboratory test. 20 48 NTL 0008. BioChem agrar, Labor für biologische und chemische Analytik GmbH GLP Unpublished	N	Globachem NV
KCP 10.3.2	Röhlig, U.	2020c	Effects of Fludioxonil 25 FS on the rove beetle *Aleochara bilineata* GYLL. in an extended laboratory test. 20 48 NKE 0007 BioChem agrar, Labor für biologische und chemische Analytik GmbH GLP Unpublished	N	Globachem NV
KCP 10.3.2	Röhlig, U.	2018a	Effect of Fludioxonil 480 FS on the rove beetle *Aleochara bilineata* GYLL. in an extended laboratory test. 18 48 NKE 0003 Labor für biologische und chemische Analytik GmbH GLP Unpublished	N	Globachem NV
KCP 10.3.2	Röhlig, U.	2020d	Effects of Fludioxonil 25 FS on the carabid beetle *Poecilus cupreus* L. in an extended laboratory test. 20 48 NLE 0005 BioChem agrar, Labor für biologische und chemische Analytik GmbH GLP Unpublished	N	Globachem NV
KCP 10.3.2	Röhlig, U.	2018b	Effects of Fludioxonil 480 FS on the carabid beetle *Poecilus cupreus* L. in an extended laboratory test 18 48 NLE 0001 BioChem agrar Labor für biologische und chemische Analytik GmbH GLP Unpublished	N	Globachem NV

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	Vertebrate study Y/N	Owner
KCP 10.4.1.1	Friedrich, S.	2017a	Sublethal effects of Fludioxonil 25 FS on the earthworm *Eisenia andrei* in artificial soil. 17 48 TEC 0025 BioChem agrar, Labor für biologische und chemische Analytik GmbH GLP Unpublished	N	Globachem NV
KCP 10.4.1.1	Friedrich, S.	2017b	Sublethal effects of Fludioxonil 480 FS on the earthworm *Eisenia andrei* in artificial soil 17 48 TEC 0041 BioChem agrar, Labor für biologische und chemische Analytik GmbH GLP Unpublished	N	Globachem NV
KCP 10.4.2	Friedrich, S.	2017c	Effects of Fludioxonil 25 FS on the reproduction of the collembolan *Folsomia candida*. 17 48 TCC 0024 BioChem agrar, Labor für biologische und chemische Analytik GmbH GLP Unpublished	N	Globachem NV
KCP 10.4.2	Friedrich, S.	2017d	Effects of Fludioxonil 480 FS on the reproduction of the collembolan *Folsomia candida* 17 48 TCC 0041 BioChem agrar, Labor für biologische und chemische Analytik GmbH GLP Unpublished	N	Globachem NV
KCP 10.4.2	Schulz, L.	2017a	Effects of Fludioxonil 25 FS on the reproduction of the predatory mite *Hypoaspis aculeifer*. 17 48 THC 0020 BioChem agrar, Labor für biologische und chemische Analytik GmbH GLP Unpublished	N	Globachem NV
KCP 10.4.2	Schulz, L.	2017b	Effects of Fludioxonil 480 FS on the reproduction of the predatory mite Hypoaspis aculeifer 17 48 THC 0037 BioChem agrar, Labor für biologische und chemische Analytik GmbH GLP Unpublished	N	Globachem NV
KCP 10.5	Schulz, L.	2020	Effects of GLOB182F on the activity of soil microflora (Nitrogen transformation test). 20 48 SMN 0041 BioChem agrar, Labor für biologische und chemische Analytik GmbH GLP Unpublished	N	Globachem NV

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

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
All toxicity data for fludioxonil were taken from the EFSA Scientific Report (2007) 110 and were thus evaluated at the EU level. For list of respective studies, please refer to Vol. 2 of the monograph.					

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	Data protection claimed Y/N	Owner
There were no data submitted by the Applicant and not relied on.						

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

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

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

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

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

A 2.2 KCP 10.2 Effects on aquatic organisms

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

Comments of izRMS:	The study was performed in line with OECD 202 with no deviations. All validity criteria were met: • in the control not more than 10% of the daphnids showed immobilization (observed 0 %), • dissolved oxygen concentrations at the end of the test were ≥ 3 mg/L in control and test vessels (observed 8.47 – 8.73 mg/L). Overall, the study is acceptable with the following endpoint relevant for the risk assessment: 48h EC_{50} = 5.38 mg product/L (corresponding to 0.51 mg a.s./L, calculated with consideration of the analytical content of a.s. in formulation and density of 1.049 g/mL)

Reference:	KCP 10.2.1
Report	Acute toxicity of GLOB182F to *Daphnia magna* in a 48-hour static test. Renner P., 2021, report No. 20 48 ADL 0012
Guideline(s):	Yes (OECD 202)
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Executive summary

Purpose of this acute study was to determine possible effects of GLOB182F on *Daphnia magna* 24 and 48 hours after test item application. *Daphnia* were exposed to a series of concentrations under static test

conditions. Data on immobility were evaluated to assess effect concentrations EC_x. LOECs were determined and NOECs were derived. The test was performed according to the recommendations of the OECD Guideline 202 (2004).

Materials and methods

Test item:	GLOB182F Batch No.: PE 2004.742 analysed content: Fludioxonil 100.0 g/L density: 1.0490 g/mL
Test species:	*Daphnia magna* STRAUS
Test system:	exposure of *Daphnia* to the test item applied in test medium (dilution water)
Test conditions:	temperature: 20.4 – 20.5 °C photoperiod: 16 h light:8 h dark, daily; 20 µEm-2s-1 pH: 7.59 – 8.04 Oxygen concentration (during the test): 8.47 – 9.01 mg O_2/L Number of Daphnia/test vessel: 5 Number of test vessels/concentration: 4 Number of Daphnia/concentration: 20 Test volume: 10 mL Loading: 2 mL test solution per Daphnia
Treatments:	control (untreated test medium) test item (GLOB182F)
Test concentrations:	1.00, 1.60, 2.54, 4.06, 6.51 mg/L test item nominal
Exposure time:	48 hours (static test procedure)
Biological observations:	number of immobilised *Daphnia*: at 24 and 48 hours
Statistics:	LOEC: Step-down Cochran-Armitage Test Procedure ($p \leq 0.05$, one-sided smaller) ECx: Probit analysis ToxRat Professional 3.3.0 (RATTE, 2018)
Dates of work:	experimental start: 20.10.2020 experimental completion: 22.10.2020

Results and discussions

Daphnia in control treatments showed neither mortality nor any visible signs of abnormality during the course of the test. Daphnia in control groups were not trapped at the surface of the water. 24 hours after test start, effects of GLOB182F were not found. After 48 hours, a LOEC of 4.06 mg/L test item was determined. The NOEC was 2.54 mg/L test item nominal. Effect concentration of EC_{10} = 3.14, EC_{20} = 3.89 and EC_{50}= 5.38 mg/L test item nominal were found.
Recoveries of Fludioxonil in 'fresh' and 'spent' test solutions were within 80 to 120 % of nominal concentrations (mean recovery values for Fludioxonil ranged from 104.8 to 107.2 % of nominal Fludioxonil in 'fresh' samples and from 103.9 to 109.6 % of nominal Fludioxonil in 'spent' samples).

Toxicity results are based on test item nominal concentration.

Table A1: Observations

time after application	GLOB182F (mg/L)					
	control	1.00	1.60	2.54	4.06	6.51
	Immobility (%)					
3 h	0.0	0.0	0.0	0.0	0.0	0.0
24 h	0.0	0.0	0.0	0.0	0.0	0.0
48 h	0.0	0.0	0.0	0.0	35.0+	70.0+

+ significantly different to control, Step-down Cochran-Armitage Test Procedure ($p \leq 0.05$, one-sided smaller)

Table A2: Effects of GLOB182F on immobility of *Daphnia magna*

effect concentration	GLOB182F (mg/L)					
	24 h			48 h		
	after application					
LOEC test item nominal	> 6.51			4.06		
NOEC test item nominal	≥ 6.51			2.54		
EC_x test item nominal (CI)	**EC_{10}** n.d.*	**EC_{20}** n.d.*	**EC_{50}** n.d.*	**EC_{10}** 3.14 (2.04 – 3.82)	**EC_{20}** 3.89 (2.90 – 4.52)	**EC_{50}** 5.38 (4.65 – 6.20)

CI - confidence intervals, lower – upper; calculations performed using unrounded values; n.d. not determined due to mathematical issues; * no effect after 24 h

Table A1: Effects on growth rate and yield 72 hours after exposure start

effect concentration	GLOB182F (Błąd! Nie można odnaleźć źródła odwołania.)					
	average specific growth rate			yield		
	0 – 72 h after application					
LOEC test item nominal	5.71			2.04		
NOEC test item nominal	2.04			0.728		
EC test item nominal (CI)	E_rC_{10} 4.55 (4.08 – 4.91)	E_rC_{20} 5.45 (5.07 – 5.79)	E_rC_{50} 7.70 (7.18 – 8.46)	E_yC_{10} 1.69 (1.34 – 2.13)	E_yC_{20} 2.36 (1.99 – 2.81)	E_yC_{50} 4.46 (3.96 – 5.03)

CI – 95 % confidence intervals (upper – lower); calculations done using unrounded values

Table A2: Observations 72 hours after exposure start

test concentration Błąd! Nie można odnaleźć źródła odwołania. test item nominal	% Inhibition	
	average specific growth rate	yield
	0 – 72 h after application	
control	-	-
0.260	0.0	0.0
0.728	-0.8[1]	-3.0[1]
2.04	4.8	17.1
5.71	23.3	60.2
16.0	96.5	99.6

[1] negative values might indicate a higher growth relative to the untreated control

Conclusion

In a static daphnia acute test in which GLOB182F was tested at nominal concentrations of 1.00, 1.60, 2.54, 4.06, 6.51 mg/L, an EC50 of 5.38 mg/L GLOB182F was determined 48 hours after test start.
Validity criteria were fulfilled.

*****

Comments of izRMS:	The study was performed fully in line with OECD 201 with no deviations. All validity criteria were met: • the biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period (observed factor of 45.8), • the mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35 % (observed 20.3 %), • the coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7 % (observed 1.4 %). Overall, the study is considered acceptable and the following endpoints are relevant for the risk assessment: 72 h E_rC_{50} = 7.70 mg product/L (corresponding to 0.73 mg a.s./L) 72 h E_yC_{50} = 4.46 mg product/L (corresponding to 0.43 mg a.s./L) 72 h NOE_rC = 2.04 mg product/L (corresponding to 0.19 mg a.s./L) Endpoints expressed in term of the a.s. were calculated with consideration of the analytical content of a.s. in formulation and density of 1.049 g/mL

Reference:	KCP 10.2.1
Report	Effect of GLOB182F on *Pseudokirchneriella subcapitata* in an algal growth inhibition test test. Renner P., 2021, report No. 20 48 AAL 0015
Guideline(s):	Yes (OECD 201)
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Executive summary

Purpose of this study was to determine possible effects of GLOB182F on *Pseudokirchneriella subcapitata* growth under static conditions. Exponentially growing cultures were exposed to various concentrations of the test item under defined conditions. Related to the inhibition of growth rate and yield over a period of 72 hours, effect concentrations of ErCx (growth rate) and EyCx (yield) were determined. LOECs and NOECs were determined as well. The test was performed according to the recommendations of the OECD Guideline 201 (2011).

Materials and methods

Test item: GLOB182F
Batch No.: PE 2004.742
analysed content: Fludioxonil 100.0 g/L
density: 1.0490 g/mL

Test species: *Pseudokirchneriella subcapitata, HILSE*

Test system: exposure of *Pseudokirchneriella subcapitata* to GLOB182F applied once in test medium (static conditions)
Initial biomass: 5 x 10^3 cells/mL test solution
Number of replicates (= test vessel): control group: 6, treated group: 3

Test conditions: temperature: 22.7 – 23.0 °C, lighting: continuous illumination (on average 67 µE m-2 s-1)

pH: 7.96 – 8.50

Treatments:	control (untreated test medium) test item (GLOB182F)
Test concentrations:	0.260, 0.728, 2.04, 5.71, 16.0 mg/L test item
Exposure time:	72 hours (static test procedure)
Biological observations:	biomass (number of cells): after 24, 48 and 72 hours
Statistics:	LOEC: Williams and Welch's t-test ($p \leq 0.05$, one-sided smaller) EC_x: 3-parameter sigmoidal (non-linear regression) and probit analysis ToxRat Professional Version 3.3.3 (RATTE, 2018))
Dates of work:	experimental start: 20.10.2020 experimental completion: 23.10.2020

The study was performed in compliance with the principles of GLP.

Results and discussions
Recoveries of Fludioxonil in 'fresh' and 'spent' test solutions were within 80 to 120 % of nominal concentrations (98.5 – 103.0 % of nominal in 'fresh' and 92.5 – 99.5 % of nominal in 'spent' test solutions).

Toxicity results are based on test item nominal concentration.

Table A3: Effects on growth rate and yield 72 hours after exposure start

effect concentration	**GLOB182F (**Błąd! Nie można odnaleźć źródła odwołania.**)**					
	average specific growth rate			**yield**		
	0 - 72 h after application					
LOEC test item nominal	5.71			2.04		
NOEC test item nominal	2.04			0.728		
EC test item nominal (CI)	**E_rC_{10}** 4.55 (4.08 – 4.91)	**E_rC_{20}** 5.45 (5.07 – 5.79)	**E_rC_{50}** 7.70 (7.18 – 8.46)	**E_yC_{10}** 1.69 (1.34 – 2.13)	**E_yC_{20}** 2.36 (1.99 – 2.81)	**E_yC_{50}** 4.46 (3.96 – 5.03)

CI – 95 % confidence intervals (upper – lower); calculations done using unrounded values

Table A4: Observations 72 hours after exposure start

test concentration **Błąd! Nie można odnaleźć źródła odwołania.** test item nominal	**% Inhibition**	
	average specific growth rate	**yield**
	0 - 72 h after application	
control	-	-
0.260	0.0	0.0
0.728	-0.8[1]	-3.0[1]
2.04	4.8	17.1
5.71	23.3	60.2
16.0	96.5	99.6

[1] negative values might indicate a higher growth relative to the untreated control

Conclusion
In an algae growth inhibition test, in which *Pseudokirchneriella subcapitata* was exposed to GLOB182F at nominal concentrations of 0.260, 0.728, 2.04, 5.71, 16.0 mg/L test item, an E_rC50 of 7.70 mg/L test item and an E_yC50 of 4.46 mg/L test item was determined. All validity criteria were met.

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

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

A 2.2.4 KCP 10.3 Effects on arthropods

A 2.2.5 KCP 10.3.1 Effects on bees

A 2.2.5.1 KCP 10.3.1.1 Acute toxicity to bees

Comments of izRMS:	The study was performed fully in line with OECD 213 and OECD 214 with a minor deviation. It was noted that the lowest recorded relative humidity during the test was 49 % which is slightly lower than the recommended minimum of 50 %. However, this deviation is considered to have no effect on the study since all validity criteria were met: • the average mortality for the total number of controls must not exceed 10 % at the end of the test (48h) (actually no oral or contact mortality observed), • the oral 24h LD_{50} of the toxic standard is in the range 0.10 – 0.35 µg a.s./bee (observed 0.110 µg a.s./bee), • the contact 24h LD_{50} of the toxic standard is in the range 0.10 – 0.30 µg a.s./bee (observed 0.148 µg a.s./bee). Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment: 48h oral LD_{50} > 1100 µg product/bee (corresponding to 104.9 µg a.s./bee) 48h contact LD_{50} > 1100 µg product/bee (corresponding to 104.9 µg a.s./bee)

Reference:	KCP 10.3.1.1
Report	Acute toxicity of GLOB182F to the honeybee *Apis mellifera* L. under laboratory conditions. Franke M., 2020, report No. 20 48 BAA 0079.
Guideline(s):	OECD 213 (1998), OECD 214 (1998)
Deviations:	Minor deviation (see table above for details) ~~No~~
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Executive summary

The purpose of this study was to determine the acute toxicity of GLOB182F to the honeybee *Apis mellifera* L. in a laboratory test after oral and contact exposure. The selected test design corresponds to the recommendations of the OECD Guidelines 213 and 214 (1998).
Data on the toxicity to *Apis mellifera* L. were generated to comply with international regulations.

Materials and methods

Test item: GLOB182F (Fludioxonil 100 FS); Batch No.: PE 2004.742

Content of active substance (a.s.):	nominal	analysed
Fludioxonil:	100 g/L	100 g/L

Reference item: Dimethoate 400 EC was tested parallel to test item (analysed content of 411.20 ± 3.47 g/L)

Test species: Honeybee – *Apis mellifera* L. subspecies Buckfast (Hymenoptera, Apoidea): worker bees of a healthy and queen-right colony; female, adult worker bees (forager bees) were collected in the morning before use; apiary: BioChem agrar GmbH, Kupferstr. 6, 04827 Machern OT Gerichshain, Germany

Guideline(s): OECD 213 (1998), OECD 214 (1998)

Test design: Test item:
Contact test: 48-h; 2 control groups of deionised water, 1 % v/v tween solution; 5 dose rates of test item; 4 dose rates of the reference item; comprising 3 replicates per dose rate each of 10 bees, application volume: 2 µL/bee
Oral test: 48-h; 1 control group of 50 % w/v sucrose solution; 5 dose rates of test item; 4 dose rates of the reference item; comprising 3 replicates per dose rate each of 10 bees; application volume: 200 µL/cage by group feeding of 10 bees (corresponding to 20 µL/bee)
The mortality and the behaviour were assessed 4, 24, 48 hours after application for the contact and oral test

Endpoints: Mortality, behavioural impairments

Dose rates [product/bee]

Test item:	
Contact test:	1100, 550, 275, 138, 68.8 µg product/bee
Oral test (offered):	1100, 550, 275, 138, 68.8 µg product/bee
Oral test (consumed):	1100, 550, 275, 138, 68.8 µg product/bee*

Dose rates [a.s./bee] based on analysed content of a.s.

Test item	
Contact test:	104.9, 52.4, 26.2, 13.1, 6.6 µg a.s./bee
Oral test (offered):	104.9, 52.4, 26.2, 13.1, 6.6 µg a.s./bee
Oral test (consumed):	104.9, 52.4, 26.2, 13.1, 6.6 µg a.s./bee*

* based on the actual food uptake

Test conditions:

Temperature:	23.9 – 25.2 °C (contact and oral)
Relative humidity:	49 - 64 % (contact and oral)
Illumination:	constant darkness throughout the test (diffuse artificial light only during handling and assessments)
Food:	50 % (w/v) sucrose solution (after application *ad libitum*)

Statistics: Statistical program used: ToxRat Professional 3.3.0 (2018)
Calculation of LD_{50} values:
Test item: Contact: no LD_{50}-calculation (due to no mortality)
Oral: no LD_{50}-calculation (due to no mortality)
Reference item: Contact: Probit analysis (linear maximum likelihood regression)
Oral: Probit analysis (linear maximum likelihood regression)
Statistical significance of mortality values:
Test item: Fisher's Exact Binomial Test with Bonferroni Correction ($\alpha = 0.05$)
Reference item: Fisher's Exact Binominal Test with Bonferroni Correction ($\alpha = 0.05$)

Validity criteria Control mortality (48 h): ≤ 10 %

LD_{50} – value of the reference (24 h): 0.10 – 0.30 µg a.s./bee (contact)

0.10 – 0.35 µg a.s./bee (oral)

Experimental phase: 10 – 12 September 2020

Results and discussions

Contact test

After 48 hours, the control groups either treated with deionised water or 1 % tween solution demonstrated no mortality. In the test item treatment group, no mortality was observed after thoracic application of up to 1100 µg GLOB182F/bee, after 48 hours. No effects on behaviour of honeybees were observed after thoracic application of up to 1100 µg GLOB182F/bee throughout the 48-hour contact test.

Oral test

After 48 hours, the control group fed 50 % sucrose solution demonstrated no mortality. In the test item treatment group, no mortality was observed after oral consumption of up to 1100 µg GLOB182F/bee, after 48 hours. No effects on behaviour of honeybees were observed after treatment with up to 1100 µg GLOB182F/bee throughout the 48-hour oral test.

Table A5. LD_{50}-values of the contact and oral toxicity test

Treatment	**LD_{50} values**	**Contact toxicity test**		**Oral toxicity test[1]**	
		24 h	48 h	24 h	48 h
Test item	**LD_{50} [µg product/bee]**	**> 1100**	**> 1100**	**> 1100**	**> 1100**
	LD_{50} [µg a.s./bee]*	**> 104.9**	**> 104.9**	**> 104.9**	**> 104.9**
Reference item	**LD_{50} [µg a.s./bee]*** (95 % confidence limits)[2]	**0.148** (0.133 – 0.162)	**0.138** (0.123 – 0.152)	**0.110** (0.095 – 0.123)	**0.106** (0.093 – 0.117)

[1] Oral dose rates based on actual consumed doses;
[2] Median lethal dose calculated by Probit analysis (with 95 % confidence limits)
* based on analysed content of a.s.

The contact and oral LD_{50} (24 h) of the reference item was calculated to be 0.148 µg a.s./bee and 0.110 µg a.s./bee, respectively. All validity criteria have been met.

Conclusion

The acute contact and oral toxicity of GLOB182F was tested on honeybees under laboratory conditions over 48 hours.

The contact LD_{50} (48 h) was > 1100 µg GLOB182F/bee that is corresponding to >104.9 µg a.s./bee. The oral LD_{50} (48 h) was > 1100 µg GLOB182F/bee that is corresponding to >104.9 µg a.s./bee.

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Comments of izRMS:	The study was performed fully in line with OECD 246 and OECD 247 with no deviations. All validity criteria were met: • mortality in the control should be ≤ 10 % at the end of the test (actually no mortality observed in both contact and oral toxicity tests), • mortality in the toxic reference substance group should be ≥ 50 % at the end of the test (actually 100 % mortality observed in both contact and oral toxicity tests). Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment: 48 h contact LD_{50} >2129.6 µg product/bumblebee (equivalent to >203.0 µg a.s./bumlebee) 48 h oral LD_{50} > 3688.3 µg product/bumblebee (equivalent to > 351.6 µg a.s./bumblebee)

Reference:	KCP 10.3.1.1
Report	Acute toxicity of Fludioxonil 100 FS to the bumblebee *Bombus terrestris* L. under laboratory conditions, Amsel K., 2020, report No. 20 48 BBA 0026.
Guideline(s):	OECD 246, OECD 247
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Contact Toxicity Test

Executive summary

In the contact toxicity test, young adult worker bumblebees (*Bombus terrestris* L.) were exposed to Fludioxonil 100 FS. The toxicity of the test item was determined at dose rates of 2129.6, 1064.8, 532.4, 266.2 and 133.1 µg product/bumblebee (equivalent to 203.0, 101.5, 50.8, 25.4 and 12.7 µg a.s./bumblebee).
Additionally, bumblebees were treated with Dimethoate EC 400 as reference item at a dose rate of 10.0 µg a.s./bumblebee and furthermore with deionised water and TritonX solution as controls.

After 48 hours of contact exposure, no mortality occurred in the control groups treated with deionised water and 0.5% (v/v) TritonX solution. In the test item treatment, no mortalities were observed at the dose rates of 2129.6, 1064.8, 532.4 and 133.1 µg product/bumblebee after 48 hours. Mortality of 3.3% occurred at the dose rate of 266.2 µg product/bumblebee, after 48 hours.

In the acute contact toxicity test with Fludioxonil 100 FS, the resulting LD_{50} after 48 hours was > 2129.6 µg product/bumblebee (equivalent to > 203.0 µg a.s./bumblebee) and the NOED was ≥ 2129.6 µg product/bumblebee (equivalent to ≥ 203.0 µg a.s./bumblebee).

Materials and methods

Test item:	Fludioxonil 100 FS (GLOB182F), Batch No.: PE 2004.742, Density: 1.0490 g/mL Content of active substance: Fludioxonil 100 g/L (nominal), 100.0 g/L (analysed)
Test species:	*Bombus terrestris* L. (bumblebee), adult worker bumblebees derived from queen right bumblebee hives; source: Biobest Belgium N.V., Ilse Velden 18, 2260 Westerlo, Belgium delivered: Katz Biotech AG, An der Birkenpfuhlheide 10, 15837 Baruth, Germany; collected from 8 bumblebee hives under red light in the evening prior to testing.
Test design:	In a 48 hours test, adults of *Bombus terrestris* were exposed to 5 dose rates of Fludioxonil 100 FS in an appropriate carrier (0.5% (v/v) TritonX) placed on the dorsal bumblebee thorax. In total, 3 treatment groups were set up: 2 control groups, 5 dose rates of the test item and 1 dose rate of the reference item with 30 replicates per dose and one bumblebee per replicate, respectively. Assessments of bumblebee mortality and behavioural effects were done after 4, 24 and 48 hours.
Endpoints:	Mortality, behavioural abnormalities
Reference item:	Dimethoate EC 400 (analysed content of dimethoate: 411.20 g/L)
Treatments:	Water control (deionised water) TritonX control (0.5% (v/v) TritonX solution) Test item at dose rates of:

2129.6, 1064.8, 532.4, 266.2 and 133.1 µg product/bumblebee
(equivalent to 203.0, 101.5, 50.8, 25.4 and 12.7 µg a.s./bumblebee)

Reference item at a dose rate of:
10.0 µg dimethoate/bumblebee

Test conditions: Temperature: 23.5 °C – 25.0 °C; relative humidity: 52% - 69%
Photoperiod: 24 h darkness
Food: 50% (w/v) sucrose solution

Statistics: Fisher's Exact Binomial test with Bonferroni Correction for mortality data (one-sided greater, $\alpha = 0.05$)

Results and discussions

After 48 hours of contact exposure, no mortality occurred in the control groups treated with deionised water and 0.5% (v/v) TritonX solution. In the test item treatment, no mortalities were observed at the dose rates of 2129.6, 1064.8, 532.4 and 133.1 µg product/bumblebee after 48 hours. Mortality of 3.3% occured at the dose rate of 266.2 µg product/bumblebee, after 48 hours.
No behavioural effects appeared at all tested dose rates of Fludioxonil 100 FS during the contact toxicity test.

For the contact toxicity test solutions, the recoveries of fludioxonil were between 84.4 – 111%. No active substance was detected in the control sample.

The results of the contact test are summarised in the following tables.

Table A6: Contact toxicity of Fludioxonil 100 FS to *Bombus terrestris*

Treatment group [dosage unit]	**Dosage** applied	**Mean mortality [%]**	
		24 h	**48 h**
Control	Water	0.0	0.0
	0.5% (v/v) TritonX	0.0	0.0
Fludioxonil 100 FS [µg product/bumblebee]	2129.6	0.0	0.0
	1064.8	0.0	0.0
	532.4	0.0	0.0
	266.2	0.0	3.3
	133.1	0.0	0.0

Calculations are performed with non-rounded values

Mortality in the reference item treatment in the contact test was 100.0% after 48 hours.

Table A7: Contact toxicity of Fludioxonil 100 FS to *Bombus terrestris*, LD_{50} / NOED values

	Endpoint	24 h	48 h
Fludioxonil 100 FS	LD_{50} [µg product/bumblebee] LD_{50} [µg a.s./bumblebee]	**> 2129.6** **> 203.0**	**> 2129.6** **> 203.0**
	NOED [µg product/bumblebee] NOED [µg a.s./bumblebee]	**≥ 2129.6** **≥ 203.0**	**≥ 2129.6** **≥ 203.0**

Conclusion

In the acute contact toxicity test with Fludioxonil 100 FS, the resulting LD_{50} after 48 hours was > 2129.6 µg product/bumblebee (equivalent to > 203.0 µg a.s./bumblebee) and the NOED was ≥ 2129.6 µg product/bumblebee (equivalent to ≥ 203.0 µg a.s./bumblebee).

Oral toxicity Test

Executive summary

In the oral toxicity test, young adult worker bumblebees (*Bombus terrestris* L.) were exposed to Fludioxonil 100 FS. The toxicity of the test item was determined at dose rates of 4259.2, 2840.0, 1893.7, 1262.7 and 842.0 µg product/bumblebee (equivalent to 406.0, 270.7, 180.5, 120.4 and 80.3 µg a.s./bumblebee). The resulting oral uptake was 3688.3, 2602.4, 1755.4, 1213.5 and 806.2 µg product/bumblebee (equivalent to 351.6, 248.1, 167.3, 115.7 and 76.9 µg a.s./bumblebee).
Additionally, bumblebees were treated with Dimethoate EC 400 as reference item at a dose rate of 1.43 µg consumed dimethoate/bumblebee and furthermore with a 50% (w/v) sucrose solution as control.

In the oral toxicity test, no mortality occurred in the control group fed with 50% (w/v) sucrose solution. In the test item treatment, no mortality occurred in the dose rates of 3688.3, 2602.4, 1755.4, 1213.5 and 806.2 µg consumed product/bumblebee after 48 hours.

In the acute oral toxicity test with Fludioxonil 100 FS, the resulting LD_{50} after 48 hours was > 3688.3 µg consumed product/bumblebee (> 351.6 µg consumed a.s./bumblebee) and the NOED after 48 hours was ≥ 3688.3 µg consumed product/bumblebee (≥ 351.6 µg consumed a.s./ bumblebee) after 48 hours.

Materials and methods

Test item:	Fludioxonil 100 FS (GLOB182F), Batch No.: PE 2004.742, Density: 1.0490 g/mL Content of active substance: Fludioxonil 100 g/L (nominal), 100.0 g/L (analysed)
Test species:	*Bombus terrestris* L. (bumblebee), adult worker bumblebees derived from queen right bumblebee hives; source: Biobest Belgium N.V., Ilse Velden 18, 2260 Westerlo, Belgium delivered: Katz Biotech AG, An der Birkenpfuhlheide 10, 15837 Baruth, Germany; collected from 8 bumblebee hives under red light in the evening prior to testing with a starvation period of 4 hours before test begin.
Test design:	In a 48 hours test, adults of *Bombus terrestris* L. were exposed to 5 dose rates of Fludioxonil 100 FS in treated food (50% (w/v) sucrose solution). In total, 3 treatment groups were set up: 1 control group, 5 dose rates of the test item and 1 dose rate of the reference item with 30 replicates per dose and one bumblebee per replicate, respectively. Assessments of bumblebee mortality and behavioural effects were done after 4, 24 and 48 hours.
Endpoints:	Mortality, behavioural abnormalities
Reference item:	Dimethoate EC 400 (analysed content of dimethoate: 411.20 g/L)
Treatments:	Sucrose control (50% (w/v) sucrose solution) Test item at dose rates of: 4259.2, 2840.0, 1893.7, 1262.7 and 842.0 µg product/bumblebee (equivalent to 406.0, 270.7, 180.5, 120.4 and 80.3 µg a.s./bumblebee) actual uptake: 3688.3, 2602.4, 1755.4, 1213.5 and 806.2 µg product/bumblebee (equivalent to 351.6, 248.1, 167.3, 115.7 and 76.9 µg a.s./bumblebee) Reference item at a dose rate of: 1.51 µg dimethoate/bumblebee (actual uptake: 1.43 µg dimethoate/bumblebee)

Test conditions:	Temperature:	23.5 °C – 25.0 °C; relative humidity: 52% - 69%
	Photoperiod:	24 h darkness
	Food:	50% (w/v) sucrose solution

Results and discussions
In the oral toxicity test, no mortality occurred in the control group fed with 50% (w/v) sucrose solution. In the test item treatment, no mortality occurred in the dose rates of 3688.3, 2602.4, 1755.4, 1213.5 and 806.2 µg consumed product/bumblebee after 48 hours.
Bumblebees in the highest dose of 3688.3 µg consumed product/bumblebee were affected. No behavioural effects appeared at the other tested dose rates of Fludioxonil 100 FS during the oral toxicity test.

For the oral toxicity test solutions, the recoveries of fludioxonil were between 90.8 - 99.3%. No active substance was detected in the control sample.

The results of the oral test are summarised in the following Tables.

Table A8: Oral toxicity of Fludioxonil 100 FS to Bombus terrestris

Treatment group [dosage unit]	**Dosage** consumed	**Mean mortality [%]**	
		24 hours	**48 hours**
Control	Sucrose solution	0.0	0.0
Fludioxonil 100 FS [µg product/ bumblebee]	3688.3	0.0	0.0
	2602.4	0.0	0.0
	1755.4	0.0	0.0
	1213.5	0.0	0.0
	806.2	0.0	0.0

Mortality in the reference item treatment in the oral test was 100.0% after 48 hours.

Table A9: Oral toxicity of Fludioxonil 100 FS to Bombus terrestris, LD50 / NOED values

	Endpoint[1]	24 h	48 h
Fludioxonil 100 FS	LD_{50} [µg product/bumblebee] LD_{50} [µg a.s./bumblebee]	**> 3688.3** **> 351.6**	**> 3688.3** **> 351.6**
	NOED [µg product/bumblebee] NOED [µg a.s./bumblebee]	**≥ 3688.3** **≥ 351.6**	**≥ 3688.3** **≥ 351.6**

[1] based on consumed values

Conclusion
In the acute oral toxicity test with Fludioxonil 100 FS, the resulting LD_{50} after 48 hours was > 3688.3 µg consumed product/bumblebee (> 351.6 µg consumed a.s./bumblebee) and the NOED after 48 hours was ≥ 3688.3 µg consumed product/bumblebee (≥ 351.6 µg consumed a.s./ bumblebee).

A 2.2.5.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

See A 2.3.1.1.

A 2.2.5.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

See A 2.3.1.1.

A 2.2.5.2 KCP 10.3.1.2. Chronic toxicity to bees

Comments of izRMS:	The study was performed in line with OECD 245 with no deviations. All validity criteria were met: • the average mortality across replicates for the untreated control groups was ≤ 15 % at the end of the test (observed 0 % mortality in both control groups), • the average mortality in the reference substance treated group was ≥ 50 % at the end of the test (observed 66.7 %). Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment: LDD_{50} = 732 µg product/bee/day (corresponding to 69.8 µg a.s./bee/day) NOEDD = 220 µg product/bee/day (corresponding to 21.0 µg a.s./bee/day) NOEC = 6.777 g product/kg food (corresponding to 0.646 g a.s./kg food)

Reference:	KCP 10.3.1.2
Report	Chronic toxicity of GLOB182F to the honey bee *Apis mellifera* L. Under laboratory conditions. Dreßler K., 2020, report No. 20 48 BAC 0051.
Guideline(s):	OECD TG 245 (2017)
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Executive summary

In a 10-day chronic toxicity feeding test, max. 2 days old worker honey bees (*Apis mellifera* L. subspecies Buckfast) were exposed to a daily application of GLOB182F diluted in the bee food (50% (w/v) aqueous sucrose solution + 0.1% (w/v) xanthan). The chronic oral toxicity of the test item was determined at nominal doses of 2129, 1065, 532, 266 and 133 µg product/bee/day (equivalent to 203, 101, 50.7, 25.4 and 12.7 µg a.i./bee/day). The corresponding test item concentrations in the feeding solutions were 54.218, 27.109, 13.555, 6.777 and 3.390 g product/kg food (equivalent to 5.169, 2.584, 1.292, 0.646 and 0.323 g a.i./kg food). Taking into account the actual food uptake and evaporated amount of feeding solution, the bees effectively consumed doses of 817, 535, 376, 220 and 140 µg product/bee/day (equivalent to 77.9, 51.0, 35.9, 21.0 and 13.3 µg a.i./bee/day).

An additional group of honey bees was exposed to a daily application of dimethoate diluted in the bee food (50% (w/v) aqueous sucrose solution) as a reference item at a nominal dose of 27.3 ng a.i./bee/day.

Untreated 50% (w/v) aqueous sucrose solution served as blank control. Untreated 50% (w/v) aqueous sucrose solution + 0.1% (w/v) xanthan served as viscosifier control.

Materials and methods

Test item: GLOB182F, Batch: PE 2004.742
Content of a.i.:
Fludioxonil: 100 g/L (nominal); 100.0 g/L (analysed)
Density: 1.0490 g/mL

Reference item: Danadim® Progress
Content of a.i.:

Dimethoate: 400 g/L (nominal); 411.20 g/L (analysed)
Density: 1.069 g/mL

Test species: *Apis mellifera* L. subspecies Buckfast (honey bee), max. 2 days old bees; derived from healthy and queen-right colonies; source:
BioChem agrar GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany

Guideline: OECD TG 245 (2017)
Deviations: none

Test design: In a 10-day chronic toxicity feeding test, young adults of *Apis mellifera* L. (max. 2 days old) were continuously exposed to GLOB182F diluted in the bee food (50% (w/v) aqueous sucrose solution + 0.1% (w/v) xanthan). The following treatment groups were set up: 5 doses of the test item, 1 untreated control group AC fed with 50% (w/v) aqueous sucrose solution, 1 untreated control group BC fed with 50% (w/v) aqueous sucrose solution + 0.1% (w/v) xanthan and 1 dose of the reference item. For each treatment group, 3 replicates per dose and 10 bees per replicate were used. All feeding solutions were freshly prepared every day and provided *ad libitum*. Assessments of bee mortality, food consumption and behavioural abnormalities were conducted daily. The food consumption per cage was corrected for the evaporation of the test solutions. In the analytical phase of the study, the concentration of the active ingredient fludioxonil in the highest and lowest test item feeding solution applied on each day of application was determined.

Endpoints: Mortality, behavioural abnormalities

Test concentrations: Control group AC: untreated food (50% (w/v) aqueous sucrose solution)
Control group BC: untreated food (50% (w/v) aqueous sucrose solution + 0.1% (w/v) xanthan)

Test item group: treated food at nominal doses of
2129, 1065, 532, 266 and 133 µg product/bee/day
(equivalent to 203, 101, 50.7, 25.4 and 12.7 µg a.i./bee/day*)
corresponding to concentrations of
54.218, 27.109, 13.555, 6.777 and 3.390 g product/kg food
(equivalent to 5.169, 2.584, 1.292, 0.646 and 0.323 g a.i./kg food*)
Effectively consumed doses:
817, 535, 376, 220 and 140 µg product/bee/day
(equivalent to 77.9, 51.0, 35.9, 21.0 and 13.3 µg a.i./bee/day*)
*based on the nominal content of active ingredient
Reference item group: treated food at a nominal dose of
27.3 ng dimethoate/bee/day
(corresponding to a concentration of 0.694 mg dimethoate/kg food)

Test conditions:

Temperature:	32.0 – 33.3 °C
Relative humidity:	56.1 – 64.0%
Photoperiod:	darkness (diffuse artificial light of about 100 lx only during assessments)
Food:	50% (w/v) aqueous sucrose solution

Statistics: Statistical software used: ToxRat Professional 3.3.0 (2018).
Step-down Cochran-Armitage Test Procedure for mortality data and determination of NOEDD/NOEC (one-sided greater, $\alpha = 0.05$). Probit analysis using linear maximum likelihood regression for the determination of LDD_X and

LC_X values along with their 95% confidence limits.

Dates of work:	Experimental starting date (biological phase):	14 Jul 2020
	Experimental completion date (biological phase):	24 Jul 2020
	Experimental starting date (analytical phase):	11 Sep 2020
	Experimental completion date (analytical phase):	12 Sep 2020

Results and discussions

After 10 days of continuous exposure, a mortality of 0.0% was observed in both control groups. Taking into account the actual food uptake and evaporated amount of feeding solution, the bees effectively consumed doses of 817, 535, 376, 220 and 140 µg product/bee/day (equivalent to 77.9, 51.0, 35.9, 21.0 and 13.3 µg a.i./bee/day) which resulted in mortalities of 56.7, 26.7, 16.7, 0.0 and 0.0% after 10 days, respectively. Mortalities in the three highest test item doses (817, 535 and 376 µg consumed product/bee/day) were statistically significantly increased compared to the viscosifier control group BC (Step-down Cochran-Armitage Test Procedure, $\alpha = 0.05$, one-sided greater).

The 10-day LDD_{50} was determined to be 732 µg consumed product/bee/day (equivalent to 69.8 µg consumed a.i./bee/day) and the 10-day LC_{50} to be 45.259 g product/kg food (equivalent to 4.314 g a.i./ kg food), respectively.
The 10-day LDD_{20} was determined to be 458 µg consumed product/bee/day (equivalent to 43.7 µg consumed a.i./bee/day) and the 10-day LC_{20} to be 20.353 g product/kg food (equivalent to 1.940 g a.i./ kg food), respectively.
The 10-day LDD_{10} was determined to be 359 µg consumed product/bee/day (equivalent to 34.2 µg consumed a.i./bee/day) and the 10-day LC_{10} to be 13.403 g product/kg food (equivalent to 1.278 g a.i./ kg food), respectively.
The NOEDD was determined to be 220 µg consumed product/bee/day (equivalent to 21.0 µg consumed a.i./bee/day) and the NOEC to be 6.777 g product/kg food (equivalent to 0.646 g a.i./kg food), respectively.

The recovery rates of the active ingredient fludioxonil in samples of the test item feeding solutions were between ± 20% of the nominal concentrations. The recoveries for fludioxonil were between 92.8% and 102% in the feeding solutions. Therefore, the concentrations of active ingredient in the test item feeding solutions were verified and endpoints have been based on nominal concentrations. No residues of the active ingredient fludioxonil were found in the control samples.

Behavioural abnormalities were observed in the second and third highest test item treatment groups BT and CT (535 and 376 µg consumed product/bee/day). One bee out of 30 remaining bees was observed as being affected (uncoordinated movements) in test item treatment group BT on day 4. One bee out of 27 remaining bees was observed as being moribund in test item treatment group CT on day 9. No other behavioural abnormalities were observed in any test item treatment group on any other assessment day.

In the test item group, the overall mean daily food consumption ranged between 15.1 and 41.3 mg feeding solution/bee/day which is 38.4% to 105.1% of the expected daily amount. In control group AC, the bees consumed on average 38.0 mg feeding solution/bee/day (=96.8% of the expected daily amount). In viscosifier control group BC, the bees consumed on average 38.6 mg feeding solution/bee/day (=98.4% of the expected daily amount).

The mean of the daily observed evaporation of control feeding solution AC ranged between 40.7 and 53.0 mg per cage. The mean of the daily observed evaporation of viscosifier control feeding solution BC ranged between 38.7 and 51.3 mg per cage. The food consumption per cage was corrected by subtracting the respective mean evaporation figure of the respective day of application.

The effective reference dosage in the study was 15.1 ng dimethoate/bee/day which resulted in a mortality of 66.7%.

All validity criteria for the study were met. Mortality in both control groups was < 15% and mortality in the reference item group was > 50% after 10 days.

Results are summarised in table below.

Table A10: Mean mortality, behaviour of bees and toxicity of GLOB182F after 10 days

								After 10 days		
Treatment group	**Treatment group ID**	**Daily dose**		**Daily dose**		**Concentration**		**Mean mortality**		**Number of bees showing behavioural abnormalities[2]**
		nominal	**consumed[1]**	**nominal**	**consumed[1]**			**absolute [%]**	**corrected [%]**	
		[µg product/ bee/day]		**[µg a.i./ bee/day]**		**[g product/ kg food]**	**[g a.i./ kg food]**			
Blank control	AC	--	--	--	--	--	--	0.0	--	0 out of 30
Viscosifier control	BC	--	--	--	--	--	--	0.0	--	0 out of 30
Test item	AT	2129	817	203	77.9	54.218	5.169	56.7*	--	0 out of 13
	BT	1065	535	101	51.0	27.109	2.584	26.7*	--	0 out of 22
	CT	532	376	50.7	35.9	13.555	1.292	16.7*	--	0 out of 25
	DT	266	220	25.4	21.0	6.777	0.646	0.0	--	0 out of 30
	ET	133	140	12.7	13.3	3.390	0.323	0.0	--	0 out of 30
		[ng product/ bee/day]		**[ng a.i./ bee/day]**		**[mg product/ kg food]**	**[mg a.i./ kg food]**			
Reference item	AR	70.9	39.3	27.3	15.1	1.805	0.694	66.7	--	0 out of 10

	Endpoints	**After 10 days**
Test item doses	LDD_{50} [µg consumed product/bee/day[1]][3]	732 (622 – 957)
	LDD_{20} [µg consumed product/bee/day[1]][3]	458 (370 – 534)
	LDD_{10} [µg consumed product/bee/day[1]][3]	359 (259 – 429)
	LDD_{50} [µg consumed a.i./bee/day[1]][3]	69.8 (59.3 – 91.2)
	LDD_{20} [µg consumed a.i./bee/day[1]][3]	43.7 (35.3 – 50.9)
	LDD_{10} [µg consumed a.i./bee/day[1]][3]	34.2 (24.7 – 40.9)
	NOEDD [µg consumed product/bee/day[1]][4]	220
	NOEDD [µg consumed a.i./bee/day[1]][4]	21.0
Test item concentrations	LC_{50} [g product/kg food][3]	45.259 (34.289 – 70.868)
	LC_{20} [g product/kg food][3]	20.353 (14.458 – 26.397)
	LC_{10} [g product/kg food][3]	13.403 (8.051 – 18.014)
	LC_{50} [g a.i./kg food][3]	4.314 (3.269 – 6.756)
	LC_{20} [g a.i./kg food][3]	1.940 (1.378 – 2.516)
	LC_{10} [g a.i./kg food][3]	1.278 (0.767 – 1.717)
	NOEC [g product/kg food][4]	6.777
	NOEC [g a.i./kg food][4]	0.646

Results are averages based on 3 replicates, containing 10 bees each; Calculations are performed with non-rounded values.
corrected: corrected mortality (according to SCHNEIDER-ORELLI 1947); Due to 0% mortality in both control groups, no correction is needed.

* Statistically significant difference in pairwise comparison between treatment and untreated viscosifier control group BC (Step-down Cochran-Armitage Test Procedure; $\alpha = 0.05$; one-sided greater)

[1] Taking into account the actual food uptake and evaporation

[2] Number of bees showing behavioural abnormalities referring to the number of remaining bees

[3] Lethal dietary doses/concentrations (95%-cl lower – upper) were calculated using Probit analysis (linear max. likelihood regression)

[4] No observed effect dietary doses/concentrations were calculated using Step-down Cochran-Armitage Test Procedure ($\alpha = 0.05$; one-sided greater)

Conclusion
The chronic oral toxicity of GLOB182F to young adult honey bees (*Apis mellifera* L.) was investigated in a 10-day chronic, dose-response feeding study under laboratory conditions.

The 10-day LDD_{50} was determined to be 732 µg consumed product/bee/day (equivalent to 69.8 µg consumed a.i./bee/day) and the 10-day LC_{50} to be 45.259 g product/kg food (equivalent to 4.314 g a.i./kg food), respectively.
The 10-day LDD_{20} was determined to be 458 µg consumed product/bee/day (equivalent to 43.7 µg consumed a.i./bee/day) and the 10-day LC_{20} to be 20.353 g product/kg food (equivalent to 1.940 g a.i./kg food), respectively.
The 10-day LDD_{10} was determined to be 359 µg consumed product/bee/day (equivalent to 34.2 µg consumed a.i./bee/day) and the 10-day LC_{10} to be 13.403 g product/kg food (equivalent to 1.278 g a.i./kg food), respectively.
The NOEDD was determined to be 220 µg consumed product/bee/day (equivalent to 21.0 µg consumed a.i./bee/day) and the NOEC to be 6.777 g product/kg food (equivalent to 0.646 g a.i./kg food), respectively.

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Comments of izRMS:	The study was performed in line with OECD 239 with a minor deviation. It was noted that the relative humidity between D8 and D15 was above the recommended 80 ± 5 % (observed 79.2 – 92.6%) because of a malfunction of the climate chamber. However, this deviation is considered to have no impact on the study outcome as no effects occurred in the untreated control and all validity criteria were met. Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment: ED_{50} = 207.1 µg product/larva NOED = 40.3 µg product/larva (corresponding to 3.8 µg a.s./larvae) EC_{50} = 1309.0 mg product/kg food NOEC = 254.9 mg product/kg food (corresponding to 24.3 mg a.s./kg food)

Reference:	KCP 10.3.1.2
Report	GLOB182F - Repeated exposure of honey bee (Apis mellifera L.) larvae under laboratory conditions, Schmidt K., 2021, report No. 20 48 BLC 0050.
Guideline(s):	OECD 239 (2016)
Deviations:	Minor deviation (see table above for details)~~No~~
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Executive summary
In a test under laboratory conditions, honey bee larvae (*Apis mellifera* L.) were repeatedly exposed to GLOB182F. The toxicity of the test item was determined at cumulative doses of 630.0, 252.0, 100.8, 40.3 and 16.1 µg product/larva (corresponding to 60.1, 24.0, 9.6, 3.8 and 1.5 µg a.i./larva). The concentrations of test item in the diets were 3982.6, 1593.0, 637.2, 254.9 and 102.0 mg product/kg food (corresponding to 379.7, 151.9, 60.7, 24.3 and 9.7 mg a.i./kg food). Additionally, honey bee larvae were treated with Dimethoate tech. as reference item at a total dose of 7.6 µg a.i./larva or with an untreated diet as control.

Materials and methods

Test item:	GLOB182F, Batch: PE 2004.742 Content of a.i.: Fludioxonil: 100 g/L (nominal); 100.0 g/L (analysed) Density: 1.0490 g/mL
Reference item:	Dimethoate tech. (analysed purity: 98.8% ± 0.5%)
Test species:	Honey bee – *Apis mellifera* L., ssp: *Buckfast* (Hymenoptera, Apoidea): First instar larvae (L1 during grafting) of queen-right colonies in good health conditions are used for the test. For each test, larvae were collected from at least three different colonies, each representing a replicate, to ensure the results are representative. source: BioChem agrar GmbH, Machern OT Gerichshain, Germany.
Guideline:	OECD TG 239 (2016) Deviations: none
Test design:	One day old honey bee larvae (D1) of *Apis mellifera* L., ssp: *Buckfast* were transferred from brood combs to polystyrene grafting cells in 48-well cell culture plates 2 days before start of the treatment. On 4 successive days (D3 to D6) the larvae were repeatedly exposed to GLOB182F diluted in the larval food (aqueous sugar solution mixed with royal jelly). After the applications, no additional feedings of the larvae took place. In total, 7 treatment groups were set up: 5 doses of the test item, one untreated control group and 1 dose of the reference item with 3 replicates per dose and 12 larvae per replicate, each. Assessments of cumulative larval mortality were performed on D4, D5, D6, D7 and D8. Additionally, other observations such as small body size or large quantities of remaining food on D8 were noted. Pupal mortality was assessed on D15 and emergence of adults was evaluated on D22. In an analytical phase of the study the concentration of the active ingredient in the test item stock solutions and in the control will be determined.
Endpoints:	Successful adult emergence, mortality, qualitative observations: *e.g.* body size, remaining food
Test concentrations:	Controls: AC untreated diet B/C (aqueous sugar solution + royal jelly) Test item: AT treated diet B/C at a concentration of 3982.6 mg product/kg food BT treated diet B/C at a concentration of 1593.0 mg product/kg food CT treated diet B/C at a concentration of 637.2 mg product/kg food DT treated diet B/C at a concentration of 254.9 mg product/kg food ET treated diet B/C at a concentration of 102.0 mg product/kg food Reference: AR treated diet B/C at a concentration of 48 mg a.i./kg food
Test conditions:	Temperature: 34.0 – 34.8 °C Relative humidity: D1 - D8: 90.6 – 99.9% D8-D15: 79.2 – 92.6% D15-D22: 63.7 – 72.4% Photoperiod: Darkness (except during assessments) Food: aqueous sugar solution with royal jelly
Statistics:	Descriptive statistics, Step-down Cochran-Armitage Test (one-sided greater, alpha = 0.05) for determination of NOED/NOEC, Trimmed Spearman-Karber procedure for determination of ED/EC_{50} values; The dataset does not allow for calculation of reliable $ED_{10/20}$ and $EC_{10/20}$.

Dates of work:	Experimental starting date:	24 Aug 2020
	Experimental completion date (biological phase):	14 Sep 2020
	Experimental completion date (analytical phase):	12 Dec 2020

Results and discussions
On D8, a larval mortality of 0.0% was observed in the control (AC). Pupal mortality (between D8 and D15) was 13.9% in the control. The control group showed a total mortality of 22.2% on D22. In the test item treated groups, larval mortalities ranged between 0.0 and 88.9% on D8. Pupal mortalities (D8-D15) ranged between 11.1 and 83.3% in the test item treatment groups. Total mortalities ranged between 25.0 and 97.2% on D22. Mortality in the reference item treated group (AR) was above 50% across all replicates on D8, being 100.0%.

On D5 and D6, larvae treated with 630.0 µg product/larvae were observed to have a small body size. ~~On D7, one larva treated with 100.8 µg product/larva were observed to have food left.~~ On D8, three of the remaining larvae treated with 630.0 µg product/larvae and one larva treated with 100.8 µg product/larva were observed to have food left.

In the final assessment on D22, an adult emergence rate of 77.8% was determined for the honey bees in the control group (AC). In the test item treated groups, the adult honey bees emerged at rates ranging between 2.8% and 75.0% following an application of 630.0, 252.0, 100.8, 40.3 and 16.1 µg product/larva, respectively, during the larval stages. On D22, larvae treated with 630.0, 252.0 and 100.8 µg product/larva showed emergence rates, which were statistically significantly decreased if compared to the control.

Based on the observed emergence rates the ED_{50} was determined to be 207.1 µg product/larva, which is equivalent to an EC_{50} of 1309.0 mg product/kg food. The respective NOED was 40.3 µg product/larva and the corresponding NOEC was 254.9 mg product/kg food.

The recoveries of active ingredient in the test item stock solutions A and E ranged between 91.7% and 101%. No test item was detected in the control specimen.

Because control mortality was ≤ 15% on D8 (observed 0 %), cumulative mortality in the reference item treatment group was ≥ 50% on D8 (observed 100 %) and adult emergence in the control was ≥ 70% on D22 (observed 77.8 %), the study can be regarded as valid.

Results are summarised in table below.

Table A10: Toxicity of GLOB182F to larvae of *Apis mellifera* L. after repeated exposure

Treatment group	Treatment ID	Dose	Concentration	On D8			On D15		On D22		
				Larval mortality D3 to D8		Mean OO	Pupal mortality D8-D15		Total mortality D3-D22		Adult emergence rate
				[%]		[%]	[%]		[%]		[%]
		[µg product/ larva]	[mg product/ kg food]	abs.	corr.		abs.	corr.	abs.	corr.	abs.
Control	AC	-	-	0.0	-	0.0	13.9	0.0	22.2	0.0	77.8
Test item	AT	630.0	3982.6	88.9	-	83.3	83.3	80.6	97.2	96.4	2.8*
	BT	252.0	1593.0	5.6	-	0.0	32.1	21.1	52.8	39.3	47.2*
	CT	100.8	637.2	2.8	-	3.0	20.5	7.6	44.4	28.6	55.6*
	DT	40.3	254.9	0.0	-	0.0	22.2	9.7	25.0	3.6	75.0
	ET	16.1	102.0	0.0	-	0.0	11.1	0.0	25.0	3.6	75.0
Reference item	AR	[µg a.i./ larva]	[mg a.i./ kg food]								
		7.6	48	100.0	-	-	-	-	100.0	100.0	0.0

Treatment	Endpoint: Successful adult emergence	Up to D22
Test item doses	ED_{50} [µg product/larva] [2]	207.1 (165.3 – 259.5)
	NOED [µg product/larva] [1]	40.3
Test item concentrations	EC_{50} [mg product/kg food] [2]	1309.0 (1044.6 – 1640.4)
	NOEC [mg product/kg food] [1]	254.9

Results are averages based on 3 replicates, containing 12 larvae each; see Appendix 4 for details
corr.: corrected mortality (according to SCHNEIDER-ORELLI 1947): test and reference item treated goups were corrected by AC; negative values were set to "0"; calculations were performed with non-rounded values; CL: confidence limit; abs.: absolute mortality as counted from the results; OO: Other observations (*e.g.* remaining food);
* Statistically significant if compared to the control (Step-down Cochran-Armitage Test)
Average% of pupal mortality: Sum of dead larvae between D8 and D15 / Sum of living larvae on D8 x 100% (replicate wise)
[1] Step-down Cochran-Armitage Test; alpha=0.05; one sided greater
[2] Trimmed Spearman-Karber procedure

Conclusion

In a test under laboratory conditions, honey bee larvae (*Apis mellifera* L.) were repeatedly exposed to GLOB182F.

All validity criteria were met (cumulative mortality in control and reference treatment groups and adult emergence in the control).

In a repeated exposure larval toxicity study with GLOB182F, the ED_{50} (adult emergence up to D22) was determined to be 207.1 µg product/larva, which is equivalent to an EC_{50} of 1309.0 mg product/kg food. The dataset does not allow for calculation of reliable $ED_{10/20}$ and $EC_{10/20}$.

The NOED was 40.3 µg product/larva and the corresponding NOEC was 254.9 mg product/kg food.

A 2.2.5.3 KCP 10.3.1.3 Effects on honey bee development and other honey bee life stages

A 2.2.5.4 KCP 10.3.1.4 Sub-lethal effects

A 2.2.5.5 KCP 10.3.1.5 Cage and tunnel tests

A 2.2.5.6 KCP 10.3.1.6 Field tests with honeybees

A 2.2.6 KCP 10.3.2 Effects on arthropods other than bees

Comments of izRMS:	The study was performed in line with the respective guideline with a minor deviation. It was noted that the food source provided during the study was 25 % w/w aqueous fructose solution while the recommended food in the respective guideline is a 1:3 v/v solution of honey and water. However, this deviation is considered to have no impact on the outcome of the study since all validity criteria were met: • mortality in the control was ≤ 13 % after 48 h (observed 2.5 %), • the corrected mortality in the reference item was ≥ 50% after 48 h (observed 100 %), • mean reproduction per female in the control was ≥ 5 mummies per female (observed 19.7), • number of surviving wasps in the control producing zero values for reproduction was ≤2 (observed 2). Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment: LR_{50} > 1.523 L product/ha ER_{50} > 1.523 L product/ha

Reference:	KCP 10.3.2
Report	Effects of GLOB182F on the parasitic wasp *Aphidius rhopalosiphi* (DESTEFANI-PEREZ) in a laboratory test, Röhlig U., 2020, 20 48 NAL 0009.
Guideline(s):	IOBC (Mead-Briggs *et al.* 2000)
Deviations:	Minor deviation (see the table above for details)~~No~~
GLP:	Yes
Acceptability:	Acceptable

Executive Summary

In a worst-case laboratory study, adults of *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) were exposed to GLOB182F sprayed on glass plates. Endpoint of the study was the mortality after 48 hours of exposure, including determination of the LR_{50}. The condition of the wasps was assessed 2, 24 and 48 hours after treatment. Effects on reproduction were then assessed by the number of parasitised aphids (mummies) produced per female.

The study encompassed 7 treatment groups (5 test item rates, control, reference item), each with 4 replicates. Seven females and 3 males per replicate were exposed to GLOB182F sprayed on glass plates at application rates of 0.0952 – 0.190 – 0.381 – 0.761 – 1.523 L product/ha. Additional test units were treated with deionised water for the water control and with DANADIM PROGRESS (active substance 411.2 g Dimethoate/L) as the reference item. The main endpoint of the study was mortality (including estimation of the LR_{50} and NOER) and, in addition, effects on reproduction were assessed (including estimation of the ER_{50} and NOER).

After 48 hours, in the water-treated control a mortality of 2.5 % was observed. In the test item treatments, mortality ranged between 0 % and 5.0 %. This resulted in corrected mortality rates of -2.6 % and 2.6 %. No statistically significant effects on mortality were determined at all test item rates (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$). The LR_{50} was estimated to be > 1.523 L product/ha. The NOER (no observed effect rate) for mortality was determined to be ≥ 1.523 L product/ha.

The mean number of mummies per female per day in the test item treatment groups ranged between 19.2 and 22.1, compared to the control with 19.7 mummies/female. No statistically significant effects on reproductive capacity were determined at all test item treatment groups (Williams-t-test, $\alpha = 0.05$). The ER_{50} was estimated to be > 1.523 L product/ha. The NOER (no observed effect rate) for reproduction was determined to be ≥ 1.523 L product/ha.

In a worst-case laboratory study with GLOB182F, the LR_{50} for *Aphidius rhopalosiphi* was estimated to be > 1.523 L product/ha. The NOER (no observed effect rate) for mortality was determined to be ≥ 1.523 L product/ha.
The ER_{50} was estimated to be > 1.523 L product/ha. The NOER (no observed effect rate) for reproduction was determined to be ≥ 1.523 L product/ha.

Materials and methods

Test item:	GLOB182F, batch No.: PE 2004.742 analysed content of a.s.: Fludioxonil: 100.0 g/L (nominal: 100 g/L)
Test species:	Parasitic wasp *Aphidius rhopalosiphi* (DeStefani-Perez), adults (< 48 hours old); source (in the stage of mummies): "Katz Biotech AG", An der Birkenpfuhlheide 10, 15837 Baruth, Germany
Test design:	Exposure of the wasps was achieved via dried spray residues on glass plates. Seven treatment groups (5 test item rates, water treated control, reference item) were set up with 4 replicates (consisting of 7 females and 3 males, each) per treatment. Mortality assessments were carried out 2, 24 and 48 hours after test initiation. At 48 hours, surviving wasps (15 females per treatment) were removed and their reproductive capacity was assessed by confining them individually over untreated wheat plants infested with adult and nymphal aphids (*Rhopalosiphum padi*). Assessment of reproduction capacity i.e. number of mummies per female, was made for the control and all test item treatment groups product/ha test item groups (1 assessment, 14 days after application).
Endpoints:	Mortality after exposure over 48 hours including the determination of the LR_{50} (Lethal Rate 50 %, rate resulting in 50 % mortality) Reproductive capacity: number of mummies per female including the determination of an ER_{50} (if possible).
Reference item:	DANADIM PROGRESS (Dimethoate 411.2 g/L, nominal: 400 g/L)
Test rates:	Control (deionised water) Test item (GLOB182F): 0.0952 – 0.190 – 0.381 – 0.761 – 1.523 L product/ha Reference item (DANADIM PROGRESS): 0.3 mL product/ha

All substances were applied in 200 L water/ha, sprayed on glass plates, via laboratory spraying equipment and air dried afterwards.

Test conditions:	Temperature:	19 °C - 22 °C
	Relative humidity:	66 % - 72 %
	Light-dark-cycle:	16 hours light : 8 hours dark
	Light intensity:	1070 lx (exposure phase)
		2510 lx (parasitisation phase)
		6590 lx (reproduction phase)
	Food:	25 % w/w aqueous fructose solution

Statistics: Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm ($\alpha = 0.05$) for mortality (test item)
Fisher's Exact Binomial Test ($\alpha = 0.05$) for mortality (reference item)
Williams-t-test for reproductive capacity ($\alpha = 0.05$)

Results and discussion

After 48 hours, in the water-treated control a mortality of 2.5 % was observed. In the test item treatments, mortality ranged between 0 % and 5.0 %. This resulted in corrected mortality rates of -2.6 % and 2.6 %. No statistically significant effects on mortality were determined at all test item rates (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$). The LR_{50} was estimated to be > 1.523 L product/ha. The NOER (no observed effect rate) for mortality was determined to be ≥ 1.523 L product/ha.

The mean number of mummies per female per day in the test item treatment groups ranged between 19.2 and 22.1, compared to the control with 19.7 mummies/female. No statistically significant effects on reproductive capacity were determined at all test item treatment groups (Williams-t-test, $\alpha = 0.05$). The ER_{50} was estimated to be > 1.523 L product/ha. The NOER (no observed effect rate) for reproduction was determined to be ≥ 1.523 L product/ha.

The results are summarised below.

Table A11. Effects on the parasitic wasp *Aphidius rhopalosiphi* exposed to GLOB182F in a worst-case laboratory test

Treatment	**Rate** [1] **[L product/ha]**	**Mortality** [2] **[%]**	**Corrected Mortality** [3] **[%]**	**Reproduction** [4] **[mean number of mummies/female]**	**Effects on reproduction** [5] **[%]**
Control	-	2.5	-	19.7	-
Test item	0.0952	2.5 (n.s.)	0	19.2 (n.s.)	2.5
Test item	0.190	0 (n.s.)	-2.6	20.3 (n.s.)	-3.0
Test item	0.381	5.0 (n.s.)	2.6	20.5 (n.s.)	-4.1
Test item	0.761	0 (n.s.)	-2.6	22.1 (n.s.)	-12.2
Test item	1.523	2.5 (n.s.)	0	21.1 (n.s.)	-7.1
	Endpoint [L product/ha]				
LR_{50}	**> 1.523**			-	
NOER	**≥ 1.523**			-	
ER_{50}	-			**> 1.523**	
NOER	-			**≥ 1.523**	

[1] Application rate in 200 L water/ha
[2] Mortality after 48 hours of exposure to the test item on treated glass plates. The results for mortality in individual treatments were compared to that in the control using Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm ($\alpha = 0.05$).
[3] Corrected mortality according to Abbott (1925).
[4] Reproduction: mean number of parasitised aphids (mummies) per surviving female. The results for the test item treatments and control were compared by Williams-t-test ($\alpha = 0.05$).

[5] Change in mean number of mummies per female, relative to control. A negative value indicates an increase and a positive value indicates a decrease relative to the control.
n.s. not statistically significant different compared to the control

No unusual observations regarding behaviour were noted in the control and the test item treatment groups at any observation point during the test.

The reference item caused a mortality of 100 % of exposed wasps, resulting in a corrected mortality of 100 %.

Conclusion
In a worst-case laboratory study with GLOB182F, the LR_{50} for *Aphidius rhopalosiphi* was estimated to be > 1.523 L product/ha. The NOER (no observed effect rate) for mortality was determined to be ≥ 1.523 L product/ha.
The ER_{50} was estimated to be > 1.523 L product/ha. The NOER (no observed effect rate) for reproduction was determined to be ≥ 1.523 L product/ha.

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Comments of izRMS:	The study was performed in line with the respective guideline with no deviations. All validity criteria were met: • the arithmetic mean mortality rate (dead and escaped mites) in the control was ≤ 20% on day 7 after treatment application (observed 2.0 %), • the cumulative mean number of eggs per female (reproduction) in the control (from day 7 to day 14) was ≥ 4 eggs/female (observed 6.42 eggs/female), • the cumulative mean mortality (control corrected) of protonymphs on day 7 exposed to the toxic reference item was between 50 and 100% (observed 74.5 %). Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment: LR_{50} > 1.523 L product/ha ER_{50} > 1.523 L product/ha

Reference:	KCP 10.3.2
Report	Effects of GLOB182F on the predatory mite *Typhlodromus pyri* SCHEUTEN in a laboratory test, Röhlig U., 2020, 20 48 NTL 0008.
Guideline(s):	IOBC (BLÜMEL et al. 2000)
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable

Executive Summary
In a worst-case laboratory study, protonymphs of the predatory mite *Typhlodromus pyri* SCHEUTEN (Acari: Phytoseiidae) were exposed to GLOB182F sprayed on glass plates. Endpoint of the study was the mortality after 7 days of exposure, including determination of the LR_{50}. Effects on reproduction were assessed by the number of eggs laid and number of juveniles per evaluation period.

The study encompassed 7 treatment groups (one water treated control, 5 test item rates, one reference item rate), each with 5 replicates. 20 protonymphs per replicate were exposed to dried residues of GLOB182F sprayed on glass plates at application rates of 0.0952 – 0.190 – 0.381 – 0.761 – 1.523 L product/ha with a water volume corresponding to 200 L/ha. Additional test units were treated with deionised water for the water control and with DANADIM PROGRESS (active substance 411.2 g/L) as

the reference item. The main endpoint of the study was mortality (including estimation of the LR_{50} and NOER) and, in addition, effects on reproduction were assessed (including estimation of the ER_{50} and NOER).
After 7 days, in the water-treated control a mortality of 2.0 % was observed. In the test item treatments, mortality ranged between 1.0 % and 4.0 %. This resulted in corrected mortality rates between -1.0 % and 2.0 %.No statistically significant effects on mortality were determined at all rates tested, compared to the control (Multiple Sequentially-rejective Chi^2-2x2 Table test after BONFERRONI-HOLM, $\alpha = 0.05$). The LR_{50} was estimated to be > 1.523 L product/ha. The NOER (no observed effect rate) for mortality was determined to be ≥ 1.523 L product/ha.
The reproductive capacity of the mites was assessed in the control group and at all test item rates. The reproduction rate amounted to 6.42 eggs/female in the control treatment. The reproduction rate in the test item treated groups were between 6.01 eggs/female and 6.92 eggs/female. Thus, an effect on reproduction between 6.4 % and -7.8 % was calculated for the test item treated groups compared to the control. No statistically significant effects on reproduction were determined at all test item rates (WILLIAMS-t-test, $\alpha = 0.05$). The ER_{50} was estimated to be > 1.523 L product/ha. The NOER (no observed effect rate) for reproduction was determined to be ≥ 1.523 L product/ha.
In a worst-case laboratory study with GLOB182F, the LR_{50} for *Typhlodromus pyri* was estimated to be > 1.523 L product/ha. The NOER (no observed effect rate) for mortality was determined to be ≥ 1.523 L product/ha.
The ER_{50} was estimated to be > 1.523 L product/ha. The NOER (no observed effect rate) for reproduction was determined to be ≥ 1.523 L product/ha.

Materials and methods

Test item:	GLOB182F, batch No.: PE 2004.742 analysed content of a.s.: Fludioxonil: 100.0 g/L (nominal: 100 g/L)
Test species:	Predatory mite *Typhlodromus pyri* Scheuten, protonymphs (< 24 hours old); source (in the stage of eggs): "Katz Biotech AG", An der Birkenpfuhlheide 10, 15837 Baruth, Germany
Test design:	Protonymphs were exposed to dried spray residues of different application rates of the test item applied on glass plates. 7 treatment groups (5 test item rates, water treated control, reference item) were set up with 5 replicates (consisting of 20 protonymphs) per treatment. Exposure lasted until 14 days after application. Mortality assessments were carried out 3 and 7 days after exposure of the mites and additionally after 9, 11 and 14 days. In addition, for the control and all test item treatment groups, the reproduction, i.e. number of eggs per female, was determined (3 assessments, 9, 11 and 14 days after application).
Endpoints:	Mortality after exposure over 7 days, including determination of a LR_{50} (Lethal Rate 50 %, rate resulting in 50 % mortality) Reproductive capacity of the surviving mites from day 7-14 including determination of a ER_{50} (Effect Rate 50 %, rate resulting in 50 % effect on reproduction)
Reference item:	DANADIM PROGRESS (Dimethoate 411.2 g/L, nominal: 400 g/L)

Test rates:	Control (deionised water) Test item (GLOB182F): 0.0952 – 0.190 – 0.381 – 0.761 – 1.523 L product/ha Reference item (DANADIM PROGRESS): 15 mL/ha All substances were applied in 200 L water/ha, sprayed on glass plates, via laboratory spraying equipment and air dried afterwards.
Test conditions:	Temperature: 23 °C - 25 °C Relative humidity: 66 % - 75 % Light-dark-cycle: 16 hours light, 8 hours dark; Light intensity: 2070 lx Food: pollen: pine (*Pinus nigra*) and birch (*Betula pendula*), 1:1
Statistics:	Multiple Sequentially-rejective Chi^2-2x2 Table test after Bonferroni-Holm ($\alpha = 0.05$) for mortality (test item) Chi^2 2x2 Table test ($\alpha = 0.05$) for mortality (reference item) Williams-t-test for reproductive capacity ($\alpha = 0.05$)

Results and discussions

After 7 days, in the water-treated control a mortality of 2.0 % was observed. In the test item treatments, mortality ranged between 1.0 % and 4.0 %. This resulted in corrected mortality rates between -1.0 % and 2.0 %. No statistically significant effects on mortality were determined at all rates tested, compared to the control (Multiple Sequentially-rejective Chi^2-2x2 Table test after BONFERRONI-HOLM, $\alpha = 0.05$). The LR_{50} was estimated to be > 1.523 L product/ha. The NOER (no observed effect rate) for mortality was determined to be ≥ 1.523 L product/ha.

The reproductive capacity of the mites was assessed in the control group and at all test item rates. The reproduction rate amounted to 6.42 eggs/female in the control treatment. The reproduction rate in the test item treated groups were between 6.01 eggs/female and 6.92 eggs/female. Thus, an effect on reproduction between 6.4 % and -7.8 % was calculated for the test item treated groups compared to the control. No statistically significant effects on reproduction were determined at all test item rates (WILLIAMS-t-test, $\alpha = 0.05$). The ER_{50} was estimated to be > 1.523 L product/ha. The NOER (no observed effect rate) for reproduction was determined to be ≥ 1.523 L product/ha.

The results are summarized below.

Table A12. Effects on predatory mite Typhlodromus pyri exposed to fresh dry residues of GLOB182F in a worst-case laboratory test

Treatment	Rate [1] [L product/ha]	Mortality [2] [%]	Corrected mortality [3] [%]	Mean number of eggs per female [4] [7-14 Day]	Effect on Reproduction [5] [%]
Control	-	2.0	-	6.42	-
Test item	0.0952	1.0 (n.s.)	-1.0	6.59 (n.s.)	-2.6
Test item	0.190	2.0 (n.s.)	0	6.27 (n.s.)	2.3
Test item	0.381	1.0 (n.s.)	-1.0	6.01 (n.s.)	6.4
Test item	0.761	2.0 (n.s.)	0	6.34 (n.s.)	1.2
Test item	1.523	4.0 (n.s.)	2.0	6.92 (n.s.)	-7.8
	Endpoint [L product/ha]				
LR_{50}	**> 1.523**			-	
NOER	≥ 1.523			-	
ER_{50}	-			**> 1.523**	
NOER	-			≥ 1.523	

[1] Application rate in 200 L water/ha

[2] Mortality after 7 days of exposure to residues on treated glass plates. The results for mortality in individual test item treatments were compared to that in the control using Multiple Sequentially-rejective Chi²-2x2 Table test after BONFERRONI-HOLM ($\alpha = 0.05$).
[3] Corrected mortality according to ABBOTT (1925)
[4] Results for reproduction compared by WILLIAMS-t-test ($\alpha = 0.05$)
[5] Change in mean number of eggs per female, relative to control. A positive value indicates a decrease, relative to the control.
n.s. not statistically significant different compared to the control

No unusual observations regarding behaviour were noted in the control and the test item treatment groups at any observation point during the test.
The reference item caused a mortality of 75.0 % of exposed mites, resulting in a corrected mortality of 74.5 %.

Conclusion
In a worst-case laboratory study with GLOB182F, the LR_{50} for *Typhlodromus pyri* was estimated to be > 1.523 L product/ha. The NOER (no observed effect rate) for mortality was determined to be ≥ 1.523 L product/ha.
The ER_{50} was estimated to be > 1.523 L product/ha. The NOER (no observed effect rate) for reproduction was determined to be ≥ 1.523 L product/ha.

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Comments of izRMS:	The study was performed in line with the respective guideline with no deviations. All validity criteria were met: • average number of beetles emerging from the fly pupae was > 400 (observed 626) (i.e. > 26.7 % of the 6000 introduced fly pupae was parasitized) (observed 41.7 %), • reduction of the reproductive capacity in the reference item treatment relative to the control was ≥ 50 % (observed 99.7 %). Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment: EC_{50} > 9.0234 mg a.s./kg soil d.w.

Reference:	KCP 10.3.2-01
Report	Effects of Fludioxonil 25 FS on the rove beetle *Aleochara bilineata* GYLL. in an extended laboratory test, Röhlig U., 2020, 20 48 NKE 0007.
Guideline(s):	Grimm *et al.* (2000)
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable

Executive Summary
An extended laboratory study was carried out to determine the effects of the test item Fludioxonil 25 FS on the rove beetle *Aleochara bilineata* (Coleoptera: Staphylinidae). For determination of the reproductive capacity adults were exposed to different concentrations of Fludioxonil 25 FS incorporated into sandy soil (LUFA 2.1). Effects on reproduction were assessed by the number of emerged beetles compared to the control group.
The study encompassed 7 treatment groups (5 test item concentrations, control, reference item), each with 4 replicates. 10 females and 10 males (10 pairs) per replicate were exposed to the test item incorporated into sandy soil at concentrations of 0.1114 – 0.3342 – 1.0026 – 3.0078 – 9.0234 mg a.s./kg soil dry weight (d.w.). Additional test units were treated with deionised water as control or with DANADIM

PROGRESS (active substance 411.2 g Dimethoate/L) as reference item. The endpoint of the study was the reproductive capacity.
In the water-treated control the average number of hatched beetles of the F_1 generation was 626. At test concentrations a reproductive capacity between 577 and 619 hatched beetles, was observed. This resulted in effects on reproduction between 7.8 % and 1.1 % inhibition. No statistically significant differences compared to the control were observed at concentrations up to and including 9.0234 mg a.s./kg soil d.w. (DUNNETT's Multiple t-test, $\alpha = 0.05$). The EC_{50} for Fludioxonil 25 FS was estimated to be > 9.0234 mg a.s./kg soil d.w. The NOEC (no observed effect concentration) for reproduction was determined to be ≥ 9.0234 mg a.s./kg soil d.w.

Materials and methods

Test item:	Fludioxonil 25 FS, batch No.: PE 2002.715 analysed content of a.i.: Fludioxonil: 25.6 g/L (nominal 25 g/L) Density: 1.06 g/mL
Test species:	Rove beetle *Aleochara bilineata* GYLL., adults (1-7 days old); source: reared in the laboratory of the test facility
Test design:	The test item concentrations and control were incorporated into the soil via a laboratory mixer (Mixing machine KitchenAid). The reference item was sprayed via a laboratory spray applicator (tracksprayer) on the soil surface. Exposure of the beetles was reached via treated sandy soil (LUFA 2.1). Seven treatment groups (5 test item concentrations, water-treated control, reference item) were set up with 4 replicates (consisting of 10 females and 10 males (10 pairs) per treatment. On day 7, 14 and 21 approx. 500 pupae of *Delia antiqua* were buried in the sandy soil (LUFA 2.1) of each replicate to be parasitised by the larvae of the beetles. On day 28 the adults were separated from the soil and the sandy soil with the pupae was allowed to dry for seven days. On day 35 the pupae were removed from the soil by a sieve and transferred into a hatching unit. After hatching, the test endpoint reproductive capacity (average number of hatched beetles of the F1 generation) was determined (daily assessments during 5 weeks).
Endpoint:	Reproductive capacity (average number of hatched beetles of the F_1 generation)
Test item concentrations:	Fludioxonil 25 FS: 0.1114 – 0.3342 – 1.0026 – 3.0078 – 9.0234 mg a.s./kg soil dry weight (d.w.). (incorporated into soil)
Control:	deionised water (incorporated into soil)
Reference item:	DANADIM PROGRESS (Dimethoate 411.2 g/L, nominal: 400 g/L) application rate: 1.5 L product/ha in 400 L water/ha (spray application)
Test conditions:	Temperature: 19°C - 22 °C; relative humidity: 66 % - 73 % light-dark-cycle: 16 hours light : 8 hours dark; light intensity: 1760 lx Food: *Chironomus* spp. larvae (thawed)

Statistics: DUNNETT's Multiple t-test, (α = 0.05) for reproductive capacity (test item)
STUDENT-t-test (α = 0.05) for reproductive capacity (reference item)

Results and discussion

In the water-treated control the average number of hatched beetles of the F_1 generation was 626. At test concentrations a reproductive capacity between 577 and 619 hatched beetles, was observed. This resulted in effects on reproduction between 7.8 % and 1.1 % inhibition. No statistically significant differences compared to the control were observed at concentrations up to and including 9.0234 mg a.s./kg soil d.w. (DUNNETT's Multiple t-test, α = 0.05). The EC_{50} for Fludioxonil 25 FS was estimated to be > 9.0234 mg a.s.kg soil d.w. The NOEC (no observed effect concentration) for reproduction was determined to be ≥ 9.0234 mg a.s./kg soil d.w.

The results are summarised below.

Table A13: Effects on reproductive capacity of the rove beetle (*Aleochara bilineata* GYLL.) exposed to Fludioxonil 25 FS in an extended laboratory test

Treatment	Concentration[1] [mg a.s./kg dry soil weight]	Reproduction [mean number of emerged beetles per replicate]	Parasitisation rate [%]	Reproduction [absolute number of emerged beetles per treatment group]	Effect on Reproduction[2] [%]
Control	-	626	41.7	2503	
Test item	0.1114	582 (n.s.)	38.8	2327	7.0
Test item	0.3342	594 (n.s.)	39.6	2376	5.1
Test item	1.0026	619 (n.s.)	41.3	2476	1.1
Test item	3.0078	582 (n.s.)	38.8	2327	7.0
Test item	9.0234	577 (n.s.)	38.5	2309	7.8
Reference item	1.5 L product/ha	2*	0.1	8	99.7
	Endpoint [mg a.s./kg soil d.w.]				
EC_{50}		> 9.0234			
NOEC		≥ 9.0234			

[1] incorporated into soil
[2] Effect on reproduction according to the following formula: (1-Pt/Pc) * 100% calculated on the absolute number of emerged beetles (positive values represent a decreased reproduction compared to the control)
n.s. not significantly different compared to the control: DUNNETT's Multiple t-test, α = 0.05 (test item)
* statistically significantly different compared to the control: STUDENT -t-test, α = 0.05 (reference item)

The reference item caused an effect on reproduction of 99.7 % of exposed beetles.
No unusual observations regarding behaviour were noted in the control and the test item treatment groups at any observation point during the test.

Conclusion

In an extended laboratory study with Fludioxonil 25 FS the EC50 for *Aleochara bilineata* was estimated to be > 9.0234 mg a.s./kg soil d.w. The NOEC (no observed effect concentration) for reproduction was determined to be ≥ 9.0234 mg a.s./kg soil d.w.

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Comments of izRMS:	The study was performed in line with the respective guideline with no deviations. It was noted that there was a typing error in Table A14: Effects on reproductive capacity of the rove beetle (*Aleochara bilineata* Gyll.) exposed to Fludioxonil 480 FS in an extended laboratory test. In the case of the reference item the parasitisation rate should be 0.55 % not 6.2 %. The error was transferred from the original study report. However, this typing error is considered to have no impact on the outcome of the study. All validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment: EC_{50} > 40 mg product/kg soil dw

Reference:	KCP 10.3.2-04
Report	Effects of Fludioxonil 480 FS on the rove beetle *Aleochara bilineata* GYLL. in an extended laboratory test, Röhlig U., 2018, 18 48 NKE 0003.
Guideline(s):	Yes, IOBC Grimm *et al.* (2000)
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable

Executive Summary

An extended laboratory study was carried out to determine the effects of the test item Fludioxonil 480 FS on the rove beetle *Aleochara bilineata* (Coleoptera: Staphylinidae). For determination of the reproductive capacity adults were exposed to different concentrations of Fludioxonil 480 FS incorporated into sandy soil (LUFA 2.1). Effects on reproduction were assessed by the number of emerged beetles compared to the control group.

The study encompassed 7 treatment groups (5 test item concentrations, control, reference item), each with 4 replicates. 10 females and 10 males (10 pairs) per replicate were exposed to the test item incorporated into sandy soil at concentrations of 2.5 – 5 – 10 – 20 – 40 mg product/kg soil dry weight (d.w.). Additional test units were treated with deionised water as control or with Dimethoate EC 400 (active substance 429.0 g/L) as reference item. The endpoint of the study was the reproductive capacity.

In the water-treated control the average number of hatched beetles of the F1 generation was 554. At test concentrations up to and including 40 mg product/kg soil d.w. a reproductive capacity between 443 and 550 hatched beetles, was observed. This resulted in effects on reproduction between 20.0 % and 0.6 % inhibition. No statistically significant differences compared to the control were observed at concentrations up to and including 10 mg product/kg soil d.w. (Multiple Sequentially-rejective Welsh-t-test after Bonferroni-Holm, $\alpha = 0.05$). The values of the two highest rates (20 mg and 40 mg product/kg soil d.w.) are statistically significant different to the control, but above the validity criterion given by the IOBC guideline of average number of hatched beetles per replicate of the F1 generation in the control > 400.

In an extended laboratory study with Fludioxonil 480 FS the EC50 for *Aleochara bilineata* was estimated to be > 40 mg product/kg soil d.w. No unacceptable effects on reproduction were observed, when the test item was incorporated into sandy soil (LUFA 2.1) at concentrations up to and including 40 mg product/kg soil d.w.

Materials and methods

Test item:	Fludioxonil 480 FS, batch No.: 3602 analysed content of a.i.: Fludioxonil: 496.9 g/L (nominal 480 g/L) Density: 1.2026 g/cm3
Control item:	Deionized water

Reference item:	Dimethoate EC 400: 1.5 L product/ha in 400 L water/ha (spray application)
Test species:	Rove beetle *Aleochara bilineata* Gyll., adults (1-7 days old); source: reared in the laboratory of the test facility
Method according to:	IOBC Grimm *et al.* (2000)
Test design:	The test item concentrations and control were incorporated into the soil via a laboratory mixer (Mixing machine KitchenAid). The reference item was sprayed via a laboratory spray applicator (tracksprayer) on the soil surface. Exposure of the beetles was reached via treated sandy soil (LUFA 2.1). Seven treatment groups (5 test item concentrations, water-treated control, reference item) were set up with 4 replicates (consisting of 10 females and 10 males (10 pairs) per treatment. On day 7, 14 and 21 approx. 500 pupae of Delia antiqua were buried in the sandy soil (LUFA 2.1) of each replicate to be parasitised by the larvae of the beetles. On day 28 the adults were separated from the soil and the sandy soil with the pupae was allowed to dry for seven days. On day 35 the pupae were removed from the soil by a sieve and transferred into a hatching unit. After hatching, the test endpoint reproductive capacity (average number of hatched beetles of the F1 generation) was determined (daily assessments during 5 weeks).
Endpoints:	Reproductive capacity (average number of hatched beetles of the F1 generation)
Test rates:	Fludioxonil 480 FS: 2.5 - 5 - 10 - 20 - 40 mg product/kg soil d.w.(incorporated into soil) Control: deionised water (incorporated into soil) Reference item: Dimethoate EC 400 (Dimethoate 429.0 g/L, nominal: 400 g/L) application rate: 1.5 L product/ha in 400 L water/ha (spray application)
Test conditions:	Temperature: 18 °C - 22 °C; relative humidity: 62 % - 74 % light-dark-cycle: 16 hours light : 8 hours dark; light intensity: 1750 lx Food: *Chironomus* spp. larvae (thawed)
Dates of work:	Experimental starting date: 11 January 2018 Experimental completion date: 19 March 2018
Statistics:	Multiple sequentially-rejective WELSH-t-test after Bonferroni-Holm ($\alpha = 0.05$) for reproductive capacity (test item) STUDENT-t-test ($\alpha = 0.05$) for reproductive capacity (reference item)

Results and discussion

The results of the control group indicated that the organisms were in a good condition (mean number of hatched beetles in the F1 generation: 554).
The results of the reference item group indicated that the test system was sensitive to harmful substances (reduction of reproductive performance: 98.5 %)

In the water-treated control the average number of hatched beetles of the F1 generation was 554. At test concentrations up to and including 40 mg product/kg soil d.w. a reproductive capacity between 443 and 550 hatched beetles, was observed. This resulted in effects on reproduction between 20.0 % and 0.6 % inhibition. No statistically significant differences compared to the control were observed at concentrations up to and including 10 mg product/kg soil d.w. (Multiple Sequentially-rejective Welsh-t-test after Bonferroni-Holm, $\alpha = 0.05$). The values of the two highest rates (20 mg and 40 mg product/kg soil d.w.) are statistically significant different to the control, but above the validity criterion given by the IOBC guideline of average number of hatched beetles per replicate of the F1 generation in the control > 400.

Concerning the reproductive capacity in the control group as well as the susceptibility of the test organisms to the reference item, the study is proved to be valid.

All validity criteria were met:

- average number of hatched beetles per replicate of the F1-generation
  in the control: > 400 (observed: 554)
  (parasitisation rate in the control) > 26.7 % (observed: 36.9 %)

- reduction of the reproductive capacity in the reference item
  treatment relative to control: ≥ 50 % (observed: 98.5 %)

The results are summarised in Tables 10.3.2-05.

Table A14: Effects on reproductive capacity of the rove beetle (*Aleochara bilineata* Gyll.) exposed to Fludioxonil 480 FS in an extended laboratory test

Treatment	Concentration [1] [mg product/kg soil d.w.]	Reproduction [mean number of emerged beetles per replicate]	Parasitisation rate [%]	Reproduction [absolute number of emerged beetles per treatment group]	Effect on Reproduction [2] [%]
Control	-	554	36.9	2214	-
Test item	2.5	547 (n.s.)	36.5	2188	1.2
Test item	5	550 (n.s.)	36.7	2200	0.6
Test item	10	521 (n.s.)	34.8	2085	5.8
Test item	20	501*	33.4	2003	9.5
Test item	40	443*	29.5	1772	20.0
Reference item	1.5 L product/ha [3]	8*	0.55	33	98.5

Test item: Fludioxonil 480 FS
[1] incorporated into soil
[2] Effect on reproduction according to the following formula: (1-Pt/Pc) * 100% calculated on the absolute number of emerged beetles (positive values represent a decreased reproduction compared to the control)
[3] Application rate in 400 L water/ha (spray application)
n.s. not significantly different compared to the control: Multiple Sequentially-rejective WELSH-t-test after Bonferroni-Holm, $\alpha = 0.05$ (test item)
* statistically significantly different compared to the control: Multiple Sequentially-rejective WELSH-t-test after Bonferroni-Holm, $\alpha = 0.05$ (test item) or STUDENT-t-test, $\alpha = 0.05$ (reference item)

The reference item caused an effect on reproduction of 98.5 % of exposed beetles.
No unusual observations regarding behaviour were noted in the control and the test item treatment groups at any observation point during the test.

Conclusion
In an extended laboratory study with Fludioxonil 480 FS the EC50 for *Aleochara bilineata* was estimated to be > 40 mg product/kg soil d.w. No unacceptable effects on reproduction were observed, when the test item was incorporated into sandy soil (LUFA 2.1) at concentrations up to and including 40 mg product/kg soil d.w.

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Comments of izRMS:	The study was performed in line with the respective guideline with no deviations. It was noted that the reference item was sprayed onto the soil while the test item was incorporated into the soil. Nevertheless, toxic effects of the reference item were observed after the exposure of carabid beetles to it and the corrected mortality of the tested organisms in the reference item group (after 14 days) was 100 % which fulfilled the validity criteria of the test (required 65 ± 35 %). Therefore, the different method of application of the reference item compared to the test item is considered acceptable. All validity criteria were met: • mortality in the control group (after 14 days) was ≤ 6.7 % (observed 0 %), • corrected mortality in the reference item group (after 14 days) was 65 ± 35 % (observed 100 %). Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment: LC_{50} > 9.0234 mg a.s./kg soil d.w. EC_{50} > 9.0234 mg a.s./kg soil d.w.

Reference:	KCP 10.3.2-02
Report	Effects of Fludioxonil 25 FS on the carabid beetle *Poecilus cupreus* L. in an extended laboratory test, Röhlig U., 2020, 20 48 NLE 0005.
Guideline(s):	IOBC (HEIMBACH *et al*. 2000)
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable

Executive Summary

An extended laboratory study was carried out to determine the effects of the test item (Fludioxonil 25 FS) on the carabid beetle *Poecilus cupreus* L. (Coleoptera: Carabidae). For determination of the mortality adult beetles were exposed to fresh residues of the test item incorporated into sandy soil (LUFA 2.1). Effects on mortality were assessed by the number of surviving beetles, additionally behavioural impacts (food uptake) were assessed.

The study encompassed 7 treatment groups (5 test item concentrations, control, reference item), each with 5 replicates. Three females and three males per replicate were exposed to dried residues of Fludioxonil 25 FS incorporated into sandy soil at concentrations of 0.1114, 0.3342, 1.0026, 3.0078 and 9.0234 mg a.s./kg soil dry weight. Additional test units were treated with deionised water as control and with DANADIM PROGRESS (active substance 411.2 g/L Dimethoate) as reference item. Endpoints of the study were mortality (including determination of the LC_{50}) and additionally effects on the food uptake (including determination of the EC_{50}).

After 14 days, in the water-treated control a mortality of 0 % was observed. In all test item treatments mortality was 0 % and 3.3 %. This resulted in corrected mortality rates of 0 % and 3.3 %.

No statistically significant effects on mortality were observed at all tested concentrations (Multiple sequentially-rejective FISHER test after BONFERRONI-HOLM, $\alpha = 0.05$). The LC_{50} for Fludioxonil 25 FS was estimated to be > 9.0234 mg a.s./kg soil dry weight. The NOEC for mortality was determined to be ≥ 9.0234 mg a.s./kg soil dry weight.

The mean food uptake (consumed fly pupae per surviving beetle) ranged between 1.22 and 1.26 fly pupae in the test item treatment groups in comparison to the control with 1.24 fly pupae. No statistically significant effects on food uptake were determined (DUNNETT's-t-test, $\alpha = 0.05$) at all tested concentrations. The NOEC for food uptake was determined to be ≥ 9.0234 mg a.s./kg soil dry weight.

Materials and methods

Test item: Fludioxonil 25 FS, batch No.: PE 2002.715

	analysed content of a.s.: Fludioxonil: 25.6 g/L (nominal 25 g/L)
Test species:	Carabid beetle *Poecilus cupreus* L. adults (3-6 weeks old); source (in-house culture): in the laboratory of the test facility BioChem agrar GmbH
Test design:	Exposure of the adults was achieved via incorporated residues into sandy soil (LUFA 2.1). Seven treatment groups (5 test item rates, water-treated control, reference item) were set up with 5 replicates (consisting of 3 females and 3 males) per treatment. Mortality and behavioural assessments were carried out 2 hours, 1, 2, 4, 7, 11 and 14 days after application. Assessment of food uptake, i.e. number of consumed fly pupae, was made for the control and the test item groups on 1, 2, 4, 7, 11 and 14 days after application.
Endpoints:	Mortality: number of dead beetles, including the estimation of a LC_{50} Food uptake: number of consumed fly pupae per surviving beetle, including estimation of an EC_{50}
Test rates:	Control (deionised water) Test item (Fludioxonil 25 FS): 0.1114 – 0.3342 – 1.0026 – 3.0078 – 9.0234 mg a.s./kg soil dry weight (incorporated into soil) Reference item (DANADIM PROGRESS): 2.25 L product/ha in 400 L water/ha (spray application)
Test conditions:	Temperature: 19 °C – 22 °C; relative humidity: 68 % - 72 % light-dark-cycle: 16 hours light : 8 hours dark; light intensity: 1050 lx Food: defrosted pupae of onion fly *Delia antiqua*
Statistics:	Mortality, feeding activity
Test concentrations:	Multiple sequentially-rejective FISHER test after BONFERRONI-HOLM ($\alpha = 0.05$) for mortality (test item) FISHER's Exact Binomial test ($\alpha = 0.05$) for mortality (reference item) DUNNETT's-t-test ($\alpha = 0.05$) for food uptake (test item) STUDENT-t-test ($\alpha = 0.05$) for food uptake (reference item)

Results and discussion

After 14 days, in the water-treated control a mortality of 0 % was observed. In all test item treatments mortality was 0 % and 3.3 %. This resulted in corrected mortality rates of 0 % and 3.3 %.

No statistically significant effects on mortality were observed at all tested concentrations (Multiple sequentially-rejective FISHER test after BONFERRONI-HOLM, $\alpha = 0.05$). The LC50 for Fludioxonil 25 FS was estimated to be > 9.0234 mg a.s./kg soil dry weight. The NOEC for mortality was determined to be ≥ 9.0234 mg a.s./kg soil dry weight.

The mean food uptake (consumed fly pupae per surviving beetle) ranged between 1.22 and 1.26 fly pupae in the test item treatment groups in comparison to the control with 1.24 fly pupae. No statistically significant effects on food uptake were determined (DUNNETT's -t-test, $\alpha = 0.05$) at all tested concentrations. The NOEC for food uptake was determined to be ≥ 9.0234 mg a.s./kg soil dry weight.

The results are summarised below.

Table A15: Effects on the carabid beetle (Poecilus cupreus) exposed to fresh dry residues of Fludioxonil 25 FS in an extended laboratory trial

Treatment	Concentration[1] [mg a.s./ kg soil d.w.]	Mortality[2] [%]	Corrected mortality[3] [%]	Total number of consumed fly pupae	Food uptake[4] [mean number of consumed fly pupae/ surviving beetle]	Effect on food uptake[5] [%]
Control	-	0	-	223	1.24	-
Test item	0.1114	0 (n.s.)	0	225	1.25 (n.s.)	-0.8
Test item	0.3342	0 (n.s.)	0	219	1.22 (n.s.)	1.6
Test item	1.0026	0 (n.s.)	0	227	1.26 (n.s.)	-1.6
Test item	3.0078	3.3 (n.s.)	3.3	219	1.22 (n.s.)	1.6
Test item	9.0234	0 (n.s.)	0	222	1.23 (n.s.)	0.8
	Endpoint [mg a.s./kg soil dry weight]					
LC_{50}	**> 9.0234**					
EC_{50}				**> 9.0234**		
Reference item DANADIM PROGRESS	2.25 **L product/ha[6]**	100*	100	11	0.19*	84.7

[1] Concentration in mg product/kg soil dry weight (incorporated in soil)

[2] Mortality after 14 days of exposure to residues on sandy soil. The results for mortality in individual treatments were compared to that in the control using Multiple sequentially-rejective FISHER test after BONFERRONI-HOLM (α = 0.05) for the test item and FISHER's Exact Binomial test (α = 0.05) for the reference item

[3] Corrected mortality according to ABBOTT (1925)

[4] Food uptake: mean number of consumed fly pupae/surviving beetle. The results for the test item treatments and control and the reference item treatment and control were compared by DUNNETT'S-t-test and STUDENT-t-test, respectively (α = 0.05).

[5] Change in mean number of consumed fly pupae per treatment group, relative to control. A positive value indicates a decrease and a negative value indicates an increase, relative to the control.

[6] Application rate in 400 L water/ha (spray application)

(n.s.) not statistically significantly different compared to the control

* statistically significantly different compared to the control

The reference item caused a mortality of 100 % of exposed beetles, resulting in a corrected mortality of 100 %.

Conclusion

In an extended laboratory study with Fludioxonil 25 FS the LC_{50} for *Poecilus cupreus* was estimated to be > 9.0234 mg a.s./kg soil dry weight. The NOEC (no observed effect concentration) for mortality was determined to be ≥ 9.0234 mg a.s./kg soil dry weight.

The EC_{50} for Fludioxonil 25 FS was estimated to be > 9.0234 mg a.s./kg soil dry weight.

The NOEC (no observed effect concentration) for food uptake was estimated to be ≥ 9.0234 mg a.s./kg soil dry weight.

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Comments of izRMS:	The study was performed in line with the respective guideline with no deviations. It was noted that the reference item was sprayed onto the soil while the test item was incorporated into the soil. Nevertheless, toxic effects of the reference item were observed after the exposure of carabid beetles to it and the corrected mortality of the tested organisms in the reference item group (after 14 days) was 66.7 % which fulfilled the validity criteria of the test (required 65 ± 35 %). Therefore, the different method of application of the reference item compared to the test item is considered acceptable. It was also noted that there was one typing error and two calculation errors in Table A17: Mean food consumption of *Poecilus cupreus* after exposure to Fludioxonil 480 FS. In the case of the reference item: - total number of pupae consumed should be 64 not 40 (typing error), - mean number of pupae consumed per surviving beetle should be 0.64 not 0.50 (calculation error), - effect on food uptake should be 63 % not 71.3 % (calculation error). Nevertheless, these errors are considered to have no impact on the overall outcome of the study. All validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: LR_{50} > 40 mg product/kg soil dry weight ER_{50} > 40 mg product/kg soil dry weight

Reference:	KCP 10.3.2-03
Report	Effects of Fludioxonil 480 FS on the carabid beetle *Poecilus cupreus* L. in an extended laboratory test, Röhlig U., 2018, 18 48 NLE 0001.
Guideline(s):	Yes, IOBC Heimbach *et al.* (2000)
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable

Executive Summary

An extended laboratory study was carried out to determine the effects of the test item (Fludioxonil 480 FS) on the carabid beetle *Poecilus cupreus* L. (Coleoptera: Carabidae). For determination of the mortality adult beetles were exposed to fresh residues of the test item incorporated into sandy soil (LUFA 2.1). Effects on mortality were assessed by the number of surviving beetles, additionally behavioural impacts (food uptake) were assessed.

The study encompassed 7 treatment groups (5 test item rates, control, reference item), each with 5 replicates. Three females and three males per replicate were exposed to dried residues of Fludioxonil 480 FS incorporated into sandy soil at concentrations of 2.5, 5, 10, 20 and 40 mg product/kg soil dry weight. Additional test units were treated with deionised water as control and with Dimethoate EC 400 (active substance 429.0 g/L) as reference item. Endpoints of the study were mortality (including determination of the LR50) and additionally effects on the food uptake.

After 14 days, in the water-treated control a mortality of 0 % was observed. In all test item treatments mortality was 0 %. This resulted in a corrected mortality rate of 0 %. No statistically significant effects on mortality were observed at all tested concentrations (Multiple sequentially-rejective FISHER test after BONFERRONI-HOLM, $\alpha = 0.05$). The LR50 for Fludioxonil 480 FS was estimated to be > 40 mg product/kg soil dry weight. The NOER for mortality was determined to be ≥ 40 mg product/kg soil dry weight.

The mean food uptake (consumed fly pupae per surviving beetle) ranged between 1.63 and 1.93 fly pupae

in the test item treatment groups in comparison to the control with 1.74 fly pupae. No statistically significant effects on food uptake were determined (DUNNETT'S-t-test, $\alpha = 0.05$) at all tested rates.

Materials and methods

Test item:	Fludioxonil 480 FS, batch No.: 3602 analysed content a.i.: Fludioxonil: 496 g/L (nominal 480 g/L)	
Control item:	Deionized water	
Reference item:	Dimethoate EC 400: 2.25 L product/ha in 400L water/ha (spray application)	
Test species:	Carabid beetle *Poecilus cupreus* L. Adults (3-6 weeks old); source (in-house culture): In the laboratory of the test facility BioChem agrar GmbH	
Method according to:	IOBC Heimbach *et al.* (2000)	
Test design:	Exposure of the adults was achieved via incorporated residues in to sandy soil (LUFA 2.1). Seven treatment groups (5 test item rates, water-treated control, reference item) were set up with 5 replicates (consisting of 3 females and 3 males) per treatment. Mortality and behavioural assessments were carried out 2 hours, 1, 2, 4, 7, 11 and 14 days after application. Assessment of food uptake, i.e. number of consumed fly pupae, was made for the control and the test item groups on 1, 2, 4, 7, 11 and 14 days after application.	
Endpoints:	Mortality including the estimation of a LR50. Food uptake: number of consumed fly pupae per surviving beetle.	
Test rates:	Control (deionised water) Test item (Fludioxonil 480 FS): 2.5 – 5 – 10 – 20 – 40 mg product/kg soil dry weight (incorporated into soil) Reference item (Dimethoate EC 400): 2.25 L product/ha in 400L water/ha (spray application)	
Test conditions:	Temperature:	19°C - 22°C
	Relative humidity:	66 - 74%
	Photoperiod:	16:8 light:dark at 1030 lux
	Food:	defrosted pupae of onion fly *Delia antiqua*
Dates of work:	Experimental starting date:	15 January 2018
	Experimental completion date:	29 January 2018
Statistics:	Multiple sequentially-rejective FISHER test after BONFERRONI-HOLM ($\alpha = 0.05$) for mortality (test item) FISHER's Exact Binomial test ($\alpha = 0.05$) for mortality (reference item) DUNNET's-t-test ($\alpha = 0.05$) for food uptake (test item) STUDENT-t-test ($\alpha = 0.05$) for food uptake (reference item)	

Results and discussion

The results of the control group indicated that the test organisms were in a good condition (mortality: 0 %; food uptake: 1.74 consumed fly pupae per beetle).

The results of the reference item group indicated that the test system was sensitive to harmful substances (corrected mortality: 66.7 %). Concerning mortality in the control group as well as the susceptibility of the test organisms to the reference item the study is proved to be valid.

After 14 days, in the water-treated control a mortality of 0 % was observed. In all test item treatments mortality was 0 %. This resulted in a corrected mortality rate of 0 %. No statistically significant effects on mortality were observed at all tested concentrations (Multiple sequentially-rejective FISHER test after BONFERRONI-HOLM, $\alpha = 0.05$). The LR50 for Fludioxonil 480 FS was estimated to be > 40 mg product/kg soil dry weight. The NOER for mortality was determined to be ≥ 40 mg product/kg soil dry weight. The mean food uptake (consumed fly pupae per surviving beetle) ranged between 1.63 and 1.93 fly pupae in the test item treatment groups in comparison to the control with 1.74 fly pupae. No

statistically significant effects on food uptake were determined (DUNNETT'S-t-test, α = 0.05) at all tested rates.

All validity criteria were met:
- Mortality in the control group (after 2 weeks): ≤ 6.7% (observed: 0%)
- Corrected mortality in the reference item group (after 2 weeks): 65 ± 35 % (observed: 66.7 %)

The results are summarised in Tables 10.3.2-03 and 10.3.2-04.

Table A16: Mortality of *Poecilus cupreus* after exposure to Fludioxonil 480 FS

3 females and 3 males per replicate (5 per treatment group) were introduced

Treatment group	dead beetles [number]		mortality[1] [%]	corrected mortality (ABBOTT) [%]
	males	females		
Control	0	0	0	-
Test item 2.5 mg product/kg soil d.w.	0	0	0 (n.s.)	0
Test item 5 mg product/kg soil d.w.	0	0	0 (n.s.)	0
Test item 10 mg product/kg soil d.w.	0	0	0 (n.s.)	0
Test item 20 mg product/kg soil d.w.	0	0	0 (n.s.)	0
Test item 40 mg product/kg soil d.w.	0	0	0 (n.s.)	0
Reference item Dimethoate EC 400 2.25 L/ha	11	9	66.7*	66.7

Test item: Fludioxonil 480 FS
[1] Mortality after 14 days. The results for mortality in individual treatments were compared to that in the control using Multiple sequentially-rejective FISHER test after BONFERRONI-HOLM (α = 0.05) for test item and FISHER`s Exact Binomial test (α = 0.05) for reference item.
(n.s.) not statistically significantly different compared to the control
* statistically significantly different compared to the control

Table A17: Mean food consumption of *Poecilus cupreus* after exposure to Fludioxonil 480 FS

Treatment group	total number of consumed fly pupae	mean number of consumed fly pupae/surviving beetle/assessment day 1	Effect on food uptake [%] [2]
Control	313	1.74	-
Test item 2.5 mg product/kg soil d.w.	347	1.93 (n.s.)	-10.9
Test item 5 mg product/kg soil d.w.	307	1.71 (n.s.)	1.7
Test item 10 mg product/kg soil d.w.	293	1.63 (n.s.)	6.3
Test item 20 mg product/kg soil d.w.	328	1.82 (n.s.)	-4.6
Test item 40 mg product/kg soil d.w.	323	1.79 (n.s.)	-2.9
Reference item Dimethoate EC 400 2.25 L/ha	40	0.50*	71.3

Test item: Fludioxonil 480 FS
[1] the mean number of consumed fly pupae/surviving beetle/assessment day was calculated from the number of consumed fly pupae per surviving beetle
[2] based on mean number of consumed fly pupae per treatment group compared to the control
(n.s.) not statistically significantly different compared to the control: DUNNETT'S-t-test (α = 0.05)
* statistically significantly different compared to the control: STUDENT-t-test (α = 0.05)

Conclusion
In an extended laboratory study with Fludioxonil 480 FS the LR50 for *Poecilus cupreus* was estimated to be > 40 mg product/kg soil dry weight. The NOER for mortality was determined to be ≥ 40 mg product/kg soil dry weight.

No statistically significant effects on the food uptake of *Poecilus cupreus* were observed at treatment concentrations up to and including 40 mg product/kg soil dry weight.

A 2.3 KCP 10.4 Effects on non-target soil meso- and macrofauna

A 2.3.1 KCP 10.4.1 Earthworms

A 2.3.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

Comments of izRMS:	The study was performed in line with OECD 222 with no deviations. The test design was relevant to derive both NOEC and ECx values (8 concentrations, 8 replicates for control, 4 replicates per treatment group). However, the ECx values could not be calculated due to no statistically significant effect of the test item at any concentration measured during the test. All validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment: NOEC = 425.48 mg product/kg soil dw (corresponding to 9.979 mg a.s./kg soil dw)

Reference:	KCP 10.4.1.1-01
Report	Sublethal effects of Fludioxonil 25 FS on the earthworm *Eisenia andrei* in artificial soil, Friedrich S., 2017, report No 17 48 TEC 0025.
Guideline(s):	Yes; OECD 222 (2016)
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable

Executive Summary

The purpose of this study was to determine potential effects of the test item on reproduction, mortality and growth of the earthworm *Eisenia andrei* by dermal and alimentary uptake using an artificial soil in a laboratory test.

Materials and methods

Test item:	Fludioxonil 25 FS, Batch No.: 170413/01 Content of a.i.: Fludioxonil: 25 g/L (24.80 g/L analysed)
Reference item:	Maypon Flow (Carbendazim, SC 500)
Test species:	Earthworm (*Eisenia andrei*), age: approximately 4 months old with clitellum; source: in-house culture Weight of animals used in the test: 301 – 488 mg/worm
Test design:	Sublethal toxicity earthworm: 56 days; 8 test item treatment groups and an untreated control group, 8 replicates in the control group and 4 replicates in the test item treatment groups, 10 worms per replicate; Exposure of worms to different concentrations of the test item mixed into the substrate (artificial soil with 10% peat); assessments of adult mortality, behavioural effects and biomass development after 28 days, and reproduction rate after an additional 28 days (assessed 56 days after application)
Endpoints :	Mortality and biomass after 28 days, reproduction after 56 days
Treatments:	Control (untreated), test item (Fludioxonil 25 FS)
Test concentrations:	6.95, 12.51, 22.52, 40.53, 72.96, 131.32, 236.38, 425.48 mg test item/kg soil dry weight (spacing factor: 1.8)

Test conditions:	Temperature:	19.5°C – 21.2°C
	Light intensity:	610 lux
	Photoperiod:	light : dark = 16h : 8h
	Water content (g/100 g dry soil):* guideline requirement: 40-60 % of WHC	
	test start: 34.9 – 35.0 (equivalent to 55.0 – 55.2 % of WHC)	
	test end: 34.1 – 34.7 (equivalent to 53.8 – 54.7 % of WHC)	
	pH-value:* guideline requirement: 6.0 ± 0.5	
	test start: 5.95 – 5.98	
	test end: 5.73 – 5.84	
	* pooled replicates per treatment group	
Statistics:	Williams-t-test for biomass change and reproduction (α = 0.05, one-sided smaller)	
	Statistical program: ToxRat Professional 3.2.1 (2015)	
Dates of work:	Experimental start date:	01 June 2017
	Experimental completion date:	27 July 2017

Results and discussion

The mean biomass change of adult worms ranged from 23.9 % to 29.6 % in the test item treated groups and 27.6 % in the control group. The test item caused no statistically significant change in biomass (change in fresh weight after 4 weeks relative to initial fresh weight) compared to the control group at any concentration tested (Williams-t-test, α = 0.05, one-sided smaller).

No mortality was recorded in the test item treatment groups and in the control group (Table 3). No pathological symptoms and no effects on behaviour (including feeding activity) of the worms were observed during the test.

No statistically significant effects (Williams-t-test, α = 0.05, one-sided smaller) on the number of juveniles compared to the control group were found at any concentration tested.

The NOEC for mortality, biomass and reproduction was determined to be 425.48 mg test item/kg soil dry weight. The LC_{50} for mortality and the EC_{10}, EC_{20} and EC_{50} values for reproduction were estimated to be > 425.48 mg test item/kg soil dry weight.

The results are summarised below.

Table A18: Sublethal effects of Fludioxonil 25 FS on *Eisenia andrei* in a 56-day reproduction study

Endpoint	Treatment group (mg test item/kg soil d.w.)								
	Control	6.95	12.51	22.52	40.53	72.96	131.32	236.38	425.48
Mortality of adult worms after 4 weeks (%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean biomass change after 4 weeks (%)	27.6	29.6	26.7	27.0	25.5	29.1	23.9	26.5	25.8
Mean number of juveniles after 8 weeks	162.0	164.3	150.0	151.0	165.3	157.8	160.0	166.8	160.0
Reduction of reproduction compared to control (%)	-	-1.4	7.4	6.8	-2.0	2.6	1.2	-2.9	1.2
NOEC (mortality)	425.48								
NOEC (biomass)	425.48								
NOEC (reproduction)	425.48								
LC_{50} (mortality) [1]	> 425.48								
EC_{10} (reproduction) [1]	> 425.48								
EC_{20} (reproduction) [1]	> 425.48								
EC_{50} (reproduction) [1]	> 425.48								

Not statistically significantly different to control regarding biomass and reproduction (Williams-t-test, α = 0.05, one-sided

smaller)
Negative values = increase, relative to control
[1] based on estimation of the data

The validity criteria for the control group were met:

- Adult mortality: ≤ 10% (0% after 4 weeks)
- Number of juveniles per test vessel: ≥ 30 (122, 165, 141, 183, 193, 162, 176, 154)
- Coefficient of variation of reproduction: ≤ 30% (14.2%)

In a separate study with the reference item at concentrations of 5 and 10 mg product/kg soil dry weight the number of juveniles was reduced by 57 and 100 %, respectively (mean number of juveniles = 46 and 0) after 8 weeks of test duration when compared to control (mean number of juveniles = 107).

Conclusion
In a 56-day earthworm reproduction study with Fludioxonil 25 FS, no statistically significant effects on biomass and reproduction of the earthworm *Eisenia andrei* in artificial soil were determined up to and including 425.48 mg test item/kg soil dry weight, i.e. the highest concentration tested.
The NOEC for mortality, biomass and reproduction was determined to be 425.48 mg test item/kg soil dry weight. The LC_{50} and the EC_{10}, EC_{20} and EC_{50} values for reproduction were estimated to be > 425.48 mg test item/kg soil dry weight.

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Comments of izRMS:	The study was performed in line with OECD 222 with no deviations. The test design was relevant to derive both NOEC and ECx values (8 concentrations, 8 replicates for control, 4 replicates per treatment group). However, the ECx values could not be calculated due to no statistically significant effect of the test item at any concentration measured during the test. All validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment: NOEC = 97.34 mg product/kg soil dw (corresponding to 40.22 mg a.s./kg soil dw)

Reference:	KCP 10.4.1.1-01
Report	Sublethal effects of Fludioxonil 480 FS on the earthworm *Eisenia andrei* in artificial soil, Friedrich S., 2017, report No 17 48 TEC 0041.
Guideline(s):	Yes; OECD 222 (2016)
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable

Executive Summary
The purpose of this study was to determine potential effects of the test item on reproduction, mortality and growth of the earthworm *Eisenia andrei* by dermal and alimentary uptake using an artificial soil in a laboratory test.

Materials and methods

Test item:	Fludioxonil 480 FS, Batch No.: 3602 Content of a.i.: Fludioxonil: 480 g/L (496.9 g/L analysed)
Reference item:	Maypon Flow (Carbendazim, SC 500)
Test species:	Earthworm (*Eisenia andrei*), age: approximately 4 months old with clitellum; source: in-house culture

	Weight of animals used in the test: 313 – 509 mg/worm
Test design:	Sublethal toxicity earthworm: 56 days; 8 test item treatment groups and an untreated control group, 8 replicates in the control group and 4 replicates in the test item treatment groups, 10 worms per replicate; Exposure of worms to different concentrations of the test item mixed into the substrate (artificial soil with 10% peat); assessments of adult mortality, behavioural effects and biomass development after 28 days, and reproduction rate after an additional 28 days (assessed 56 days after application)
Endpoints :	Mortality and biomass after 28 days, reproduction after 56 days
Treatments:	Control (untreated), test item (Fludioxonil 480 FS)
Test concentrations:	1.59, 2.86, 5.15, 9.27, 16.69, 30.04, 54.08, 97.34 mg test item/kg soil dry weight (spacing factor: 1.8)
Test conditions:	Temperature: 19.5°C – 20.7°C Light intensity: 610 lux Photoperiod: light : dark = 16h : 8h Water content (g/100 g dry soil):* guideline requirement: 40-60 % of WHC test start: 34.9 – 35.0 (equivalent to 55.9 – 56.1 % of WHC) test end: 34.3 – 34.9 (equivalent to 55.0 – 55.9 % of WHC) pH-value:* guideline requirement: 6.0 ± 0.5 test start: 6.01 – 6.14 test end: 5.79 – 5.83 * pooled replicates per treatment group
Statistics:	Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm for mortality (α = 0.05, one-sided greater), Williams-t-test for biomass change and reproduction (α = 0.05, one-sided smaller) Statistical program: ToxRat Professional 3.2.1 (2015)
Dates of work:	Experimental start date: 18 July 2017 Experimental completion date: 12 September 2017

Results and discussion

The mean biomass change of adult worms ranged from 25.1 % to 29.6 % in the test item treated groups and 26.9 % in the control group. The test item caused no statistically significant change in biomass (change in fresh weight after 4 weeks relative to initial fresh weight) compared to the control group at any concentration tested (Williams-t-test, α = 0.05, one-sided smaller).

Mortality rates of 0 % - 2.5 % were recorded in the test item treatment groups and 0 % mortality was observed in the control group.

No statistically significant mortality compared to the control was observed at any concentration tested (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, α = 0.05, one-sided greater).

No pathological symptoms and no effects on behaviour (including feeding activity) of the worms were observed during the test.

No statistically significant effects (Williams-t-test, α = 0.05, one-sided smaller) on the number of juveniles compared to the control group were found at any concentration tested.

The NOEC for mortality, biomass and reproduction was determined to be 97.34 mg test item/kg soil dry weight. The LC50 for mortality and the EC10, EC20 and EC50 values for reproduction were estimated to be > 97.34 mg test item/kg soil dry weight.

The results are summarised below.

Table A19: Sublethal effects of Fludioxonil 480 FS on *Eisenia andrei* in a 56-day reproduction study

Endpoint	Treatment group (mg test item/kg soil d.w.)								
	Control	1.59	2.86	5.15	9.27	16.69	30.04	54.08	97.34
Mortality of adult worms after 4 weeks (%)	0.0	2.5	0.0	2.5	0.0	2.5	0.0	0.0	2.5
Mean biomass change after 4 weeks (%)	26.9	28.2	25.2	26.4	29.6	25.1	28.4	28.1	26.4
Mean number of juveniles after 8 weeks	152.9	158.0	148.3	143.8	154.3	147.0	151.8	153.5	141.8
Reduction of reproduction compared to control (%)	-	-3.4	3.0	6.0	-0.9	3.8	0.7	-0.4	7.3
NOEC (mortality)	97.34								
NOEC (biomass)	97.34								
NOEC (reproduction)	97.34								
LC_{50} (mortality) [1]	> 97.34								
EC_{10} (reproduction) [1]	> 97.34								
EC_{20} (reproduction) [1]	> 97.34								
EC_{50} (reproduction) [1]	> 97.34								

Not statistically significantly different to control regarding biomass and reproduction (Williams-t-test, α = 0.05, one-sided smaller)
Negative values = increase, relative to control
[1] based on estimation of the data

The validity criteria for the control group were met:
- Adult mortality: ≤ 10% (0% after 4 weeks)
- Number of juveniles per test vessel: ≥ 30 (140, 151, 183, 117, 127, 194, 173 and 138)
- Coefficient of variation of reproduction: ≤ 30% (18.1%)

In a separate study with the reference item at concentrations of 5 and 10 mg product/kg soil dry weight the number of juveniles was reduced by 57 and 100 %, respectively (mean number of juveniles = 46 and 0) after 8 weeks of test duration when compared to control (mean number of juveniles = 107).

Conclusion
In a 56-day earthworm reproduction study with Fludioxonil 480 FS, no statistically significant effects on mortality, biomass and reproduction of the earthworm *Eisenia andrei* in artificial soil were determined up to and including 97.34 mg test item/kg soil dry weight, i.e. the highest concentration tested. The NOEC for mortality, biomass and reproduction was determined to be 97.34 mg test item/kg soil dry weight. The LC50 and the EC10, EC20 and EC50 values for reproduction were estimated to be > 97.34 mg test item/kg soil dry weight.

A 2.3.1.2 KCP 10.4.1.2 Earthworms - field studies

A 2.3.2 KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

Comments of izRMS:	The study was performed in line with OECD 232 with no deviations. The test design was relevant to derive both NOEC and ECx values (8 concentrations, 8 replicates for control, 4 replicates per treatment group). However, the ECx values could not be calculated due to no statistically significant effect of the test item at any concentration measured during the test. All validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment: NOEC = 425.48 mg product/kg soil dw (corresponding to 9.979 mg a.s./kg soil dw)

Reference:	KCP 10.4.2-01
Report	Effects of Fludioxonil 25 FS on the reproduction of the collembolan *Folsomia candida*, Friedrich S., 2017, report No 17 48 TCC 0024.
Guideline(s):	Yes; OECD 232 (2016)
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable

Executive Summary

The purpose of this study was to determine potential effects of the test item on the reproductive output of the collembolan *Folsomia candida* as a representative of soil micro-arthropods during a test period of 28 days. After 4 weeks the number of offspring (juveniles) and surviving parental collembolans were counted.

Materials and methods

Test item: Fludioxonil 25 FS, Batch No.: 170413/01
Content of a.i.: Fludioxonil: 25 g/L (24.80 g/L analysed)

Reference item: Boric acid

Test species: Collembola (*Folsomia candida*), age: 9 - 12 days; source: in-house culture

Test design: Chronic toxicity *Folsomia candida*: 28 days;
8 test item treatment groups and an untreated control group,
8 replicates in the control group and 4 replicates in the test item treatment groups, each containing 10 collembolans (9-12 days old);
Exposure of collembolans to different concentrations of the test item mixed into the substrate (artificial soil with 5 % peat);
assessments of adult mortality and reproduction 28 days after application

Endpoints : Mortality and reproduction after 28 days

Treatments: Control (untreated), test item (Fludioxonil 25 FS)

Test concentrations: 6.95, 12.51, 22.52, 40.53, 72.96, 131.32, 236.38, 425.48 mg test item/kg soil dry weight (spacing factor: 1.8)

Test conditions:

Temperature:	19.7 °C – 20.7 °C
Light intensity:	615 lux
Photoperiod:	light : dark = 16h : 8h

Water content (g/100 g soil d.w.): guideline requirement: 40 - 60 % of WHC
test initiation: 24.9 – 25.1 (equivalent to 59.1 – 59.6 % of WHC)
test completion: 24.4 – 24.7 (equivalent to 58.0 – 58.7 % of WHC)

pH-value: guideline requirement: 6.0 ± 0.5
test initiation: 6.01 – 6.06
test completion: 5.82 – 5.89

Statistics: Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, Williams-t-test (α = 0.05, one-sided), Statistical program: ToxRat Professional 3.2.1 (2015)

Dates of work: Experimental start date: 30 May 2017
Experimental completion date: 27 June 2017

Results and discussion

Mortality rates of 0 % - 5.0 % were recorded in the test item treatment groups. 3.8 % parental mortality was observed in the control. No statistically significant effects on parental mortality (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, α = 0.05, one-sided greater) was found for any concentration tested. No effects on behaviour of the collembolans were observed during the test.

The mean number of juvenile collembolans counted four weeks after introduction of the parental collembolans into the test vessels was 712 in the control and 740, 714, 700, 730, 727, 686, 695 and 730 at concentrations of 6.95, 12.51, 22.52, 40.53, 72.96, 131.32, 236.38 and 425.48 mg test item/kg soil d.w., respectively. No statistically significant effects (Williams-t-test, α = 0.05, one-sided smaller) on the number of juveniles compared to the control group were found at any concentration tested.

The results are summarised below.

Table A20: Chronic effects of Fludioxonil 25 FS on *Folsomia candida* in a 28-day reproduction study

Endpoint	**Treatment group [mg test item/kg soil dry weight]**								
	Control	6.95	12.51	22.52	40.53	72.96	131.32	236.38	425.48
Mean adult mortality [%]	3.8	5.0	5.0	5.0	5.0	2.5	5.0	0.0	5.0
Mean number of juveniles	712	740	714	700	730	727	686	695	730
Reduction of reproduction [%] compared to control	-	-4	0	2	-3	-2	4	2	-3
	Endpoints [mg test item/kg soil dry weight]								
NOEC (mortality)	425.48								
NOEC (reproduction)	425.48								
LC_{50} (mortality) [1]	> 425.48								
EC_{10} (reproduction) [1]	> 425.48								
EC_{20} (reproduction) [1]	> 425.48								
EC_{50} (reproduction) [1]	> 425.48								

Not statistically significantly different to control regarding mortality (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, α = 0.05, one-sided greater) and reproduction (Williams-t-test, α = 0.05, one-sided smaller)
Negative values = increase, relative to control
[1] based on estimation of the data

The validity criteria for the control group were met:

- Mean adult mortality: ≤ 20 % (observed: 3.8 %)
- Mean number of juveniles per test vessel: ≥100 (observed: average of 712/vessel)
- Coefficient of variation for the mean number of juveniles: < 30 % (observed: 11.9 %)

The reference item boric acid was tested in a separate study at concentrations of 44, 67, 100, 150 and 225 mg/kg soil dry weight. The EC_{50} was determined to be 104 mg/kg soil dry weight. The LC_{50} was determined to be 165 mg/kg soil dry weight. The NOEC for mortality and for reproduction was determined to be 67 and 44 mg/kg soil dry weight, respectively. The EC_{50} value for the reproduction was close to the value of 100 mg/kg soil dry weight as stated in OECD 232 (2016). The EC_{50} therefore showed that the test system was sensitive.

Conclusion
In a 28-day *Folsomia candida* reproduction study with Fludioxonil 25 FS, the NOEC for mortality of the parental collembolans was determined to be 425.48 mg test item/kg soil dry weight. The LC_{50} could not be calculated, but it can be concluded that the LC_{50} is higher than 425.48 mg test item/kg soil d.w., the highest concentration tested.
The NOEC for reproduction was determined to be 425.48 mg test item/kg soil dry weight. The EC_{10}, EC_{20} and EC_{50} values for reproduction could not be calculated, but it can be concluded that these values are higher than 425.48 mg test item/kg soil d.w., the highest concentration tested.

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Comments of izRMS:	The study was performed in line with OECD 232 with no deviations. The test design was relevant to derive both NOEC and ECx values (8 concentrations, 8 replicates for control, 4 replicates per treatment group). However, the ECx values could not be calculated due to no statistically significant effect of the test item at any concentration measured during the test. All validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment: NOEC = 97.34 mg product/kg soil dw (corresponding to 40.22 mg a.s./kg soil dw)

Reference:	KCP 10.4.2
Report	Effects of Fludioxonil 480 FS on the reproduction of the collembolan *Folsomia candida*, Friedrich S., 2017, report No 17 48 TCC 0041.
Guideline(s):	Yes; OECD 232 (2016)
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable

Executive Summary
The purpose of this study was to determine potential effects of the test item on the reproductive output of the collembolan *Folsomia candida* as a representative of soil micro-arthropods during a test period of 28 days. After 4 weeks the number of offspring (juveniles) and surviving parental collembolans were counted.

Materials and methods

Test item:	Fludioxonil 480 FS, Batch No.: 3602 Content of a.i.: Fludioxonil: 480 g/L (496.9 g/L analysed)
Reference item:	Boric acid
Test species:	Collembola (*Folsomia candida*), age: 9 - 12 days; source: in-house culture
Test design:	Chronic toxicity *Folsomia candida*: 28 days; 8 test item treatment groups and an untreated control group, 8 replicates in the control group and 4 replicates in the test item treatment

	groups, each containing 10 collembolans (9-12 days old); Exposure of collembolans to different concentrations of the test item mixed into the substrate (artificial soil with 5 % peat); assessments of adult mortality and reproduction 28 days after application
Endpoints :	Mortality and reproduction after 28 days
Treatments:	Control (untreated), test item (Fludioxonil 480 FS)
Test concentrations:	1.59, 2.86, 5.15, 9.27, 16.69, 30.04, 54.08, 97.34 mg test item/kg soil dry weight (spacing factor: 1.8)
Test conditions:	Temperature: 18.0 °C – 21.2 °C Light intensity: 640 lux Photoperiod: light : dark = 16h : 8h Water content (g/100 g soil d.w.): guideline requirement: 40 - 60 % of WHC test initiation: 24.9 – 25.1 (equivalent to 57.8 – 58.2 % of WHC) test completion: 24.3 – 24.7 (equivalent to 56.4 – 57.3 % of WHC) pH-value: guideline requirement: 6.0 ± 0.5 test initiation: 6.07 – 6.11 test completion: 5.93 – 6.00
Statistics:	Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, Williams-t-test (α = 0.05, one-sided), Statistical program: ToxRat Professional 3.2.1 (2015)
Dates of work:	Experimental start date: 24 July 2017 Experimental completion date: 21 August 2017

Results and discussion

Mortality rates of 0 % - 5.0 % were recorded in the test item treatment groups. 3.8 % parental mortality was observed in the control. No statistically significant effect (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, α = 0.05, one-sided greater) on parental mortality was found for any concentration tested. No effects on behaviour of the collembolans were observed during the test.

The mean number of juvenile collembolans counted four weeks after introduction of the parental collembolans into the test vessels was 725 in the control and 734, 700, 742, 696, 740, 704, 709 and 668 at concentrations of 1.59, 2.86, 5.15, 9.27, 16.69, 30.04, 54.08 and, 97.34 mg test item/kg soil d.w., respectively. No statistically significant effects (Williams-t-test, α = 0.05, one-sided smaller) on the number of juveniles compared to the control group were found at any concentration tested.

The results are summarised below.

Table A21: Chronic effects of Fludioxonil 25 FS on *Folsomia candida* in a 28-day reproduction study

Endpoint	Treatment group [mg test item/kg soil dry weight]								
	Control	1.59	2.86	5.15	9.27	16.69	30.04	54.08	97.34
Mean adult mortality [%]	3.8	0.0	2.5	5.0	2.5	5.0	2.5	2.5	5.0
Mean number of juveniles	725	734	700	742	696	740	704	709	668
Reduction of reproduction [%] compared to control	-	-1	3	-2	4	-2	3	2	8
	Endpoints [mg test item/kg soil dry weight]								
NOEC (mortality)	97.34								
NOEC (reproduction)	97.34								
LC_{50} (mortality) [1]	> 97.34								
EC_{10} (reproduction) [1]	> 97.34								
EC_{20} (reproduction) [1]	> 97.34								
EC_{50} (reproduction) [1]	> 97.34								

Not statistically significantly different to control regarding mortality (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$, one-sided greater) and reproduction (Williams-t-test, $\alpha = 0.05$, one-sided smaller)
Negative values = increase, relative to control
[1] based on estimation of the data

The validity criteria for the control group were met:
- Mean adult mortality: ≤ 20 % (observed: 3.8 %)
- Mean number of juveniles per test vessel: ≥100 (observed: average of 725/vessel)
- Coefficient of variation for the mean number of juveniles: < 30 % (observed: 13.4 %)

The reference item boric acid was tested in a separate study at concentrations of 44, 67, 100, 150 and 225 mg/kg soil dry weight. The EC_{50} was determined to be 107 mg/kg soil dry weight. The LC_{50} was determined to be 158 mg/kg soil dry weight. The NOEC for mortality and for reproduction was determined to be 67 and 44 mg/kg soil dry weight, respectively. The EC_{50} value for the reproduction was close to the value of 100 mg/kg soil dry weight as stated in OECD 232 (2016). The EC_{50} therefore showed that the test system was sensitive.

Conclusion
In a 28-day Folsomia candida reproduction study with Fludioxonil 480 FS, the NOEC for mortality of the parental collembolans was determined to be 97.34 mg test item/kg soil dry weight. The LC50 could not be calculated, but it can be concluded that the LC50 is higher than 97.34 mg test item/kg soil d.w., the highest concentration tested. The NOEC for reproduction was determined to be 97.34 mg test item/kg soil dry weight. The EC10, EC20 and EC50 values for reproduction could not be calculated, but it can be concluded that these values are higher than 97.34 mg test item/kg soil d.w., the highest concentration tested.

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Comments of izRMS:	The study was performed in line with OECD 226 with no deviations. The test design was relevant to derive both NOEC and ECx values (8 concentrations, 8 replicates for control, 4 replicates per treatment group). However, the ECx values could not be calculated due to no statistically significant effect of the test item at any concentration measured during the test. All validity criteria were met: • mean adult female mortality was ≤ 20 % (observed 0 %), • mean number of juveniles per replicate was ≥ 50 (observed 307.6), • coefficient of variation for the number of juveniles per replicate was ≤ 30 (observed 5.1 %). Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment: NOEC = 425.48 mg product/kg soil dw (corresponding to 9.979 mg a.s./kg soil dw)

Reference:	KCP 10.4.2-02
Report	Effects of Fludioxonil 25 FS on the reproduction of the predatory mite *Hypoaspis aculeifer*, Schulz L., 2017, report No 17 48 THC 0020.
Guideline(s):	Yes, OECD 226
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable

Executive Summary

The purpose of this study was to determine potential effects of the test item on mortality and reproductive output of the soil mite species *Hypoaspis aculeifer* (CANESTRINI) as a representative species of soil microarthropods during a test period of 14 days.

Material and methods

Test item: Fludioxonil 25 FS, Batch No.: 170413/01
Content of a.i.: Fludioxonil: 25 g/L (24.80 g/L analysed)

Reference item: Dimethoate (EC 400 g/L, nominal)

Test species: *Hypoaspis aculeifer* (CANESTRINI)
age: adult female mites with an age difference of 2 days
source: in-house culture

Test system: Exposure of female mites to different concentrations of the test item mixed into artificial soil substrate

Test design: The effects of the test item on mortality and reproduction of the soil mite species *Hypoaspis aculeifer* (CANESTRINI) were investigated in a chronic laboratory experiment over a time period of 14 days according to OECD 226.
Each of the eight different test item concentrations were homogeneously mixed into artificial soil and filled into glass vessels. Subsequently, the soil mites were introduced on top of the soil and the vessels were covered. Four replicates were performed for the test item groups and eight replicates for the control group; each replicate consisted of ten female soil mites. The mites were fed with *Tyrophagus putrescentiae* (SCHRANK) at the beginning and every two to three days during the whole test period.
For the main measured variable, the number of juveniles per test vessel and

	additionally the mortality of the adult female mites were determined. The reproductive output of the mites exposed to the test item was compared to that of the control in order to determine the no observed effect concentration (NOEC). Assessment of adult mortality and reproduction effects was carried out after 14 days.
Endpoints:	Mortality and number of juveniles
Test conditions:	Artificial soil according to OECD 226, pH 6.1 - 6.2 at test start, pH 5.8 – 5.9 at test end; water content at test start 47.53 – 50.29% of maximum water holding capacity (WHC) and 45.67 – 48.12% of maximum WHC at test end; temperature 20.0 - 20.3°C; photoperiod: 16 h light : 8 h dark; light intensity: 707 lx.
Test concentrations:	6.95, 12.51, 22.52, 40.53, 72.96, 131.32, 236.38, 425.48 mg test item/kg soil dry weight (spacing factor: 1.8)
Dates of work:	Experimental start date: 7 June 2017 Experimental completion date: 27 June 2017
Statistics:	Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm ($\alpha = 0.05$, one-sided greater) for mortality; Multiple Sequentially-rejective U-test after Bonferroni-Holm ($\alpha = 0.05$, one-sided smaller) for reproduction Statistical program: ToxRat Professional 3.2.1 (2015)

Results and discussion
All validity criteria for the study were met.

Mortality rates of 0.0 - 5.0 % were recorded in the test item treatment groups. In the control group the mortality rate was 0.0 %.
The observed mortality rates in the test item treatment groups compared to control were not statistically significant (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$, one-sided greater).
Differences in the behaviour and the morphology of the mites between the control and the test item treatment groups could not be observed.

Fourteen days after introduction of the parental mites into the test vessels, the mean number of juveniles was 310.5, 315.8, 313.0, 299.5, 317.3, 307.5, 291.5 and 300.5 at concentrations of 6.95, 12.51, 22.52, 40.53, 72.96, 131.32, 236.38 and 425.48 mg test item/kg soil d.w., respectively. The mean reproduction in the control reached 307.6 juveniles. The test item showed no statistically significantly adverse effects on reproduction at all tested concentrations (Multiple Sequentially-rejective U-test after Bonferroni-Holm, $\alpha = 0.05$, one-sided smaller).

In a separate study (BioChem project No. R 16 10 48 006 S, experimental start date: 13.01.2017), the EC_{50} (reproduction) of the reference item dimethoate (EC 400 g/L, nominal) was calculated to be 5.8 mg a.s./kg soil d.w. The results of the reference test demonstrate the sensitivity of the test system.

Table A22: ` Effects of Fludioxonil 25 FS on *Hypoaspis aculeifer* mortality and reproduction (day 14)

Test item [mg/kg soil dry weight]	Treatment group								
	Control	6.95	12.51	22.52	40.53	72.96	131.32	236.38	425.48
Mean adult mortality (day 14) [%]	0.0	0.0	5.0	2.5	2.5	2.5	0.0	2.5	0.0
Mean number of juveniles (day 14)	307.6	310.5	315.8	313.0	299.5	317.3	307.5	291.5	300.5
Coefficient of variation [%]	5.1	11.7	5.6	4.3	8.7	5.0	2.3	8.3	12.9
Reproduction in [%] of control	**100**	**101**	**103**	**102**	**97**	**103**	**100**	**95**	**98**
	Endpoints [mg test item/kg soil dry weight]								
NOEC (mortality)	425.48								
NOEC (reproduction)	425.48								
LC_{50} (mortality)	> 425.48								
EC_{10} (reproduction)	> 425.48								
EC_{20} (reproduction)	> 425.48								
EC_{50} (reproduction)	> 425.48								

Not statistically significantly different compared to the control (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm for mortality, $\alpha = 0.05$, one-sided greater and Multiple Sequentially-rejective U-test after Bonferroni-Holm for reproduction, $\alpha = 0.05$, onesided smaller)

Conclusion

In a 14-day *Hypoaspis aculeifer* reproduction study with Fludioxonil 25 FS, the LC_{50} and the EC_{10}, EC_{20} and EC_{50} values could not be calculated, but it can be concluded that these values are higher than 425.48 mg test item/kg soil dry weight. The NOEC for mortality and for reproduction was determined to be 425.48 mg test item/kg soil dry weight, the highest concentration tested.

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Comments of izRMS:	The study was performed in line with OECD 226 with no deviations. The test design was relevant to derive both NOEC and ECx values (8 concentrations, 8 replicates for control, 4 replicates per treatment group). However, the ECx values could not be calculated due to no statistically significant effect of the test item at any concentration measured during the test. All validity criteria were met: • mean adult female mortality was ≤ 20 % (observed 0 %), • mean number of juveniles per replicate was ≥ 50 (observed 317.3), • coefficient of variation for the number of juveniles per replicate was ≤ 30 (observed 5.7 %). Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment: NOEC = 97.34 mg product/kg soil dw (corresponding to 40.22 mg a.s./kg soil dw)

Reference:	KCP 10.4.2
Report	Effects of Fludioxonil 480 FS on the reproduction of the predatory mite *Hypoaspis aculeifer*, Schulz L., 2017, report No 17 48 THC 0037.
Guideline(s):	Yes, OECD 226 (2016)
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable

Executive Summary

The purpose of this study was to determine potential effects of the test item on mortality and reproductive output of the soil mite species *Hypoaspis aculeifer* (CANESTRINI) as a representative species of soil microarthropods during a test period of 14 days.

Material and methods

Test item: Fludioxonil 480 FS, Batch No.: 3602
Content of a.i.: Fludioxonil: 480 g/L (496.9 g/L analysed)

Reference item: Dimethoate (EC 400 g/L, nominal)

Test species: *Hypoaspis aculeifer* (CANESTRINI)
age: adult female mites with an age difference of 2 days
source: in-house culture

Test system: Exposure of female mites to different concentrations of the test item mixed into artificial soil substrate

Test design: The effects of the test item on mortality and reproduction of the soil mite species *Hypoaspis aculeifer* (CANESTRINI) were investigated in a chronic laboratory experiment over a time period of 14 days according to OECD 226. Each of the eight different test item concentrations were homogeneously mixed into artificial soil and filled into glass vessels. Subsequently, the soil mites were introduced on top of the soil and the vessels were covered. Four replicates were performed for the test item groups and eight replicates for the control group; each replicate consisted of ten female soil mites. The mites were fed with *Tyrophagus putrescentiae* (SCHRANK) at the beginning and every two to three days during the whole test period.
For the main measured variable, the number of juveniles per test vessel and additionally the mortality of the adult female mites were determined. The reproductive output of the mites exposed to the test item was compared to that of the control in order to determine the no observed effect concentration (NOEC).
Assessment of adult mortality and reproduction effects was carried out after 14 days.

Endpoints: Mortality and number of juveniles

Test conditions: Artificial soil according to OECD 226, pH 5.9 - 6.1 at test start, pH 5.5 – 5.8 at test end; water content at test start 48.82 – 50.45% of maximum water holding capacity (WHC) and 45.86 – 47.77% of maximum WHC at test end; temperature 19.5 - 20.2°C; photoperiod: 16 h light : 8 h dark; light intensity: 507 lx.

Test concentrations: 1.59, 2.86, 5.15, 9.27, 16.69, 30.04, 54.08 and 97.34~~6.95, 12.51, 22.52, 40.53, 72.96, 131.32, 236.38, 425.48~~ mg test item/kg soil dry weight (spacing factor:

1.8)

Dates of work: Experimental start date: 3 July 2017
Experimental completion date: 24 July 2017

Statistics: Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm ($\alpha = 0.05$, one-sided greater) for mortality; Dunnett-t-test ($\alpha = 0.05$, one-sided smaller) for reproduction.
Statistical program: ToxRat Professional 3.2.1 (2015)

Results and discussion
All validity criteria for the study were met.

Mortality rates of 0.0 - 2.5 % were recorded in the test item treatment groups. In the control group the mortality rate was 0.0 %. The observed mortality rates in the test item treatment groups compared to control were not statistically significant (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$, one-sided greater). Differences in the behaviour and the morphology of the mites between the control and the test item treatment groups could not be observed.

Fourteen days after introduction of the parental mites into the test vessels, the mean number of juveniles was 324.5, 308.8, 301.5, 324.8, 294.8, 321.5, 319.5 and 318.0 at concentrations of 1.59, 2.86, 5.15, 9.27, 16.69, 30.04, 54.08 and 97.34 mg test item/kg soil d.w., respectively. The mean reproduction in the control reached 317.3 juveniles. The test item showed no statistically significantly adverse effects on reproduction at all tested concentrations (Dunnett-t-test, $\alpha = 0.05$, one-sided smaller).

In a separate study (BioChem project No. R 16 10 48 006 S, experimental start date: 13.01.2017), the EC_{50} (reproduction) of the reference item dimethoate (EC 400 g/L, nominal) was calculated to be 5.8 mg a.s./kg soil d.w. The results of the reference test demonstrate the sensitivity of the test system.

Table A23: Effects of Fludioxonil 480 FS on *Hypoaspis aculeifer* mortality and reproduction (day 14)

Endpoint	Treatment group [mg test item/kg soil dry weight]								
	Control	**1.59**	**2.86**	**5.15**	**9.27**	**16.69**	**30.04**	**54.08**	**97.34**
Mean adult mortality [%]	0.0	0.0	0.0	2.5	0.0	2.5	5.0	0.0	0.0
Mean number of juveniles (day 14)	317.3	324.5	308.8	301.5	324.8	294.8	321.5	319.5	318.0
Coefficient of variation [%]	5.7	5.7	8.2	11.5	2.6	12.9	6.4	8.3	6.8
Reproduction in [%] of control (day 14)	**100**	**102**	**97**	**95**	**102**	**93**	**101**	**101**	**100**
	Endpoints [mg test item/kg soil dry weight]								
NOEC (mortality)	97.34								
NOEC (reproduction)	97.34								
LC_{50} (mortality)	> 97.34								
EC_{10} (reproduction)	> 97.34								
EC_{20} (reproduction)	> 97.34								
EC_{50} (reproduction)	> 97.34								

Not statistically significantly different compared to the control (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm for mortality, $\alpha = 0.05$, one-sided greater and Multiple Sequentially-rejective U-test after Bonferroni-Holm for reproduction, $\alpha = 0.05$, onesided smaller)

Conclusion
In a 14-day *Hypoaspis aculeifer* reproduction study with Fludioxonil 480 FS, the LC50 and the EC10,

EC20 and EC50 values could not be calculated, but it can be concluded that these values are higher than 97.34 mg test item/kg soil dry weight. The NOEC for mortality and for reproduction was determined to be 97.34 mg test item/kg soil dry weight, the highest concentration tested.

A 2.3.2.1 KCP 10.4.2.1 Species level testing

A 2.3.2.2 KCP 10.4.2.2 Higher tier testing

A 2.4 KCP 10.5 Effects on soil nitrogen transformation

Comments of izRMS:	The study was performed fully in line with OECD 216 with no deviations. All validity criteria were met: • the variation between replicate control samples was ≤ 15 % (observed max. 6.2 %). Overall, the study is considered acceptable. It may be concluded that the effects of the test item on soil nitrogen formation rates were < 25 % at the end of the study period (28 days) up to 10.0 mg product/kg soil dw.

Reference:	KCP 10.5
Report	Effects of GLOB182F on the activity of soil microflora (Nitrogen transformation test). Schulz L., 2020, report No. 20 48 SMN 0041.
Guideline(s):	OECD 216 (2000)
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Executive summary

The purpose of this study was to determine the effects of the test item on the activity of soil microflora with regard to nitrogen transformation (mineralization) in a laboratory test over a period of 28 days of exposure.
The test was performed in accordance with the OECD Guideline 216 (2000) by measuring the nitrogen turnover.

Materials and methods

Test item:	GLOB182F
Study title:	Effects of GLOB182F on the activity of soil microflora (Nitrogen transformation test)
Guideline(s):	OECD 216 (2000)
Name of the test item:	GLOB182F
Batch No.:	PE 2004.742
Formulation type:	FS (flowable concentrate for seed treatment)
Active ingredient/content:	fludioxonil 100 g/L (nominal); 100.0 g/L (analysed)
Test soil:	Biologically active agricultural soil: loamy sand (DIN 4220) / loam (USDA), pH 6.3, 1.42 % C_{org}, WHC: 38.20 g/100 g dry soil.
Lucerne meal:	C/N ratio of 13.2/1

Test design:	The test was performed in accordance with the OECD Guideline 216 (2000). Aim of the study was the determination of the nitrogen transformation (NO_3-nitrogen-production) in soil enriched with lucerne meal (concentration in soil 0.5 %) by comparison of nitrogen transformation in test item treated soil with a non-treated soil. Three replicates per treatment and concentration. NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined by using the Autoanalyzer (SEAL Analytical). Sampling scheme: 0, 7, 14 and 28 days after treatment.
Test concentrations:	Control, 1 mg test item/kg soil dry weight and 10 mg test item/kg soil dry weight. Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3.
Endpoints:	Effects on NO_3-nitrogen-production after 28 days of exposure.
Reference item:	Dinoterb (purity: 99.28 % (g/g) analysed). The reference item was tested in a separate study (20 48 SMO 0001) at concentrations of 6.80, 13.60 and 27.20 mg/kg soil dry weight.
Test conditions:	Water content: approximately 45 % of its maximum water holding capacity; water content: 17.01 - 17.62 g/100 g dry soil; pH: 6.1 - 6.2 Soil samples were incubated at 19.7 - 20.7 °C, while stored in test vessels in the dark.
Statistics: coefficients	Calculation of mean values per treatment, standard deviations, of variation.
Dates of work:	Experimental start: 30.09.2020 Experimental end: 28.10.2020

Results and discussions

No adverse effects of the test item on nitrogen transformation in soil could be observed at both test concentrations (1 mg/kg soil dry weight and 10 mg/kg soil dry weight) after 28 days (time interval 14-28). The results are summarised in the table below.

Table A24: Effects on nitrogen transformation in soil after treatment with the test item

Time Interval (days)	**Control**	**1 mg GLOB182F/ kg soil dry weight**		**10 mg GLOB182F/ kg soil dry weight**	
	NO_3-N/day [mg/kg soil d.w.]	**NO_3-N/day [mg/kg soil d.w.]**	**% difference to control [1)]**	**NO_3-N/day [mg/kg soil d.w.]**	**% difference to control [1)]**
0-7	4.74	4.85	+2.4	4.79	+1.1
7-14	1.92	1.84	-4.2	2.07	+7.7
14-28	1.77	1.87	+5.5	1.75	-0.9

The calculations were performed with unrounded values

[1)] based on NO_3-N-production; - = inhibition; + = stimulation

In a separate study the reference item Dinoterb caused stimulations of nitrogen transformation of +59.9 %, +216.3 % and +238.5 % (required $\geq$ 25 %) at 6.80, 13.60 and 27.20 mg Dinoterb per kg soil dry weight, respectively, determined 28 days after application (time interval 14-28) and thus demonstrated the sensitivity of the test system.

Conclusion
The test item GLOB182F (tested at 1 mg/kg soil dry weight and 10 mg/kg soil dry weight) caused no adverse effects (deviation from control <25 %, OECD 216) on soil nitrogen transformation (measured as NO_3-N-production) at the end of the 28-day incubation period.

A 2.5 KCP 10.6 Effects on terrestrial non-target higher plants

A 2.5.1 KCP 10.6.1 Summary of screening data

A 2.5.2 KCP 10.6.2 Testing on non-target plants

A 2.5.3 KCP 10.6.3 Extended laboratory studies on non-target plants

A 2.6 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

A 2.7 KCP 10.8 Monitoring data