

FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: **FLUDIO 025 GF**

Product names: **FLUDIO ŽEL 025 FS /**

FUNABEN® ŽEL 025 FS

Chemical active substance:

Fludioxonil, 25 g/L

Central Zone

Zonal Rapporteur Member State: **Poland**

CORE ASSESSMENT

(authorization)

Applicant: **Synthos Agro Sp. z o.o.**

Submission date: **01/2023**

MS Finalisation date: **06/2023; 10/2023**

Version history

When	What
01/2023	Initial dRR submmited by Aplicant
06/2023	Initial zRMS assessment
10/2023	Corrected afler comments

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9 Ecotoxicology (KCP 10)

Justification regarding the difference in the formulation type between the product code name - FLUDIO 025 GF and the product trade names - FLUDIO ŽEL 025 FS, FUNABEN® ŽEL 025 FS is presented in Part C.

The product code name FLUDIO 025 GF is used in all draft Registration Report.

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

Use- No. (e)	Mem- ber state(s)	Crop and/ or situation (crop destina- tion / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I	Pests or Group of pests con- trolled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g safen- er/synergist per ha (f)	conclusion							
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. inter- val between applica- tions (days)	kg or L product / ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max			Birds	Mammals	Aquatic organisms	Bees	Non-target	Soil organisms	Non-target plants	
Zonal uses (field or outdoor uses, certain types of protected crops)																					
1	PL	Winter rye	F	<i>Fusarium spp.</i> <i>Urocystis occulta</i>	Seed treatment	BBCH 00	1	-	0.2-0.4 L/ha	Fludioxonil 5 – 10 g	-		200 mL/100 kg seeds Sowing rate: 100 – 200 kg seeds/ha	A	A	A	A	A	A	A	
2	PL	Winter wheat	F	<i>Fusarium spp.</i> <i>Monographella nivalis</i> <i>Tilletia caries</i>	Seed treatment	BBCH 00	1	-	0,3-0,5 L/ha	Fludioxonil: 7,5-12,5g	-		200 ml/100 kg seeds Sowing rate: 150-250 kg seeds/ha	A	A	A	A	A	A	A	
3	PL	Winter barley	F	<i>Fusarium spp.</i> <i>Monographella nivalis</i> <i>Pyrenophora graminea</i>	Seed treatment	BBCH 00	1	-	0,24-0,4 L/ha	Fludioxonil: 6-10g	-		200 ml/100 kg seeds Sowing rate: 120-200 kg	A	A	A	A	A	A	A	

Use- No. (e)	Mem- ber state(s)	Crop and/ or situation (crop destina- tion / purpose of crop)	F, Fn, G, Gn, Gpn or I	Pests or Group of pests con- trolled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g safen- er/synergist per ha (f)	conclusion						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. inter- val between applica- tions (days)	kg or L product / ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max			Birds	Mammals	Aquatic organisms	Bees	Non-target	Soil organisms	Non-target plants
													seeds/ha							
4	PL	Winter triticale	F	<i>Fusarium spp.</i>	Seed treatment	BBCH 00	1	-	0.2-0.4 L/ha	Fludioxonil 5 – 10 g	-		200 ml/100 kg seeds Sowing rate (triticale): 100- 200 kg seeds/ha	A	A	A	A	A	A	A
5	PL	Spring wheat	F	<i>Fusarium spp.</i> <i>Tilletia caries</i>	Seed treatment	BBCH 00	1	-	0,3-0,5 L/ha	Fludioxonil: 7,5-12,5g	-		200 ml/100 kg seeds Sowing rate: 150-250 kg seeds/ha	A	A	A	A	A	A	A
6	PL	Spring barley	F	<i>Fusarium spp.</i>	Seed treatment	BBCH 00	1	-	0,24-0,4 L/ha	Fludioxonil: 6-10g	-		200 ml/100 kg seeds Sowing rate: 120-200 kg seeds/ha	A	A	A	A	A	A	A

**Re-
marks
table
heading:**

(a) e.g. wettable powder (WP), emulsifiable concentrate (EC), granule (GR)

(b) Catalogue of pesticide formulation types and international coding system CropLife International Technical Monograph n°2, 6th Edition Revised May 2008

(c) g/kg or g/l

(d) Select relevant

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

(f) No authorization possible for uses where the line is highlighted in grey, Use should be crossed out when the notifier no longer supports this use.

Use- No. (e)	Mem- ber state(s)	Crop and/ or situation (crop destina- tion / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I	Pests or Group of pests con- trolled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g safen- er/synergist per ha (f)	conclusion							
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. inter- val between applica- tions (days)	kg or L product / ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max			Birds	Mammals	Aquatic organisms	Bees	Non-target	Soil organisms	Non-target plants	
Re- marks col- umns:	1	Numeration necessary to allow references						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												
	2	Use official codes/nomenclatures of EU Member States																			
	3	For crops, the EU and Codex classifications (both) should be used; when relevant, the use situation should be described (e.g. fumigation of a structure)						8	The maximum number of application possible under practical conditions of use must be provided.												
	4	F: professional field use, Fn: non-professional field use, Fpn: profes- sional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non- professional greenhouse use, I: indoor application						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.												
	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 develop- mental stages of the pests and pest groups at the moment of application must be named.						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												
	6	Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench						14	Remarks may include: Extent of use/economic importance/restrictions												
		Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, be- tween the plants - type of equipment used must be indicated.																			

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

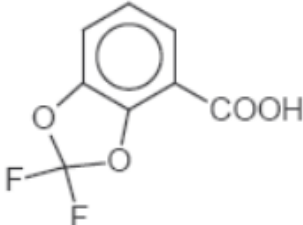
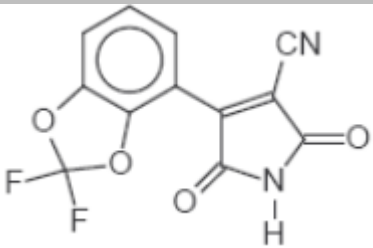
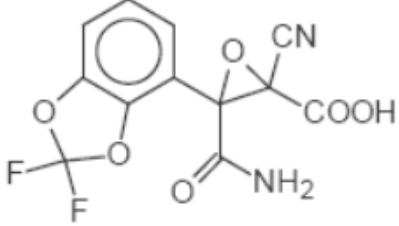
** F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application

Explanation for column 15 – 21 “Conclusion”

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

9.1.1 Overall conclusions

9.1.1.1 Table 9.1-3 Metabolites of fludioxonil

9.1.1.2 Me taboli te	Chemical structure	Molar mass [g/mol]	Maximum occurrence in compartments	Risk assessment required?
CGA 192155 (2,2-difluoro- benzo[1,3]dioxol- 4carbocyclic acid		202.1	Soil 11.7% Surface 17.3%	PEC _{soil} : risk for soil organisms PEC _{sw} : risk for aquatic organisms PEC _{gw} : leaching potential to groundwater
CGA 265378 4-(2,2-difluoro- benzo[1,3]dioxol-4-yl)- 2,5dioxo-2,5-dihydro- 1H-pyrrole-3carbonitrile		278.2	Soil 12.3 % Surface 3.8% /	PEC _{gw} : leaching potential to groundwater PEC _{soil} : risk for soil organisms
CGA 339833 3-carbamoyl-2-cyano-3- (2,2- difluorobenzo[1,3]dioxol- 4-yl)-oxirane- 2carbocyclic acid		312.2	Soil: 9.1% Surface/ groundwater: 1x10 ⁻⁶	PEC _{gw} : leaching potential to groundwater PEC _{sw} : risk for aquatic organisms

zRMS comment:

Information regarding to metabolites are in line with EU agreed data reported in EFSA Scientific Report (2007) 110. The metabolites CGA265378 and CGA339833 were formed via photodegradation in soil. However, due to the type of application as seed treatment photolysis will not play a major role in degradation of fludioxonil in soil. This metabolites formed exclusively via photolysis in soil may not be taken into account in exposure assessment for the intended uses of Fludio Žel 0.25 GF.

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)

An estimation of risk indicate low risk for birds and other terrestrial vertebrates of each range of assessed issues. Calculations conducted due to the influence of FLUDIO 025 GF due to the acute and long-term toxicity and reproductive did not indicate any hazardous properties and danger. There was also no negative effects regarding to drinking water exposure and effect of secondary poisoning. There is no influence

to evaluated organism regarding to dangerous to food poisoning. To protect birds/mammals the product must be entirely incorporated in the soil; ensure that the product is also fully incorporated at the end of rows.

9.1.1.3 Effects on aquatic organisms (KCP 10.2)

An estimation of risk indicate low risk for aquatic organism in each range of assessed issues. Calculations conducted due to the influence of FLUDIO 025 GF due to the acute and long-term toxicity did not indicate any hazardous properties and danger for aquatic organisms.

9.1.1.4 Effects on bees (KCP 10.3.1)

An estimation of risk indicate low risk for bees of each range of assessed issues. Calculations conducted due to the influence of FLUDIO 025 GF due to the acute oral and contact toxicity did not indicate any hazardous properties and danger. According to Commission regulation (EU) No 284/2013, point 10.3.1. (Effects on bees): Applicant should provide chronic test on bees and evaluation of effects on honey bee development with formulated product. The chronic studies weren't performed, therefore, for Poland, the deficiencies need to be fill by 31.12.2021.

9.1.1.5 Effects on arthropods other than bees (KCP 10.3.2)

Aleochara bilineata, spiders of genus *Pardosa* are organisms used to designation the initial assessment. The calculations present an acceptable risk to non-target arthropods, after application of FLUDIO 025 GF.

Aleochara bilineata, *Pardosa* spiders, are organisms used to designation the initial assessment.

9.1.1.6 The calculations present an acceptable risk to non-target arthropods, after application of FLUDIO 025 GF.

zRMS comment:

Studies on toxicity of Fludio Žel 0.25 FS formulation for seed treatment to non-target arthropods were evaluated by the RMS and considered acceptable.

The in-field risk is acceptable for relevant soil dwelling species. Therefore, an acceptable overall risk can be concluded for all uses.

In case of Fludioxonil 0.25 FS the formulation is applied as a seed treatment 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.

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

An estimation of risk indicate low risk for earthworms, other non-target soil organisms and microbial activity in soil in each range of assessed issues. Calculations conducted due to the influence of FLUDIO 025 GF due to the chronic toxicity did not indicate any hazardous properties and danger.

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

FLUDIO 025 GF is a fungicidal plant protection product and is to be used as a seed treatment product. Therefore there is no exposure risk for non-target terrestrial plants.

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

Not relevant.

9.1.2 Grouping of intended uses for risk assessment

The following table documents the grouping of the intended uses to support application of the risk envelope approach (according to SANCO/11244/2011).

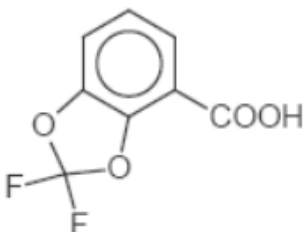
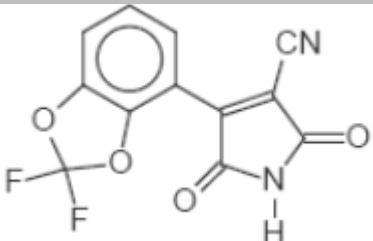
Table 0-1: Critical use pattern of FLUDIO 025 GF

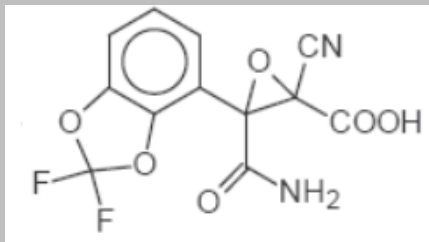
Grouping according to application rate		
Group	Intended uses	Recommended dose rate
Bare soil (treated seed)	Winter and spring cereals (Wheat, Barley, Triticale, Rye)	0.12 – 0.5 L/ha

9.1.3 Consideration of metabolites

A list of metabolites found in environmental compartments is provided below. The need for conducting a metabolite-specific risk assessment in the context of the evaluation of FLUDIO 025 GF is indicated in the table.

Table 9.1-3 Metabolites of fludioxonil

Metabolite	Chemical structure	Molar mass [g/mol]	Maximum occurrence in compartments	Risk assessment required?
CGA 192155 (2,2-difluoro-benzo[1,3]dioxol-4-carboxylic acid)		202.1	Soil 11.7% Surface 17.3%	PEC _{soil} : risk for soil organisms PEC _{sw} : risk for aquatic organisms PEC _{gw} : leaching potential to groundwater
CGA 265378 4-(2,2-difluoro-benzo[1,3]dioxol-4-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrole-3-carbonitrile		278.2	Soil 12.3 % Surface 3.8% /	PEC _{gw} : leaching potential to groundwater PEC _{soil} : risk for soil organisms

Metabolite	Chemical structure	Molar mass [g/mol]	Maximum occurrence in compartments	Risk assessment required?
CGA 339833 3-carbamoyl-2-cyano-3-(2,2-difluorobenzo[1,3]dioxol-4-yl)-oxirane-2carboxylic acid		312.2	Soil: 9.1% Surface/ groundwater: 1×10^{-6}	PEC _{gw} : leaching potential to groundwater PEC _{sw} : risk for aquatic organisms

zRMS comment:

Information regarding to metabolites are in line with EU agreed data reported in EFSA Scientific Report (2007) 110. The metabolites CGA265378 and CGA339833 were formed via photodegradation in soil. However, due to the type of application as seed treatment photolysis will not play a major role in degradation of fludioxonil in soil. This metabolites formed exclusively via photolysis in soil may not be taken into account in exposure assessment for the intended uses of Fludio Žel 0.25 GF.

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with difenoconazole and fludioxonil. Full details of these studies are provided in the respective EU Draft Assessment Report and related documents.

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

Species	Substance	Exposure System	Results	Reference
Mallard duck	fludioxonil	Oral, 1 d, Acute	LD ₅₀ > 2000 mg/kg bw	EFSA Journal Scientific Report (2007) 110
Bobwhite quail	fludioxonil	Dietary, 8 d, Short-term	LDD ₅₀ > 833 mg/kg bw/d	EFSA Journal Scientific Report (2007) 110
Bobwhite quail	fludioxonil	Dietary, Reproductive toxicity	NOAEL=62.8 mg/kg bw/d	EFSA Journal Scientific Report (2007) 110

9.2.1.1 Justification for new endpoints

9.2.2 Risk assessment for spray applications

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

9.2.2.1 Screening assessment (screening/generic focal species)

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

Since expected exposure is mostly dietary, calculations for acute as well as dietary toxicity is performed.

Since Screening assessment shows no risk for birds, no further calculations are performed.

According to EFSA Journal 2009; 7(12):1438 guideline, when consumption of seeds and newly emerged crop shoots (including roots and remaining seed) is likely to occur, it is necessary to conduct a risk assessment for granivorous and herbivorous birds using NAR, what is nominal loading/application rate of active substance in mg/kg seed.

NAR for the worst case scenario (0.5l/ha) is 50 mg a.s./kg seed

$TER_{acute} = LD50 / (NAR \times FIR/bw)$

$FIR/bw = 0.3$

Residues in seedling = $NAR/5 = 10$ mg a.s./kg

Table 9.2-2: Screening assessment of the acute and long-term/reproductive risk for birds due to the presence of FLUDIO 025 GF in seedling (winter and spring cereals)

Intended use		Bare soil (winter and spring cereals)					
Active substance/product		Fludioxonil					
Application rate (g/ha)		1 × 12.5					
Acute toxicity (mg/kg bw)		2000					
TER criterion		10					
Crop scenario Growth stage	Indicator/generic focal species		MAF	FIR/bw	NAR ×FIR/bw		TER _a
BBCH 00	Small granivorous bird		1	0.3	15		133
BBCH >10	Small omnivorous bird		1	0.5	5		400
Dietary toxicity (mg/kg bw)		833					
TER criterion		10					
Crop scenario Growth stage	Indicator/generic focal species		MAF	FIR/bw	NAR ×FIR/bw		TER _a
BBCH 00	Small granivorous bird		1	0.3	15		56
BBCH >10	Small omnivorous bird		1	0.5	5		167
Reprod. toxicity (mg/kg bw/d)		62.8					
TER criterion		5					
Crop scenario Growth stage	Indicator/generic focal species		MAF	FIR/bw	TWA	NAR ×FIR/bw× TWA	TER _{lt}
BBCH 00	Small granivorous bird		1	0.3	0.53	7.95	7.9
BBCH >10	Small omnivorous bird		1	0.5	0.53	2.65	23.69

TER value below the trigger are bolded.

Since TER_{lt} is over the trigger value, no further calculations have to be performed.

9.2.2.2 Higher-tier risk assessment

Not relevant.

9.2.2.3 Drinking water exposure

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

Leaf scenario

Since FLUDIO 025 GF is not a product for spray applications the leaf scenario does not have to be considered.

Puddle scenario

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

With a $K(f)_{oc}$ of 145000, fludioxonil belongs to the group of more sorptive substances.

Effective application rate (g/ha)=	12.5	
Acute toxicity (mg/kg bw) =	2000	quotient = 0.006
Reprod. toxicity (mg/kg bw/d) =	62.8	quotient = 0.20

9.2.2.4 Effects of secondary poisoning

The log P_{ow} of difenoconazole and fludioxonil are 4.4 and 4.12 respectively and thus exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

Risk assessment for earthworm-eating birds via secondary poisoning

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

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 bare soil (winter and spring cereals)

Parameter	fludioxonil	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.014 0.016	
$\log P_{ow} / P_{ow}$	4.4 4.12	
K_{oc}	145000 145600	
foc	0.02	Default
BCF_{worm}	0.057	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times P_{ow}) / foc \times K_{oc}$
PEC_{worm}	0.0008	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$

Parameter	fludioxonil	comments
Daily dietary dose (mg/kg bw/d)	0.0008	$DDD = PEC_{\text{worm}} \times 1.05$
NOEL (mg/kg bw/d)	62.8	
TER _{lt}	78 500	

TER values shown in bold fall below the relevant trigger.

Since TER_{lt} are above the trigger value of 5, low risk for earthworm-eating birds is considered

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 based on the regulatory acceptable concentration for aquatic organisms as a limit value for admissible concentrations of fludioxonil in 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 bare soil (winter and spring cereals)

Parameter	fludioxonil	comments
RAC (mg/L)	0.0005	
BCF _{fish}	0.57 366	
BMF	-	biomagnification factor (relevant for $BCF \geq 2000$)
PEC _{fish}	0.000285	$PEC_{\text{fish}} = RAC \times BCF_{\text{fish}}$
Daily dietary dose (mg/kg bw/d)	0.000045	$DDD = PEC_{\text{fish}} \times 0.159$
NOEL (mg/kg bw/d)	62.8	
TER _{lt}	1 395 556	

TER values shown in bold fall below the relevant trigger.

Since TER_{lt} are above the trigger value of 5, low risk for fish-eating birds is considered.

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Full risk assessment was performed in point 9.2.2.

9.2.4 Overall conclusions

An estimation of risk indicate low risk for birds of each range of assessed issues. Calculations conducted due to the influence of FLUDIO 025 GF due to the acute and long-term toxicity and reproductive did not indicate any hazardous properties and danger for birds. There was also no negative effects regarding to drinking water exposure and effect of secondary poisoning. There is no influence to evaluated organism regarding to dangerous to food poisoning.

zRMS comment:

Birds toxicity data are in line with EU agreed endpoints reported in EFSA Scientific Report (2007) 110. The risk assessment presented in Table 9.2.3 is accepted by the zRMS for uses of Fludio 025 GF as a seed treatment according uses in GAP for which acceptable acute and long-term risk is concluded. For metabolites formed via photolysis in soil not be their taken into account in the risk assessment for the intended in GAP.

The drinking water scenario is not required for seed treatment uses, however the Applicant performed assessment for precautionary reasons. The drinking water risk assessment provided by the Applicant in table above is agreed by the zRMS.

The approach of the Applicant in the evaluation of the risk of secondary poisoning for earthworm-eating birds presented above is correct performed calculations was in line with value reported in EFSA Scientific Report (2007) 110. Overall, the risk of secondary poisoning is concluded to be low.

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. Full details of these studies are provided in the respective EU Draft Assessment Report and related documents as well as in Section 6 (Mammalian Toxicology) of this report.

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

Species	Substance	Exposure System	Results	Reference
Rat SD	fludioxonil	Oral, 1 d, Acute	LD ₅₀ > 5000 mg/kg bw	EFSA Journal Scientific Report (2007) 110
Rats SD	fludioxonil	Dietary, Reproductive toxicity Two-generation study	NOAEL = 200 mg/kg bw/d	Draft Assessment Report for Fludioxonil (Denamrk 2006)

9.3.1.1 Justification for new endpoints

9.3.2 Risk assessment for spray applications

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

9.3.2.1 Screening assessment (screening/generic focal species)

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

According to EFSA Journal 2009; 7(12):1438 guideline, when consumption of seeds and newly emerged crop shoots (including roots and remaining seed) is likely to occur, it is necessary to conduct a risk as-

assessment for granivorous and herbivorous mammals using NAR, what is nominal loading/application rate of active substance in mg/kg seed.

NAR for the worst case scenario (0.5l/ha) is 50 mg/kg seed

$$TER_{acute} = LD50 / (NAR \times FIR/bw)$$

$$FIR/bw = 0.24$$

$$\text{Residues in seedling} = NAR/5 = 10 \text{ mg a.s./kg}$$

Table 9.3-2: Screening assessment of the acute and long-term/reproductive risk for mammals due to presence of FLUDIO 025 GF in seedlings (winter and spring cereals)

Intended use		Bare soil (winter and spring cereals)				
Active substance/product		Fludioxonil				
Application rate (g/ha)		1 × 12.5				
Acute toxicity (mg/kg bw)		5000				
TER criterion		10				
Crop scenario Growth stage	Indicator/generic focal species	MAF	FIR/bw	NAR ×FIR/bw		TER _a
BBCH 00	Small omnivorous mammal	1	0.24	12		417
BBCH >10	Small omnivorous mammal	1	0.24	2.4		2083
Reprod. toxicity (mg/kg bw/d)		200				
TER criterion		5				
Crop scenario Growth stage	Indicator/generic focal species	MAF	TWA	FIR/bw	NAR ×FIR/bw× TWA	TER _{lt}
BBCH 00	Small omnivorous mammal	1	0.53	0.24	6.36	31
BBCH >10	Small omnivorous mammal	1	0.53	0.24	1.27	157

Since TER_{lt} is over the trigger value, no further calculations have to be performed.

9.3.2.2 Higher-tier risk assessment

Not relevant

9.3.2.3 Drinking water exposure

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

Leaf scenario

Since FLUDIO 025 GF is not a product for spray applications the leaf scenario does not have to be considered.

Puddle scenario

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

With a $K(f)_{oc}$ of 145000, fludioxonil belongs to the group of more sorptive substances.

Effective application rate (g/ha) =	12.5	
Acute toxicity (mg/kg bw) =	5000	quotient = 0.0025
Reprod. toxicity (mg/kg bw/d) =	200	quotient = 0.063

9.3.2.4 Effects of secondary poisoning

The $\log P_{ow}$ of fludioxonil is 4.12 and thus exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

Risk assessment for earthworm-eating mammals via secondary poisoning

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

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 bare soil (winter and spring cereals)

Parameter	fludioxonil	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.014	
$\log P_{ow} / P_{ow}$	4.14/13804	
K_{oc}	145000	
foc	0.02	Default
BCF_{worm}	0.57 366	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.12 \times P_{ow}) / foc \times K_{oc}$
PEC_{worm}	0.008	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.01	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	200	
TER_{lt}	20 000	

TER values shown in bold fall below the relevant trigger.

Since TER_{lt} are above the trigger value of 5, low risk for earthworm-eating mammals is considered.

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 based on the regulatory acceptable concentration for aquatic organisms as a limit value for admissible concentrations of difenconazole and fludioxonil in water.

Table 9.3-4: Assessment of the risk for fish-eating mammals due to ~~exposure to difenoecon-azole-and~~ fludioxonil via bioaccumulation in fish (secondary poisoning) for the intended use in bare soil (winter and spring cereals)

Parameter	fludioxonil	comments
RAC	0.0005	
BCF _{fish}	366	
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	0.000285	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	0.00004	DDD = PEC _{fish} × 0.142
NOEL (mg/kg bw/d)	200	
TER _{lt}	5 000 000	

TER values shown in bold fall below the relevant trigger.

Since TER_{lt} are above the trigger value of 5, low risk for fish-eating mammals is considered.

9.3.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.3.4 Overall conclusions

An estimation of risk indicate low risk for mammals of each range of assessed issues. Calculations conducted due to the influence of FLUDIO 025 GF due to the acute and long-term toxicity and reproductive did not indicate any hazardous properties and danger for birds. There was also no negative effects regarding to drinking water exposure and effect of secondary poisoning. There is no influence to evaluated organism regarding to dangerous to food poisoning.

zRMS comment:

Mammals toxicity data are in line with EU agreed endpoints reported in EFSA Scientific Report (2007) 110. The risk assessment presented in Tables above is accepted by the zRMS for uses of Fludio 025 GF as a seed treatment according uses in GAP for which acceptable acute and long-term risk is concluded. For metabolites formed via photolysis in soil not be their taken into account in the risk assessment for the intended in GAP.

The drinking water scenario is not required for seed treatment uses, however the Applicant performed assessment for precautionary reasons. The drinking water risk assessment provided by the Applicant in table above is agreed by the zRMS.

The approach of the Applicant in the evaluation of the risk of secondary poisoning for earthworm-eating birds presented above is correct performed calculations was in line with value reported in EFSA Scientific Report (2007) 110. Overall, the risk of secondary poisoning is concluded to be low.

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

Not relevant.

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 FLUDIO 025 GF 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.

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
<i>Oncorhynchus mykiss</i>	fludioxonil	96 h, f	LC ₅₀ = 0.23 mg a.s./L _{mm}	EFSA Scientific Report (2007) 110
<i>Oncorhynchus mykiss</i>	CGA 339833	96 h, s	LC ₅₀ >100 mg a.s./L _{mm}	EFSA Scientific Report (2007) 110
<i>Oncorhynchus mykiss</i>	CGA 192155	96 h, s	LC ₅₀ >100 mg a.s./L _{mm}	EFSA Scientific Report (2007) 110
<i>Pimephales promelas</i>	fludioxonil	28 d, f	NOEC = 0.039 mg a.s./L _{mm}	EFSA Scientific Report (2007) 110
<i>Mysidopsis bahia</i>	fludioxonil	96h, s	EC ₅₀ = 0.27 mg a.s./L _{mm}	EFSA Scientific Report (2007) 110
<i>Daphnia magna</i>	fludioxonil	48 h, f	EC ₅₀ = 0.4 mg a.s./L _{mm}	EFSA Scientific Report (2007) 110
<i>Daphnia magna</i>	CGA339833	48 h, s	EC ₅₀ > 100 mg a.s./L _{mm}	EFSA Scientific Report (2007) 110
<i>Daphnia magna</i>	CGA192155	48 h, s	EC ₅₀ > 100 mg a.s./L _{mm}	EFSA Scientific Report (2007) 110
<i>Daphnia magna</i>	fludioxonil	21 d, ss	NOEC = 0.005 mg a.s./L _{mm}	EFSA Scientific Report (2007) 110
<i>Chironomus riparius</i>	fludioxonile	28 d, spiked sediment	NOEC _{water} = 0.2 mg a.s./L _{nom} NOEC _{sediment} = 40 mg a.s./kg sed	EFSA Scientific Report (2007) 110
<i>Selenastrum capricornutum</i>	fludioxonil	120 h, s	E _r C ₅₀ = 0.33 mg a.s./L _{mm} E _b C ₅₀ = 0.024 mg a.s./L _{mm}	EFSA Scientific Report (2007) 110
<i>Scenedesmus subspicatus</i>	CGA 192155	96 h, s	ErC50 > 100 mg a.s./L	EFSA Scientific Report (2007) 110

Species	Substance	Exposure System	Results	Reference
<i>Selenastrum capricornutum</i>	CGA 339833	72 h, s	E _r C ₅₀ = 104.7 mg a.s./L _{mm} E _b C ₅₀ = 95.8 mg a.s./L _{mm}	EFSA Scientific Report (2007) 110

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 – FLUDIO 025 GF

Species	Substance	Exposure System	Results	Reference
<i>Oncorhynchus mykiss</i>	FLUDIO 025 GF	96 h, ss	LC ₅₀ = 8.92 mg/L _{nom} LC ₅₀ = 0.195 a.s. mg/L _{mm}	Nierzędska E., 2022, W-33-21
<i>Daphnia magna</i>	FLUDIO 025 GF	48 h, s	EC ₅₀ = 104.66 mg/L _{nom}	Hodorek G., 2022, W-31-21
<i>Pseudokirchneriella subcapitata</i>	FLUDIO 025 GF	72 h, s	E _r C ₅₀ = 19.7 mg/L _{nom} E _y C ₅₀ = 9.8 mg/L _{nom} E _r C ₅₀ = 0.443 mg a.s./L _{mm} E _y C ₅₀ = 0.212 mg a.s./L _{mm}	Hodorek G., 2022, W-32-21

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

zRMS comment:

The studies performed with *Oncorhynchus mykiss*, *Daphnia magna* and *Pseudokirchneriella subcapitata* demonstrated that formulation 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 was not required for fungicides.

9.5.1.1 Justification for new endpoints

Not relevant.

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

Since TER calculations proposed in SANCO/10553/2012 for treated seeds is less rigorous than condi-

tions for spray application risk assessment, it was not used for calculations.

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

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

Table 9.5-3: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for fludioxonil for each organism group based on FOCUS Steps 1 calculations for the use of FLUDIO 025 GF in bare soil

Group		Fish acute	Fish pro- longed	Inverteb. acute		Inverteb. prolonged	Algae	Sed. dwell. prolonged
Test species		<i>Oncorhynchus mykiss</i>	<i>Pimephales promelas</i>	<i>Daphnia magna</i>	<i>Mysidopsis bahia</i>	<i>Daphnia magna</i>	<i>Pseudokirchn. subcapitata</i>	<i>Chironomus riparius</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	EC ₅₀	NOEC	E _r C ₅₀	NOEC
(µg/L)		495 230	39	400	270	5	330	200
AF		100	10	100	100	10	10	10
RAC (µg/L)		4.95 2.30	3.9	4.0	2.7	0.5	33	20
FOCUS Scenario	PEC _{gl-max} (µg/L)							
Step 1								
	0.02	0.01	0.0051	0.005	0.007	0.04	0.0006	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

Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for CGA 192155 for each organism group based on FOCUS Steps 1 calculations for the use of FLUDIO 025 GF in bare soil

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Scenedesmus subspicatus</i>
Endpoint		LC ₅₀	EC ₅₀	E _r C ₅₀
(µg/L)		100000	100000	100000
AF		100	100	10
RAC (µg/L)		1000	1000	10000
FOCUS Scenario	PEC _{gl-max} (µg/L)			
Step 1				
	0.95	0.00095	0.00095	0.000095

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

Table 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for CGA 339833 for each organism group based on FOCUS Steps 1 calculations for the use of FLUDIO 025 GF in bare soil

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Scenedesmus subspicatus</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₅₀ 100000	E _r C ₅₀ 104700
AF		100	100	10
RAC (µg/L)		1000	1000	10470
FOCUS Scenario	PEC _{gl-max} (µg/L)			
Step 1				
	0.47	0.0005	0.0005	0.00004

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 bare soil, calculated PEC/RAC ratios indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for prolonged exposure of *Daphnia magna*) in all FOCUS Steps 1 scenarios. Therefore, no further assessment is necessary.

9.5.3 Overall conclusions

An estimation of risk indicate low risk for aquatic organism of each range of assessed issues. Calculations conducted due to the influence of FLUDIO 025 GF due to the acute and long-term toxicity did not indicate any hazardous properties and danger for aquatic organisms.

zRMS comment:

The risk assessment provided by the Applicant in tables above is accepted by the zRMS. In performed calculations the overall maximum PEC_{sw}/sed values were used to covering all intended uses. It is also noted that in the risk assessment for algae from fludioxonil the E_rC₅₀ value was used according recommended by EFSA aquatic guidance (2013).

The acceptable risk could be concluded for fludioxonil and metabolite CGA192155 following application of Fludio Žel 025 GF as seed treatment for proposed GAP and no need for risk mitigation measures.

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 Draft Assessment Report and related documents.

Effects on bees of FLUDIO 025 GF 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.

Since FLUDIO 025 GF is to be used as a seed treatment, bees cannot be directly exposed, but they can have contact with active substance in dust particles. Therefore calculations for product as well as for active substances are performed.

Table 9.6-1: Endpoints and effect values relevant for the risk assessment for bees

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i>	fludioxonil	Oral	LD ₅₀ > 100 µg/bee	EFSA Scientific Report (2007) 110
<i>Apis mellifera</i>	fludioxonil	Contact	LD ₅₀ > 100 µg/bee	EFSA Scientific Report (2007) 110
<i>Apis mellifera</i>	FLUDIO 025 GF	Oral	LD ₅₀ > 200 µg/bee	Kulec-Płoszczyca, E. 2022, B-03-22
<i>Apis mellifera</i>	FLUDIO 025 GF	Contact	LD ₅₀ > 200 µg/bee	Kulec-Płoszczyca, E. 2022, B-04-22
<i>Apis mellifera</i>	FLUDIO 025 GF	Chronic oral	LDD ₅₀ > 20 µg/bee	Kulec-Płoszczyca, E. 2022, B-02-22
<i>Apis mellifera</i>	FLUDIO 025 GF	Larval repeated	NOED ₅₀ > 100 µg/bee	Kulec-Płoszczyca, E. 2022, B-01-22

Species	Substance	Exposure System	Results	Reference
<i>Bombus terrestris</i>	FLUDIO 025 GF	Oral	LD ₅₀ > 200 µg/bee	Kulec-Płoszczyca, E. 2022, B-67-21
<i>Bombus terrestris</i>	FLUDIO 025 GF	Contact	LD ₅₀ > 200 µg/bee	Kulec-Płoszczyca, E. 2022, B-68-21

9.6.1.1 Justification for new endpoints

9.6.2 Risk assessment

Formulas used to calculation:

$$Q_{HO} = \frac{\text{Single application rate [g/ha]}}{LD_{50}}$$

$$Q_{HC} = \frac{\text{Single application rate [g/ha]}}{LD_{50}}$$

Table 9.6-2: First-tier assessment of the risk for bees due to the use of formulation in bare soil

Intended use	Bare soil (Winter and spring cereals)		
Active substance/ product	FLUDIO 025 GF		
Application rate (g/ha)	12.5 × 1		
Test design	LD ₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Fludio Žel 025 GF			
Oral toxicity	>100	12.5	0.125
Contact toxicity	>100	12.5	0.125
FLUDIO 025 GF			
Oral toxicity	>200	25 12.5	0.125 0.0625
Contact toxicity	>200	25 12.5	0.125 0.0625

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

The Q_{HO}, Q_{HC} values for FLUDIO 025 GF are below the trigger value recommended by the EFSA Journal 2012; 10(5):2668, and therefore the risk for bees is regarded as acceptable.

According to Environmental risk assessment scheme for plant protection products - Chapter 10: Honey-bees – Proposed scheme, FLUDIO 025 GF is to be used as a seed treatment, therefore bees cannot be directly exposed. Moreover, each crop proposed in the GAP, is not attractive to bees, then even in case of systemic plant protection products, bees are not exposed.

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

Not relevant.

9.6.3 Effects on bumble bees

Not relevant.

9.6.4 Effects on solitary bees

Not relevant.

9.6.5 Overall conclusions

An estimation of risk indicate low risk for bees of each range of assessed issues. Calculations conducted due to the influence FLUDIO 025 GF due to the acute oral and contact toxicity did not indicate any hazardous properties and danger.

zRMS comment:

Data on the toxicity to bees have been carried out with fludioxonil. Full details of these studies are provided in the respective EU Draft Assessment Report and related documents.
Studies on toxicity of Fludio Žel 0.25 FS were evaluated by the zRMS and considered acceptable.
No separate acute risk assessment scheme for bees for seed treatment is provided in SANCO/10329/2002 rev. 2 final. The evaluation of Fludio Žel 0.25 FS has been carried out using assumptions for spray treatments and is considered acceptable by the zRMS since extremely worst case assumptions were made that bees. This approach covers assumptions made for the acute risk assessment according to EFSA (2013). No additional calculations were performed since acceptable risk could be concluded based on these worst case.

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

Effects on non-target arthropods of FLUDIO 025 GF 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.

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

Species	Substance	Exposure System	Results	Reference
<i>Aleochara bilineata</i>	FLUDIO 025 GF	Product mixed with sand 9 weeks exposure	ER ₅₀ > 4.5 L/ha NOER ≥ 4.5 L/ha	Nácarová, J., 2022, 21/350
<i>Pardosa</i> spider	FLUDIO 025 GF	Coated seeds placed in sand (14 d)	ER ₅₀ > 4.5 L/ha NOER ≥ 4.5 L/ha	Diáková, K. 2022, 21/349

9.7.1.1 Justification for new endpoints

No deviation from EU agreed endpoints.

9.7.2 Risk assessment

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

9.7.2.1 Risk assessment for in-field exposure

Table 9.7-2: First-tier assessment of the in-field risk for non-target arthropods due to the use of FLUDIO 025 GF in bare soil (winter and spring cereals)

Intended use	Bare soil (seed treatment)		
Active substance/product	FLUDIO 025 GF		
Application rate (mL/ha)	1 × 500		
MAF	1		
Test species Tier I	NOER (lab.) (mL /ha)	PER_{in-field} (mL/ha)	Acceptable risk
<i>Aleochara bilineata</i>	> 4500	500	Yes
<i>Pardosa</i> spider	> 4500		Yes

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

The risk for non-target arthropods after application of FLUDIO 025 GF is considered to be low.

9.7.2.2 Additional higher-tier risk assessment

Not relevant.

9.7.2.3 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

Aleochara bilineata, *Pardosa* spiders, are organisms used to designation the initial assessment.

The calculations present an acceptable risk to non-target arthropods, after application of FLUDIO 025 GF.

zRMS comment:

Studies on toxicity of Fludio Žel 0.25 FS formulation for seed treatment to non-target arthropods were evaluated by the RMS and considered acceptable.

The in-field risk is acceptable for relevant soil dwelling species. Therefore, an acceptable overall risk can be concluded for all uses.

In case of Fludioxonil 0.25 FS the formulation is applied as a seed treatment 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.

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

9.8.1 Toxicity data

Studies on the toxicity to earthworms have been carried out with fludioxonil and its relevant metabolites. Full details of these studies are provided in the respective EU Draft Assessment Report and related documents.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of FLUDIO 025 GF 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.

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

Species	Substance	Exposure System	Results	Reference
<i>Eisenia foetida</i>	fludioxonil	Mixed into substrate 56 d, chronic 10 % peat content	NOEC > 20 mg a.s./kg NOEC _{corr} > 10 mg/kg dw*	EFSA Scientific Report (2007) 110
<i>Eisenia foetida</i>	fludioxonil	Mixed into substrate 14 d, acute 10 % peat content	LC ₅₀ > 1000 mg/kg	EFSA Scientific Report (2007) 110
<i>Eisenia foetida</i>	CGA 265378	Mixed into substrate 14 d, acute 10 % peat content	LC ₅₀ > 1000 mg/kg	EFSA Scientific Report (2007) 110
<i>Eisenia foetida</i>	CGA 192155	Mixed into substrate 14 d, acute 10 % peat content	LC ₅₀ = 794 mg/kg	EFSA Scientific Report (2007) 110
<i>Eisenia fetida</i>	FLUDIO 025 GF	Mixed into substrate / Overspray 56 d, chronic 10 % peat content	NOEC ≥ 1000 mg/kg dw NOEC _{corr} ≥ 500 mg/kg dw*	Wróbel A., 2022, G-23-21
<i>Hypoaspis aculeifer</i>	FLUDIO 025 GF	Mixed into substrate 14 d, chronic	NOEC _{reprod.} ≥ 1000 mg/kg dw NOEC _{corr} ≥ 500 mg/kg dw*	Wróbel A., 2022, G-25-21
<i>Folsomia candida</i>	FLUDIO 025 GF	Mixed into substrate 28 d, chronic	NOEC _{reprod.} ≥ 1000 mg/kg dw NOEC _{corr} ≥ 500 mg/kg dw*	Gierbuszewska A., 2022, G-24-21

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

9.8.1.1 Justification for new endpoints

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 8.7.1, According to the assessment of environmental-fate data, multi-annual accumulation in soil is 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 FLUDIO 025 GF in bare soil (winter and spring cereals)

Intended use	Bare soil (winter and spring cereals)		
Acute effects on earthworms			
Product/active substance	LC ₅₀ (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _a (criterion TER ≥ 10)
fludioxonil	1000	0.047	21277
CGA 192155	794	0.002	397000
CGA 265378	1000	0.002	500000
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
fludioxonil	40 NOEC ≥ 20 mg/kg dw NOEC _{corr} ≥ 10 mg/kg dw*	0.047	213
FLUDIO 025 GF	500	0.71**	704
Chronic effects on non-target soil organisms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
FLUDIO 025 GF (<i>Hypoaspis aculeifer</i>)	500	0.71**	704
FLUDIO 025 GF (<i>Folsomia candida</i>)	500	0.71**	704

TER values shown in bold fall below the relevant trigger.

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

**PECs for formulation

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

The calculated acute and chronic TER values for fludioxonil and its metabolites are above the trigger value of 10 (acute) and 5 (chronic). Calculated values for other non-target soil organisms also indicate acceptable risk indicating acceptable risk from the proposed uses of FLUDIO 025 GF.

zRMS comment:

Since acute toxicity to earthworms is no longer a data requirement, the acute endpoints were struck through as not considered in the risk assessment.

The long-term TER value exceed the Annex VI long-term trigger value of 5 indicating that Fludio Žel 025 GF poses long-term risk to earthworms and other non-target soil organisms (meso- and macrofauna) 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 soil microorganisms 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 soil microorganisms of FLUDIO 025 GF 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.

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	97 d, aerobic soil type	Nitrate formation rate 1.3 mg as/kg soil ± 20 %	EFSA Scientific Report (2007) 110
N-mineralisation	CGA-192155	28 d, aerobic soil type	Nitrate formation rate 0.353 mg as/kg soil <25%	EFSA Scientific Report (2007) 110
N-mineralisation	CGA-265378	28 d, aerobic soil type	Nitrate formation rate 0.37 mg/kg soil <25%	EFSA Scientific Report (2007) 110
N-mineralisation	FLUDIO 025 GF	28 d, aerobic soil type	<25% effect at day 28 at 3.55 mg/kg dw soil	Gierbuszewska A., 2022, G-26-21

9.9.1.1 Justification for new endpoints

9.9.2 Risk assessment

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

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.1 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of FLUDIO 025 GF in bare soil (winter and spring cereals)

Intended use			
N-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
formulation	3.55 (at 28 d)	0.71	yes

9.9.3 Overall conclusions

On the basis of results it was assessed that FLUDIO 025 GF in considered applications does not pose unacceptable risk to soil microorganisms.

The risk to soil micro-organisms is considered to be low for all representative uses.

zRMS comment:

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 formulation Fludio Žel 025 GF on soil nitrogen turnover was evaluated by the zRMS 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.

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

9.10.1 Toxicity data

Studies on the toxicity to non-target terrestrial plants have not been carried out with fludioxonil.

However, the provision of further data on the FLUDIO 025 GF is not considered essential, because the active substance fludioxonil is fungicide and have no impact on the terrestrial plants.

zRMS comment:

Since Fludioxonil is applied as a seed treatment, exposure via dust drift is relevant. However, no risk assessment is conducted for exposure via dust drift because there is no zonal agreement on this point.

9.10.1.1 Justification for new endpoints

9.10.2 Risk assessment

Due to the low toxicity and the low exposure from a seed treatment no further assessment is required

9.10.2.1 Tier-1 risk assessment (based screening data)

Not relevant.

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

Not relevant.

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

No risk mitigation needed.

9.10.3 Overall conclusions

Based on risk assessment regarding effects on non-target terrestrial plants, no risk mitigation needed.

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

Not relevant.

9.12 Monitoring data (KCP 10.8)

9.13 Classification and Labelling

FLUDIO 025 GF was classified and labeled according to REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.


According to result of acute toxicity studies for FLUDIO 025 GF, the product can be classified as non-acutely toxic to aquatic species (Endpoints are over 1mg of product/L).

For chronic classification of FLUDIO 025 GF mixtures classification method was used.

Chronic Category 1 (concentration of fludioxonil multiplied by its corresponding M-factor of 10 is lower than 25%).

Chronic Category 2 (concentration of fludioxonil multiplied by its corresponding M-factor of 10 and additional factor of 10 is higher than 25%).

CLASSIFICATION	
Hazard classes, categories:	Aquatic Chronic 2

LABELLING	
Hazard pictograms:	 GHS09
Signal word:	Warning
Hazard statements:	H411 – Toxic to aquatic life with long lasting effects
Precautionary statements:	P273 – Avoid release to the environment. P391 - Collect spillage. P501 - Dispose of contents/container to an approved waste disposal plant.

Standard phrases under Regulation (EU) No 547/2011

SP 1	Do not contaminate water with the product or its container (Do not clean application equipment near surface water/Avoid contamination via drains from farmyards and roads).
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Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2	Hodorek G.	2022	FLUDIO 025 GF: <i>Raphidocelis subcapitata</i> SAG 61.81 (formerly <i>Pseudokirchneriella subcapitata</i>), Growth inhibition test W-32-21 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna GLP Unpublished	N	Synthos Agro Sp. z o.o.
KCP 10.2	Hodorek G.	2022	FLUDIO 025 GF: <i>Daphnia magna</i> , acute immobilisation test W-31-21 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna GLP Unpublished	N	Synthos Agro Sp. z o.o.
KCP 10.2	Nierzędska Ewa	2022	FLUDIO 025 GF: <i>Rainbow trout</i> , acute toxicity test W-33-21 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna GLP Unpublished	Y	Synthos Agro Sp. z o.o.
KCP 10.3.1	Kulec-Płoszczyca Elżbieta	2022	FLUDIO 025 GF: Honeybees (<i>A. mellifera</i>), Acute oral toxicity test B-03-22	N	Synthos Agro Sp. z

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
			Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna GLP Unpublished		o.o.
KCP 10.3.1	Kulec-Płoszczyca Elżbieta	2022	FLUDIO 025 GF: Honeybees (<i>A. mellifera</i>), Acute contact toxicity test B-04-22 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna GLP Unpublished	N	Synthos Agro Sp. z o.o.
KCP 10.3.1	Kulec-Płoszczyca Elżbieta	2022	FLUDIO 025 GF: Bumblebees (<i>Bombus</i> spp.), Acute oral toxicity test B-67-21 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna GLP Unpublished	N	Synthos Agro Sp. z o.o.
KCP 10.3.1	Kulec-Płoszczyca Elżbieta	2022	FLUDIO 025 GF: Bumblebees (<i>Bombus</i> spp.), Acute contact toxicity test B-68-21 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna GLP Unpublished	N	Synthos Agro Sp. z o.o.
KCP 10.3.1.2	Kulec-Płoszczyca Elżbieta	2022	FLUDIO 025 GF: Honeybees (<i>A. mellifera</i>), Chronic oral toxicity test B-02-22 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna GLP Unpublished	N	Synthos Agro Sp. z o.o.
KCP 10.3.1.3	Kulec-Płoszczyca Elżbieta	2022	FLUDIO 025 GF: Honeybees (<i>A. mellifera</i>), Larval toxicity test - repeated B-01-22 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna GLP Unpublished	N	Synthos Agro Sp. z o.o.
KCP 10.3.1.3	Kulec-Płoszczyca Elżbieta	2022	FLUDIO 025 GF: Honeybees (<i>A. mellifera</i>), Acute Oral Toxicity Test B-03-22 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna GLP Unpublished	N	Synthos Agro Sp. z o.o.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.1.3	Kulec-Płoszczyca Elżbieta	2022	FLUDIO 025 GF: Honeybees (<i>A. mellifera</i>), Acute Contact Toxicity Test B-04-22 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna GLP Unpublished	N	Synthos Agro Sp. z o.o.
KCP 10.3.2	Diáková Kateřina	2022	GLP laboratory study to determine effects of a plant protection product FLUDIO 025 GF on spiders of the genus <i>Pardosa</i> (Aranea, Lycosidae) Study code: 21/349 i2L Research Europe s.r.o. GLP Unpublished	N	Synthos Agro Sp. z o.o.
KCP 10.3.2	Nácarová Jana	2022	GLP laboratory study to determine the effect of a plant protection product FLUDIO 025 GF on the entomophagous rove beetle <i>Aleochara bilineata</i> ; Study code: 21/350 i2L Research Europe s.r.o. GLP Unpublished	N	Synthos Agro Sp. z o.o.
KCP 10.4	Gierbuszewska Aneta	2022	FLUDIO 025 GF: Collembolan (<i>Folsomia candida</i>) Reproduction Test G-24-21 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna GLP Unpublished	N	Synthos Agro Sp. z o.o.
KCP 10.4	Wróbel Anna	2022	FLUDIO 025 GF: Earthworm reproduction test (<i>Eisenia andrei</i>) G-23-21 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna GLP Unpublished	N	Synthos Agro Sp. z o.o.
KCP 10.4	Wróbel Anna	2022	FLUDIO 025 GF: Predatory mite (<i>Hypoaspis (Geolaelaps) Aculeifer</i>) reproduction test in soil G-25-21 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna GLP Unpublished	N	Synthos Agro Sp. z o.o.
KCP 10.5	Gierbuszewska Aneta	2022	FLUDIO 025 GF: Soil Microorganisms: Nitrogen Transformation Test G-26-21 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna GLP Unpublished	N	Synthos Agro Sp. z o.o.

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
KCP 10.1.1	Haking, B., et al.	1990	The acute oral toxicity (LD50) of CGA173506 to the Mallard duck. Syngenta Report No. CGA173506/0064 Unpublished GLP	Y	Syngenta
KCP 10.1.1	Haking, B., et al.	1993b	The dietary toxicity (LC50) of CGA173506 to the bob-white quail. Syngenta Report CGA173506/0062 Unpublished GLP	Y	Syngenta
KCP 10.1.1	Johnson, A. et al.	1996a	Bobwhite quail dietary reproduction and tolerance studies. Syngenta report No. SGA173506/0355 Unpublished GLP	Y	Syngenta
KCP 10.1.2	Glaza, S.M.	1991	Acute oral toxicity study of CGA-173506 Technical in rats (EPA Guidelines) Report No. HW1 10200144 Published GLP	Y	Syngenta
KCP 10.1.2	Singh, A.R., et al.	1992	A two generation reproductive toxicity study in rats Report No 902001 Published GLP	Y	Syngenta
KCP 10.2	Biever, R.C.	1997a	CGA 173506 technical – acute toxicity to rainbow trout, <i>Oncorhynchus mykiss</i> , under flow thru conditions Syngenta Unpublished report No. 173506/0926 Unpublished report no. 276-89 GLP	Y	Syngenta
KCP 10.2	Grade, R.	2000a	Acute toxicity test of CGA 339833 (metabolite of CGA 173506) to the cladoceran <i>Daphnia magna</i> Straus in the static system. Syngenta unpublished report No. CGA339833/006 Unpublished report no 2003512 GLP	N	Syngenta
KCP 10.2	Grade, R	2000b	Growth inhibition of CGA 339833 (metabolite of CGA 173506) to green algae, <i>Selenastrum capricornutum</i> , under static conditions Syngenta unpublished report No. CGA339833/0004 GLP	N	Syngenta
KCP 10.2	Graves, C.W., et al.	1994	CGA-173506, An early life-stage toxicity test with the fathead minnow (<i>Pimephales promelas</i>)	Y	Syngenta

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
			Syngenta Unpublished report No. CGA173506/0416 Unpublished report No. 108A-153 GLP		
KCP 10.2	Hoberg, J.R.	1992	CGA-173506 technical – Toxicity to the fresh water green alga, <i>Selenastrum capricornutum</i> , Syngenta Unpublished report CGA173506/2043 GLP	N	Syngenta
KCP 10.2	Rufli, H.	2000	Acute toxicity test of CGA 339833 (metabolite of CGA 173506) to rainbow trout (<i>Oncorhynchus mykiss</i>) under static conditions. Syngenta Unpublished report No. CGA 339833/0005 Unpublished report 2003513 GLP	Y	Syngenta
KCP 10.2	Suprenant, D.	1990a	CGA 173506 Technical – Acute toxicity to daphnids (<i>Daphnia magna</i>) under flow-through conditions. Syngenta Unpublished report CGA173506/0059 Unpublished report No. 89-05-2990	N	Syngenta
KCP 10.3.1	Wainwright, M.	2001	Acute toxicity to Honey bee (<i>Apis mellifera</i>) Syngenta Unpublished report CGA173506/5376 GLP	N	Syngenta
KCP 10.4	Batscher, R.	2002	Acute toxicity of CGA 192155 (metabolite of CGA 173506) to the earthworm <i>Eisenia Fetida</i> in a 14-day test. Unpublished report No. 812068 GLP	N	Syngenta
KCP 10.4	Batscher, R.	2002a	Acute toxicity of CGA 265378 (metabolite of CGA 173506) to the earthworm <i>Eisenia Fetida</i> in a 14-day test. Unpublished report No. 812070 GLP	N	Syngenta
KCP 10.4	Friedrich, S.	2003	Fludioxonil (CGA 173506): Sublethal toxicity of the technical material to the earthworm <i>Eisenia fetida</i> . Syngenta Unpublished report CGA173506/5665. GLP	N	Syngenta
KCP 10.4	Rufli, H.	1989	Effect on Earthworm – Acute Toxicity to Earthworms. Syngenta Unpublished report CGA173506/0705 Report No. 953608 GLP	N	Syngenta
KCP 10.5	Schame, C. and Galicia, H.	1992	The effects of CGA 173506 on soil respiration and nitrification. Syngenta Unpublished report CGA173506/0193 Unpublished report no. 315843 GLP	N	Syngenta
KCP	Vokel, W.	2001	The effects of CGA 192155 (metabolite of CGA	N	Syngenta

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
10.5			173506) on soil respiration and nitrification. Syngenta Unpublished report CGA173506/0014 Unpublished report no. 808121 GLP		
KCP 10.5	Vokel, W.	2002	The effects of CGA 265378 (metabolite of fludioxonil) on soil respiration and nitrification. Syngenta Unpublished report CGA173506/0012 Unpublished report no. 808198 GLP	N	Syngenta

List of data submitted by the applicant and not relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner

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

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner

Appendix 2 Detailed evaluation of the new studies

KCP 10.1 Effects on birds and other terrestrial vertebrates

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

A 2.2.1.1.1

Study 1

Comments of zRMS:	<p>The study was performed in line with OECD 203. The study is acceptable.</p> <p>All validity criteria were met: - the mortality in the control was 0% at exposure termination (should not exceed 10% or 1 fish if less than 10 fish are used); - dissolved oxygen concentrations were within the range of 85 – 100% of air saturation value (obligatory above 60% of air saturation value)</p> <p>The endpoint value determined on the basis of the nominal test item concentration and mortality of fish: The LC50 value after 96 h of exposure is 8.92 mg/L (95% confidence limit: 6.90 – 11.73). The LOEC value/96 h is 13.64 mg/L and the NOEC value/96 h is 6.20 mg/L.</p>
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Report	FLUDIO 025 GF: <i>Rainbow trout</i> , acute toxicity test, Nierzędska.E., Study code: W-33-21
Guideline(s):	OECD No. 203
Deviations:	Yes (The deviation has no impact on the study results)
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Aim of the study:

The aim of the study was to determine the test item concentration causing 50% mortality of rainbow trout (*Oncorhynchus mykiss*) i.e. LC50 value after 96 h of exposure. The NOEC and LOEC values were also determined after 96 h of exposure.

Materials and methods

Test organism	Rainbow trout (<i>Oncorhynchus mykiss</i>)
Test design	Semi-static system (96 h of exposure with daily renewals), one replicate per treatment, seven fish per replicate, the ratio of fish weight per volume (10 L) was 0.58 g/L.
Nominal test item concentrations	30, 13.64, 6.20, 2.82, 1.28, 0.58 mg/L plus the control
Test conditions	Temperature: 12.8 – 14.5°C, control pH: 7.65-7.84, dissolved oxygen concentration: 85-100%, light: 16h light : 8h dark, no feeding, constant aeration, 588 lx at exposure initiation

Validity criteria

- the mortality in the control was 0% at exposure termination (should not exceed 10% or 1 fish if less than 10 fish are used);
- dissolved oxygen concentrations were within the range of 85 – 100% of air saturation value (obligatory above 60% of air saturation value).

Analytical measurements:

The concentrations of fludioxonil were chemically determined. The validated analytical method was performed according to SANTE/2020/12830 rev.1. Samples of all test item concentrations and the control collected at exposure initiation, spent samples of all test item concentrations during the first renewal and fresh samples of the highest (with alive fish) and the lowest test item concentration and the control collected at each renewal. Moreover, spent samples of the highest and lowest test item concentration and the control during renewals and the same spent samples at exposure termination were chemically determined.

Table 4. Results from analysis of active substance in test sample

Time/ date of analysis	Concentration of test item [mg/L]	Nominal concentration of fludioxonil [mg/L]	Mean concentration of fludioxonil determined (n=3) in samples [mg/L]	% ± RSD of nominal concentration
t0 (23.05.2022)	control	---	< LoD	---
	0.58	0.0136	0.0120	88.2 ± 0.8
	1.28	0.0301	0.0263	87.4 ± 0.8
	2.82	0.066	0.075	113.6 ± 0.0
	6.20	0.146	0.145	99.3 ± 0.7
	13.64	0.321	0.308	96.0 ± 0.3
	30.0	0.705	0.700	99.3 ± 0.4
t24 (24.05.2022) spent	control	---	< LoD	---
	0.58	0.0136	0.0084	61.8 ± 0.0
	1.28	0.0301	0.0170	56.5 ± 0.6
	2.82	0.066	0.056	84.8 ± 0.0
	6.20	0.146	0.131	89.7 ± 0.8
	13.64	0.321	0.258	80.4 ± 0.4
	30.0	0.705	0.587	83.3 ± 0.7
t24 (24.05.2022) fresh	control	---	< LoD	---
	0.58	0.0136	0.0126	92.6 ± 0.8
	13.64	0.321	0.325	101.2 ± 0.3
t48 (25.05.2022) spent	control	---	< LoD	---
	0.58	0.0136	0.0085	62.5 ± 2.4
	13.64	0.321	0.290	90.3 ± 0.3
t48 (25.05.2022) fresh	control	---	< LoD	---
	0.58	0.0136	0.0127	93.4 ± 6.3
	13.64	0.321	0.315	98.1 ± 0.3
t72 (26.05.2022) spent	control	---	< LoD	---
	0.58	0.0136	0.0090	66.2 ± 3.3
	13.64	0.321	0.305	95.0 ± 1.0
t72 (26.05.2022) fresh	control	---	< LoD	---
	0.58	0.0136	0.0134	98.5 ± 0.0
	6.20	0.146	0.150	102.7 ± 0.7
t96 (27.05.2022) spent	control	---	< LoD	---
	0.58	0.0136	0.0093	68.4 ± 7.5
	6.20	0.146	0.143	97.9 ± 4.2

Since concentrations of fludioxonil were not in the range of 80 – 100% of nominal concentration, geometric means of fludioxonil concentrations were calculated:

Nominal concentration of fludioxonil: 30, 13.64, 6.20, 2.82, 1.28, 0.58 mg/L plus the control.

Geometric mean: 0.641, 0.281, 0.1378, 0.0648, 0.0211, 0.010 mg/L plus the control.

Results

In the control and in the test item concentrations of 0.58, 1.28, and 2.82 mg/L nor mortality of fish and no intoxications symptoms was observed during exposure. In the test item concentration of 6.20 mg/L after 96 h of exposure, loss of balance, hyperventilation for seven fish, immobilization for four fish, swimming near bottom and head-up or head-down for three fish, as well as loss of schooling were observed. In the test item concentra-

tions of 30 and 13.64 mg/L after 24 h and 52 h of exposure, respectively, after opinion of veterinarian all survived fish were humanitarian euthanized.

Conclusion

The endpoint value determined on the basis of the nominal test item concentration and mortality of fish:
The LC50 value after 96 h of exposure is 8.92 mg/L (95% confidence limit: 6.90 – 11.73).
The LOEC value/96 h is 13.64 mg/L and the NOEC value/96 h is 6.20 mg/L.

The endpoint values based on geometric means of determined fludioxonil concentrations:
The median concentration causing 50% mortality of rainbow trout after 96 hours of exposure LC50/96 h is 0.195 mg/L (95% confidence limits: 0.159 – 0.241).
The LOEC value/96 h is 0.282 mg/L and the NOEC value/96 h is 0.138 mg/L.

Study 2

Comments of zRMS:	<p>The study was performed in line with OECD 202. The study is acceptable.</p> <p>All validity criteria were met:</p> <ul style="list-style-type: none">- the percentage of immobilized on of <i>Daphnia magna</i> in the control was 0.0% (criterion: not more than 10%),- the dissolved oxygen concentrations in the test vessels were within the range of 6.5 – 8.5 mg/L (criterion: not less than 3 mg/L). <p>The endpoint values based on nominal test item concentrations are given below: the EC50/48 h value is 104.66 mg/L (95% confidence limits: 85.12 - 128.73); the LOEC/48 h value is 100 mg/L;</p>
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Report	FLUDIO 025 GF – <i>Daphnia magna</i> , Acute immobilisation test. Hodorek G., Study code W-31-21
Guideline:	OECD No. 202
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Aim of the study

The aim of the study was to determine the test item concentration causing 50% immobilisation of *Daphnia magna* i.e. EC50 value after 48 h of exposure. The LOEC and NOEC values were also determined.

Materials and methods

Test organism	<i>Daphnia magna</i>
Test design	Static system (48 h of exposure), four replicate per treatment, five daphnids per replicate
Nominal test item concentrations	400, 200, 100, 50, 25, 12.5 mg/L
Test conditions	Temperature: 19.8 – 21.0°C; pH of the control: 7.90 – 8.02; dis-

	solved oxygen concentration in the control: 7.6 – 8.4 mg/L; daily cycle 16 h light : 8 h dark; fluorescent light source; no feeding; no aeration; medium: Elendt M7.
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Validity criteria

- the percentage of immobilized on of Daphnia magna in the control was 0.0% (criterion: not more than 10%),
- the dissolved oxygen concentrations in the test vessels were within the range of 6.5 – 8.5 mg/L (criterion: not less than 3 mg/L).

Analytical measurements

The concentrations of fludioxonil in the test item concentrations, were determined using a validated high performance liquid chromatographic (HPLC) with Diode Array Detection. Samples for chemical determination were collected from all the test item concentrations and the control at exposure initiation and at exposure termination.

At exposure initiation, the determined concentrations of fludioxonil were in the range of 102.3 – 106.0% of the nominal concentration. The results confirm that the test item concentrations were prepared correctly.

At exposure termination, the determined concentrations of fludioxonil were in the range of 91.6 – 117.7% of the nominal concentration. Therefore, the concentrations of fludioxonil were stable under test conditions.

Table 2. Data of control and fortified samples of Elendt M7 medium for fludioxon

Lab Sample Description	Fortified Analyte Concentration [mg/L]	Lab Sample Amount [mL]	Final Volume [mL]	Measured Concentration [µg/mL]	Analysed Analyte Concentration [mg/L]
Elendt M7 control	--	1	2	--	--
Elendt M7 control	--	1	2	--	--
Elendt M7 0.05 mg-L	0.05	1	2	0.0260	0.0520
Elendt M7 0.05 mg-L	0.05	1	2	0.0256	0.0512
Elendt M7 0.05 mg-L	0.05	1	2	0.0256	0.0512
Elendt M7 0.05 mg-L	0.05	1	2	0.0252	0.0504
Elendt M7 0.05 mg-L	0.05	1	2	0.0248	0.0496
Elendt M7 0.5 mg-L	0.5	1	2	0.2529	0.506
Elendt M7 0.5 mg-L	0.5	1	2	0.2535	0.507
Elendt M7 0.5 mg-L	0.5	1	2	0.2494	0.499
Elendt M7 0.5 mg-L	0.5	1	2	0.2531	0.506
Elendt M7 0.5 mg-L	0.5	1	2	0.2513	0.503

Range the calibration curve is from 0.01 to 1 µg/mL
--not detected

Table 3. Calibration Data of fludioxonil in range 0.1 µg/mL to 1 µg/mL equivalent to mg/L

Fludioxonil							
Lab Sample Description	Lab Sample ID	Nominal Concentration [µg/mL]	Peak Area	Measured Concentration [µg/mL]	Slope	Intercept	Coefficient
std 0.01 mg/L	Standard	0.01	1197	0.0103	132406	-171.205	0.9997110
std 0.05 mg/L	Standard	0.05	6469	0.0502			
std 0.1 mg/L	Standard	0.1	12676	0.0970			
std 0.5 mg/L	Standard	0.5	64771	0.4905			
std 1.0 mg/L	Standard	1.0	133824	1.0120			

Table 4. Results from analysis of active substance in test sample

Nominal test item concentration [mg/L]	Nominal concentration of fludioxonil [mg/L]	Mean concentration of fludioxonil determined (n=3) in samples collected			
		at 0h (01.02.2022) [mg/L]	% ± RSD of nominal concentration	at 48h (03.02.2022) [mg/L]	%± RSD of nominal concentration
Control	0.000	< LoD	---	< LoD	---
12.5	0.294	0.301	102.4±0.7	0.300	102.0±0.7
25	0.588	0.610	103.7±0.5	0.621	105.6±1.1
50	1.175	1.213	103.2±0.2	1.185	100.9±0.3
100	2.35	2.403	102.3±1.1	2.154	91.7±0.2
200	4.7	4.809	102.3±0.7	5.533	117.7±1.1
400	9.4	9.962	106.0±0.2	8.613	91.6±0.6

LoQ = 0.05 mg/L
LoD = 0.02 mg/L
--- not calculated

Conclusion

The endpoint values based on nominal test item concentrations are given below:
the EC50/48 h value is 104.66 mg/L (95% confidence limits: 85.12 - 128.73);
the LOEC/48 h value is 100 mg/L;

Table 7. Endpoint values based on the nominal test item concentration, definitive test

Endpoint value [mg/L]	Time of exposure	
	24 h	48 h
EC ₅₀	>400	104.66 (85.12 – 128.73)
EC ₂₀	336.95 (197.35 – 1634.24)	68.05 (49.21 – 83.83)
EC ₁₀	188.18 (75.41 – 367.47)	54.34 (35.81 – 69.13)
LOEC	>400	100
NOEC	≥400	50

Calculations were made according to [7], [SOP/W/68, SOP/OG/7]
(-) - 95% confidence interval

Comments of zRMS:	<p>The study was performed in line with OECD 201. The study is acceptable.</p> <p>All validity criteria were met:</p> <ul style="list-style-type: none">- the biomass in the control increased by a factor of 71.4 within the 72-hour test period (criterion: at least a 16-fold growth),- the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 0.5% (criterion: it must not exceed 7%).
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	<p>- the mean coefficient of variation for the section-by-section growth rate in the control culture was 30.5% (criterion: it must not exceed 35%).</p> <p>The endpoint values based on nominal test item concentrations: The ErC50/72 h value is 19.7 mg/L (95% confidence interval: 18.5 – 20.9). The EyC50/72 h value is 9.8 mg/L (95% confidence interval: 8.3 – 11.6). The LOEC/72 h value for growth rate and yield is 9.8 mg/L. The NOEC/72 h value for growth rate and yield is 3.05 mg/L.</p>
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Report	FLUDIO 025 GF - <i>Raphidocelis subcapitata</i> SAG 61.81 (formerly <i>Pseudokirchneriella subcapitata</i>) Growth inhibition test, Hodorek G., Study code: W-32-21
Guideline(s):	OECD No. 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Aim of the study

The aim of the study was to determine the test item concentrations causing 50% inhibition of growth rate and yield of the algae, *Raphidocelis subcapitata* SAG 61.81 (formerly *Pseudokirchneriella subcapitata*) (ErC50 and EyC50 after 72 hours of exposure, respectively). The LOEC and NOEC values were also determined.

Materials and methods

Test organism	<i>Pseudokirchneriella subcapitata</i>
Test design	72 h of exposure, three replicates per test item concentration, six replicates of control, initial cells concentration 104 cells/ml
Nominal test item concentrations	0.95, 3.05, 9.8, 31.3 and 100 mg/L plus the control.
Geometric means of determined concentrations of florasulam	0.014, 0.057, 0.213, 0.719, 2.345 mg/L plus the control.
Test conditions	Temperature: 22.4 – 23.0°C; pH of the control: 7.56 – 8.52; mean light intensity: 7483 - 7508 lux; constant illumination and shaking; medium: AAP.

Validity criteria

- the biomass in the control increased by a factor of 71.4 within the 72-hour test period (criterion: at least a 16-fold growth),
- the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 0.5% (criterion: it must not exceed 7%).
- the mean coefficient of variation for the section-by-section growth rate in the control culture was 30.5% (criterion: it must not exceed 35%).

Analytical measurements

Table 9. Concentration and stability of fludioxonil, definitive test

Nominal test item concentration [mg/L]	Nominal concentration of fludioxonil [mg/L]	Mean concentration of fludioxonil determined (n=3) in samples collected			
		at exposure initiation (14.02.2022) [mg/L]	% ± RSD% of nominal concentration	at exposure termination (17.02.2022) [mg/L]	% ± RSD% of nominal concentration
Control	–	< LoD	–	< LoD	–
0.95	0.0223	0.0236	105.8±0.4	0.0085	38.1±7.1
3.05	0.0717	0.0761	106.1±0.3	0.0422	58.9±4.0
9.80	0.2303	0.2434	105.7±0.2	0.1864	80.9±1.4
31.3	0.7356	0.7942	108.0±0.2	0.6507	88.5±0.7
100.0	2.35	2.597	110.5±0.3	2.1172	90.1±0.9

LoQ = 0.0005 mg/L
LoD = 0.0001 mg/L
–not calculated
RSD – relative standard deviation

Results

Table 7. Growth rate and yield, definitive test

Nominal test item concentration [mg/L]	Growth rate* [10 ⁶ cells/mL]				Yield** [10 ⁶ cells/mL]
	0-24 h	24-48 h	48-72 h	0-72 h	72 h
Control	1.526	0.777	1.981	1.428	0.715
	1.435	1.058	1.744	1.412	0.682
	1.335	0.927	2.005	1.422	0.703
	1.335	1.125	1.829	1.430	0.719
	1.194	1.231	1.859	1.428	0.715
	1.335	1.090	1.824	1.416	0.690
mean	1.360	1.034	1.874	1.423	0.704
standard deviation	0.112	0.160	0.100	0.007	0.015
0.95	0.531	1.963	1.655	1.383	0.623
	0.916	1.671	1.500	1.363	0.586
	0.916	1.737	1.515	1.389	0.636
mean	0.788	1.790	1.557	1.378	0.615
standard deviation	0.223	0.153	0.085	0.014	0.026
3.05	1.194	1.713	1.187	1.365	0.590
	0.262	2.529	1.395	1.396	0.648
	0.262	2.529	1.521	1.437	0.736
mean	0.573	2.257	1.368	1.399	0.658
standard deviation	0.538	0.471	0.168	0.036	0.074
9.8	-0.916	2.603	1.892	1.193	0.348
	-0.223	2.716	1.131	1.208	0.365
	-0.223	2.683	1.199	1.219	0.378
mean	-0.454	2.667	1.407	1.207	0.364
standard deviation	0.400	0.058	0.421	0.013	0.015
31.3	-0.916	1.981	0.129	0.398	0.023
	n.d.	n.d.	0.148	0.355	0.019
	-0.916	1.658	0.174	0.305	0.015
mean	n.d.	n.d.	0.151	0.353	0.019
standard deviation	n.d.	n.d.	0.023	0.046	0.004
100	n.d.	n.d.	0.000	-0.074	-0.002
	-0.916	1.179	0.000	0.087	0.003
	-0.916	0.693	0.000	-0.074	-0.002
mean	n.d.	n.d.	0.000	-0.020	0.000
standard deviation	n.d.	n.d.	0.000	0.093	0.003

* - Growth rate [10⁶ cells/mL] was calculated according to the following formula:

Growth rate (0 – 72 h) =
$$\frac{[\ln (\text{cell density at 72 h})] - [\ln (\text{cell density at 0 h})]}{3 \text{ days}}$$

** - Yield was calculated according to the following formula:

Yield [10⁶cells /mL] = (cell density at 72 h) – (cell density at 0 h)

n.d. – not determined due mathematical reasons

Conclusion

Table 10. Growth rate endpoint values based on the nominal test item concentrations, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E _r C ₅₀	1.53 (0.39 – 3.00)	25.1 (20.9 – 29.7)	19.7 (18.5 – 20.9)
E _r C ₂₀	0.45 (0.005 – 1.005)	13.5 (9.1 – 16.9)	11.1 (10.0 – 12.0)
E _r C ₁₀	n.d.	9.8 (5.7 – 13.0)	8.2 (7.2 – 9.1)
LOEC	≤0.95	100.0	9.8
NOEC	<0.95	31.3	3.05

(-) – 95% confidence interval

Calculations were made according to [8], [SOP/W/68]

n.d. – not determined

Table 11. Yield endpoint values based on the nominal test item concentrations, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E _y C ₅₀	0.8 (n.d. – 1.7)	16.8 (12.5 – 23.0)	9.8 (8.3 – 11.6)
E _y C ₂₀	n.d.	10.1 (5.8 – 13.4)	5.3 (3.6 – 6.5)
E _y C ₁₀	n.d.	7.7 (3.7 – 10.8)	3.8 (2.2 – 5.1)
LOEC	≤0.95	100.0	9.8
NOEC	<0.95	31.3	3.05

(-) – 95% confidence interval

Calculations were made according to [8], [SOP/W/68]

n.d. – not determined

Table 12. Growth rate endpoint values based on the geometric means of determined concentrations of fludioxonil, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E _r C ₅₀	0.025 (0.005 – 0.054)	0.569 (0.470 – 0.679)	0.443 (0.416 – 0.472)
E _r C ₂₀	0.006 (0.000 – 0.015)	0.298 (0.198 – 0.377)	0.242 (0.219 – 0.264)
E _r C ₁₀	n.d.	0.213 (0.121 – 0.288)	0.176 (0.154 – 0.197)
LOEC	≤0.014	2.345	0.213
NOEC	<0.014	0.719	0.057

(-) – 95% confidence interval

Calculations were made according to [8], [SOP/W/68]

n.d. – not determined

Table 13. Yield endpoint values based on the geometric means of determined concentrations of fludioxonil, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
EyC50	0.011 (n.d. – 0.029)	0.374 (0.274 – 0.521)	0.212 (0.176 – 0.254)
EyC20	n.d.	0.220 (0.123 – 0.296)	0.110 (0.069 – 0.140)
EyC10	n.d.	0.166 (0.076 – 0.235)	0.078 (0.040 – 0.107)
LOEC	≤0.014	2.345	0.213
NOEC	<0.014	0.719	0.057

(–) – 95% confidence interval
Calculations were made according to [8], [SOP/W/68]
n.d. – not determined

The endpoint values based on nominal test item concentrations:
The ErC50/72 h value is **19.7** mg/L (95% confidence interval: 18.5 – 20.9).
The EyC50/72 h value is 9.8 mg/L (95% confidence interval: 8.3 – 11.6).
The LOEC/72 h value for growth rate and yield is 9.8 mg/L.
The NOEC/72 h value for growth rate and yield is 3.05 mg/L.

The endpoint values based on the geometric means of determined concentrations of fludioxonil:
The ErC50/72 h value is **0.443** mg/L (95% confidence interval: 0.416 – 0.472).
The EyC50/72 h value is 0.212 mg/L (95% confidence interval: 0.176 – 0.254).
The LOEC/72 h values for growth rate and yield are 0.213 mg/L.
The NOEC/72 h values for growth rate and yield are 0.057 mg/L.

A 2.2.2	KCP 10.2.2	Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms
A 2.2.3	KCP 10.2.3	Further testing on aquatic organisms
A 2.3	KCP 10.3	Effects on arthropods
A 2.3.1	KCP 10.3.1	Effects on bees
A 2.3.1.1	KCP 10.3.1.1	Acute toxicity to bees
A 2.3.1.1.1	KCP 10.3.1.1.1	Acute oral toxicity to bees

Comments of zRMS:	<p>The study was performed in line with OECD 213.</p> <p>Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>The all validity criteria were met:</p> <ul style="list-style-type: none">- the average mortality for the control was 0.0% at the end of the experiment (criterion: it must not exceed 10%).- the LD50/24 h of the reference item (dimethoate) was 0.255 µg a.i./bee (criterion: 0.10 – 0.35 µg a.i./bee). <p>The endpoint values determined on the basis of the nominal test item concentrations are given below:</p> <p>The EC₅₀/48 h values is higher than 200 µg/bee.</p>
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Report	FLUDIO 025 GF: Honeybees (<i>A. mellifera</i>), Acute oral toxicity test, Kulec-Płoszczyca, E., Study code: B-03-22
Guideline(s):	OECD Guideline for the Testing of Chemicals No. 213 (1998) and the EU Method C.16. (2008)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Aim of the study

The aims of the study were to use a laboratory method to determine the acute oral toxicity of FLUDIO 025 GF to adult worker honeybees and to demonstrate that the LD50 values are higher than the highest dose used in the test.

Materials and methods

Test organism	<i>Apis mellifera</i>
Test design	48 h of exposure, 30 bees per treatment
Nominal test item concentrations	200, 100, 50, 25, 12.5 µg/bee
Test conditions	temperature: 23.5 – 27°C, relative air humidity: 54 – 65% place: a dark room

Validity criteria

- the average mortality for the control was 0.0% at the end of the experiment (criterion: it must not exceed 10%).
- the LD50/24 h of the reference item (dimethoate) was 0.255 µg a.i./bee (criterion: 0.10 – 0.35 µg a.i./bee).

Results

The acute oral toxicity study of the test item, DIFLUD 050 FS on honeybees (*Apis mellifera* L.) in the laboratory test are summarized below.

Dose [µg/bee]	Number of tested bees [no.]	Mortality after 48 h of exposure		LD ₅₀ [µg/bee]
		Total		
		[no.]	[%]	
0.0 (Control)	30	0	0.0	> 200.0
12.5	30	0	0.0	
25.0	30	0	0.0	
50.0	30	0	0.0	
100.0	30	1	3.3	
200.0	30	1	3.3	

Conclusion

The endpoint values determined on the basis of the nominal test item concentrations are given below:
The EC₅₀/48 h values is higher than 200 µg/bee.

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

Comments of zRMS:	<p>The study was performed in line with OECD 214.</p> <p>The all validity criteria were met:</p> <ul style="list-style-type: none">- the average mortality for the control was 0.0% after 48 h (criterion: it must not exceed 10%),- the LD50/24 h of the reference item (dimethoate) was 0.27 µg a.i./bee (criterion: 0.10 - 0.30 µg a.i./bee). <p>The study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>The endpoint values determined on the basis of the nominal test item concentrations are given below:</p> <p>The EC₅₀/48 h values is higher than 200 µg/bee.</p>
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Report	FLUDIO 025 GF: Honeybees (<i>A. mellifera</i>), Acute contact toxicity test, Kulec-Płoszczyca, E., Study code: B/04/19
Guideline(s):	OECD Guideline for the Testing of Chemicals No. 214 (1998) and the EU Method C.17. (2008)
Deviations:	Yes – deviation had no impact on the results (different method of anesthesia)
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Aim of the study

The aims of the study were to use a laboratory method to determine the acute contact toxicity of FLUDIO 025 GF to adult worker honeybees and to demonstrate that the LD50 values are higher than the highest dose used in the test.

Materials and methods

Test organism	<i>Apis mellifera</i>
Test design	48 h of exposure, 30 bees per treatment
Nominal test item concentrations	200, 100, 50, 25, 12.5 µg/L
Test conditions	temperature: 25 – 26.5°C, relative air humidity: 59 – 67% place: a dark room

Validity criteria

- the average mortality for the control was 0.0% after 48 h (criterion: it must not exceed 10%),
- the LD50/24 h of the reference item (dimethoate) was 0.27 µg a.i./bee (criterion: 0.10 - 0.30 µg a.i./bee).

Results

The acute contact toxicity study of the test item DIFLUD 050 FS on honeybees (*Apis mellifera* L.) in the laboratory test are summarized below.

Dose [µg/bee]	Number of tested bees [no.]	Mortality after 48 h of exposure		LD ₅₀ [µg/bee]
		Total		
		[no.]	[%]	
0.0 (Control)	30	0	0.0	> 200.0
12.5	30	0	0.0	
25.0	30	0	0.0	
50.0	30	0	0.0	
100.0	30	0	0.0	
200.0	30	0	0.0	

Conclusion

The endpoint values determined on the basis of the nominal test item concentrations are given below:
The EC₅₀/48 h values is higher than 200 µg/bee.

Comments of izRMS:	<p>The study was performed in line with OECD 247.</p> <p>All validity criteria were met:</p> <ul style="list-style-type: none">• 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). <p>The study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>48 h contact LD₅₀ >200 µg product/bumblebee</p>
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Reference:	KCP 10.3.1.1
Report	FLUDIO 025 GF

	Bumblebees (<i>Bombus</i> spp.), Acute Oral Toxicity Test; STUDY CODE: B-67-21
Guideline(s):	OECD 247
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

The study was conducted to determine the acute oral toxicity of FLUDIO 025 GF to bumblebees (*Bombus* spp.) with a laboratory method and to demonstrate, that the median lethal dose, i.e. the LD50 at the end of exposure, is higher than the dose used in the test (limit test). One dose of the test item, i.e. 200.0 µg test item/bumblebee, plus the control and one dose of the reference item were used. The design of the definitive test was selected on the basis of the non-GLP preliminary range-finding test results.

The bumblebees were exposed to the test item distributed in a 50% aqueous sucrose solution. The insects were selected for the exposure in terms of their sizes. The treated diet was provided in calibrated pipettes. Each pipette contained 40 µL of the sucrose solution with the test item at the tested dose. The insects were kept individually in isolators.

The sensitivity of the test bumblebees was verified using a reference item, i.e. dimethoate at the dose of 4.0 µg/bumblebee.

The insects were observed for mortality and other signs of toxicity 4-5, 24 and 48 hours after the test/ reference item administration. The acute oral toxicity test finished after the 48-hour observation.

Material and methods: Test item:	FLUDIO 025 GF content: 25.0 g/L of fludioxonil (CAS No. 131341-86-1) batch no.: SNS-F-06-22 production date: 10.2021 expiry date: 10.2023
Biological test system:	species: bumblebee, <i>Bombus</i> spp. source: Koppert Polska sp. z o.o. (a commercial supplier) age: adult worker bumblebees
Experimental design:	– a control (50% sucrose solution w/v) number of replicates: 50; number of insects: 1 insect/replicate; – test item: number of doses: 1, number of replicates: 50; number of insects: 1 insect/replicate; – the reference item: number of doses: 1, number of replicates: 30; number of insects: 1 insect/replicate
Dose of the test item:	200.0 µg test item/bumblebee
Dose of the reference item:	4.0 µg/bumblebee
Exposure duration:	48 hours
Test conditions:	temperature: 24.0 – 24.5°C (required: 25 ± 2°C) relative air humidity: 65 – 67% (required: 60 ± 20%) place: a dark climate room
Endpoints:	– bumblebee mortality after 48 hours of exposure,

	– LD50 after 48 hours of exposure
Statistical method:	statistical analysis was not needed due to the lack of mortality.

Table 2. Bumblebee mortality after 4 hours of exposure – preliminary non-GLP test

Dose [µg test item/bumblebee]	Number of tested bumblebees [no.]	Mortality	
		Number of dead bumblebees [no.]	[%]
Control	10	0	0.0
8.0	10	0	0.0
40	10	0	0.0
200	10	0	0.0

Table 3. Bumblebee mortality after 24 hours of exposure – preliminary non-GLP test

Dose [µg test item/bumblebee]	Number of tested bumblebees [no.]	Mortality	
		Number of dead bumblebees [no.]	[%]
Control	10	0	0.0
8.0	10	0	0.0
40	10	0	0.0
200	10	0	0.0

Table 4. Bumblebee mortality after 48 hours of exposure – preliminary non-GLP test

Dose [µg test item/bumblebee]	Number of tested bumblebees [no.]	Mortality	
		Number of dead bumblebees [no.]	[%]
Control	10	0	0.0
8.0	10	0	0.0
40	10	0	0.0
200	10	1	10.0

The exposure in the preliminary non-GLP test was performed between 30.11 – 02.12.2021

Table 7. Bumblebee mortality after 4 hours of exposure – definitive test

Dose [µg/bumblebee]	Number of tested bumblebees [no.]	Mortality	
		Number of dead bumblebees [no.]	[%]
Control	50	0	0.0
200.0	50	0	0.0
Reference item: dimethoate			
4.0	30	0	0.0

Table 8. Bumblebee mortality after 24 hours of exposure and LD₅₀/24 h – definitive test

Dose [µg/bumblebee]	Number of tested bumble- bees [no.]	Mortality		LD ₅₀	
		Number of dead bumble- bees [no.]	[%]	[µg test item/ bumblebee]	fludioxonil [µg a.i. / bumblebee]
Control	50	0	0.0	> 200.0	> 5.0
200.0	50	0	0.0		
Reference item: dimethoate					
4.0	30	29	96.7	–	–

Table 9. Bumblebee mortality after 48 hours of exposure and LD₅₀/48 h – definitive test

Dose [µg/bumblebee]	Number of tested bumble- bees [no.]	Mortality		LD ₅₀	
		Number of dead bumble- bees [no.]	[%]	[µg test item/ bumble bee]	fludioxonil [µg a.i. / bumble-bee]
Control	50	0	0.0	> 200.0	> 5.0
200.0	50	0	0.0		
Reference item: dimethoate					
4.0	30	29	96.7	–	–

The exposure in the definitive test was performed between 26 – 28.01.2022

6. RESULTS

6.1. Preliminary non-GLP test

Mortality is presented in Tables 2, 3 and 4. After 4, 24 and 48 hours, there were no dead bumblebee in the control group. The percentages of mortality of bumblebees exposed to the test item at all the doses, i.e. 8.0, 40.0, and 200 µg/bumblebee.

6.2. Definitive test

Mortality of the treated insects is presented in Tables 7 – 9.

Mortality in the group was 0.0% after 48 hours of exposure. The percentage of mortality after 48 h hours of exposure to the test item at the dose of 200.0 µg test item/bumblebee was 0.0%. During the experiment sublethal effects (toxic symptoms) in the group treated with the test item were not observed (Table 10).

The median lethal doses for the test item (LD50/24 h, LD50/48 h) are higher than the dose used in the test, i.e. > 200.0 µg test item/ bumblebee, i.e. > 5.0 µg fludioxonil/ bumblebee (Table 8 and 9).

Dose-effect curves showing the influence of the test item on mortality after 24 and 48 hours of exposure are not given due to the lack of mortality.

The percentage of mortality after 48 h hours of exposure to the reference item at the dose of 4.0 µg/bumblebee was 96.7%. In the reference item group fifteen affected bumblebees after 4 h of exposure were observed (Table 9).

The mean weights of the bumblebees in each group were: 0.228 g for the control group, 0.233 g for the group treated with the test item and 0.224 g for the group treated with the reference item (Table 11).

6.2.1. Results of chemical determinations

At exposure initiation, in the fresh test item sample, the concentration of fludioxonil was 103.2% of the nominal concentration. The results confirm that the test item concentration was prepared correctly.

VALIDITY OF THE STUDY

The following validity criteria were met:

- Mortality of the control groups was 0.0% at the end of the test (criterion: ≤ 10%).
- Mortality in the toxic reference item group (dimethoate) at the end of the test was 96.7% (criterion: ≥ 50%).

DEVIATIONS IN THE STUDY

The test was performed according to the OECD Guideline for the Testing of Chemicals No. 247 (2017): Bumblebee, Acute Oral Toxicity Test' [1], other references given in section 9 and the SOP's listed in section 10 of the report.

In the study following deviation occurred. According to the OECD Guideline No. 247 it is recommended to use plastic syringes for the test item administration. However, in the experiment they were replaced by calibrated glass pipettes.

This deviation had no impact on the quality, integrity and final results of the study.

Comments of zRMS:	The study was performed in line with OECD 246 with no deviations. All validity criteria were met: The study is considered acceptable with the following endpoints relevant for the risk assessment: 48 h oral LD ₅₀ > 200 µg product/bumblebee
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Reference:	KCP 10.3.1.1
Report	FLUDIO 025 GF Bumblebees (Bombus spp.), Acute Contact Toxicity Test; STUDY CODE: B-68-21
Guideline(s):	OECD 246
Deviations:	No
GLP:	Yes

Acceptability:	Acceptable
Duplication (if vertebrate study)	No

The study was conducted to determine the acute contact toxicity of FLUDIO 025 GF to bumblebees (*Bombus* spp.) with a laboratory method and to demonstrate, that the median lethal dose, i.e. the LD50 at the end of exposure, is higher than the dose used in the test (limit test). One dose of the test item, i.e. 200.0 µg test item/bumblebee, plus the controls and one dose of the reference item were used. The design of the definitive test was selected on the basis of the non-GLP preliminary range finding test results.

The bumblebees were exposed to the test item diluted in distilled water with surfactant Triton® X-100 and applied to the dorsal part of the thorax, using a microapplicator. The volume was 2 µL/bumblebee. The insects were selected for the exposure in terms of their sizes. After that, the insects were kept individually in isolators. The sensitivity of the test bumblebees was verified using a reference item, i.e. dimethoate at the dose of 10.0 µg/bumblebee. The insects were observed for mortality and other signs of toxicity 4, 24 and 48 hours after the test/ reference item administration. The acute contact toxicity test finished after the 48-hour observation.

DEVIATIONS IN THE STUDY

The test was performed according to the OECD Guideline for Testing of Chemicals No. 246 (2017): Bumblebee, Acute Contact Toxicity Test’ [1], other references given in section 9 and the SOP’s listed in section 10 of the report. FLUDIO 025 GF Bumblebees (*Bombus* spp.), Acute Contact Toxicity Test Study code: B-68-21

The median lethal doses (LD₅₀/24 h, LD₅₀/48 h) are higher than the dose used in the test, i.e. > 200.0 µg test item/bumblebee, i.e. > 5.0 µg fludioxonil/ bumblebee.

Dose		Number of tested bumblebees [no.]	Mortality after 48 h		LD ₅₀ /48 h	
test item [µg/ bumblebee]	fludioxonil [µg a.i. / bumblebee]		[no.]	[%]	[µg/ bumblebee]	fludioxonil [µg a.i. / bumblebee]
Control		50	0	0.0	> 200.0	> 5.0
Control + 1% surfactant		50	0	0.0		
200.0	5.0	50	0	0.0		
Reference item: dimethoate						
Dose [µg/ bumblebee]	10.0	30	29	96.7	–	

Comments of zRMS:	<p>The study was performed in line with OECD 245 with no deviations.</p> <p>All validity criteria were met:</p> <p>The study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>48 h oral LD₅₀ > 200 µg product/bumblebee</p>
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Reference: KCP 10.3.1.1
Repor: Honeybees (Apis mellifera L.), Chronic Oral Toxicity Test STUDY CODE: B-02-22
Guideline(s): OECD Guideline No. 245 (2017)
Deviations: No
GLP: Yes
Acceptability: Yes
Duplication (if vertebrate study) No

FLUDIO 025 GF
Honeybees (*Apis mellifera* L.), Chronic Oral Toxicity Test
Study code: B-02-22

REPORT

SUMMARY

The mortality of honeybees exposed to FLUDIO 025 GF was investigated during 10-days chronic oral toxicity test.

The design of the definitive test was selected on the basis of the preliminary range-finding non-GLP test results. One dose of the test item was used (limit test). The nominal concentration was 666.7 mg/kg of diet (corresponding to the nominal dose of 20 µg/30 mg/day).

Daily dose, consumed by the bees in the group treated with the test item at the nominal concentration of 666.7 mg/kg (20 µg/30 mg/day) was 13.6 µg/bee/day (dietary dose). Daily dose was calculated on the basis of average consumption of a treated 50% sucrose solution and the nominal dose used for the treatment.

Each group of bees (5 replicates/group; 10 bees/replicate) was fed with 2 mL of a 50% sucrose solution containing the test item at the concentration of 666.7 mg/kg or 50% sucrose solution alone (control group) for 10 days.

Dimethoate, which is a recommended reference item, was used to verify the sensitivity of the bees and the precision of the test procedure. The group treated with the reference item (3 replicates per 10 bees) was fed with 2 mL of a 50% sucrose solution containing reference item at the nominal concentration of 0.8 mg/kg (corresponding to the nominal dose of 0.024 µg/30 mg). Daily weighed feeders were used. During the experiment, the insects were caged in groups of 10. Daily dose, consumed by the bees in the group treated with the reference item at the nominal concentration of 0.8 mg/kg (0.024 µg/30 mg/day) was 0.011 µg/bee/day (dietary dose).

The insects were observed for mortality and behavioral abnormalities (signs of intoxication) at daily intervals up to 10 days of exposure.

Average consumption of a 50% sucrose solution in the control group was 24.48 mg/bee/day. Average consumption in the group treated with the test item at the concentration of 666.7 mg/kg was 20.33 mg/bee/day. Average consumption of a 50% sucrose solution containing the reference item at the concentration of 0.8 mg/kg was 13.66 mg/bee/day.

The concentrations of fludioxonil were chemically determined using the validated high performance liquid chromatographic method with DAD detection. Fresh samples of the test item concentration and the control were chemically analyzed at test initiation and at the end of the maximum storage period (i.e. after 4 days). At exposure initiation, in the fresh sample of the test item of 666.7 mg/kg, the determined concentration of fludioxonil was

FLUDIO 025 GF

Honeybees (*Apis mellifera* L.), Chronic Oral Toxicity Test

Study code: B-02-22

REPORT

104.4% of nominal concentration. The results confirm that the test item concentration was prepared correctly.

After 4 days of the storage period, in the sample of the test item of 666.7 mg/kg, the determined concentration of fludioxonil was 105.3% of nominal concentration. Based on the results of chemical analyses, the concentration of fludioxonil was stable under storage conditions.

Material and methods:

Test item:	FLUDIO 025 GF content: 25.0 g/L of fludioxonil (CAS No. 131341-86-1) batch no.: SNS-F-06-22 production date: 10.2021 expiry date: 10.2023
Biological test system:	species: the honeybee, <i>Apis mellifera</i> L.; strain: carnica, source: an apiary at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna; age: freshly emerged worker honeybees (max. 2 days old) from the same queen-right colony
Experimental design:	– the test item: number of concentrations: 1 and the control number of replicates: 5 number of insects: 10 bees/replicate – the reference item: number of concentrations: 1 number of replicates: 3 number of insects: 10 bees/replicate exposure duration: 10 days
Nominal concentration of the test item:	666.7 mg/kg
Nominal dose of the test item:	20.0 µg/bee/day
Test item dietary dose:	13.6 µg/bee/day
Nominal concentration of the reference item (dimethoate):	0.8 mg/kg
Nominal dose of the reference item (dimethoate):	0.024 µg/bee/day
Reference item dietary dose:	0.011 µg/bee/day
Test conditions:	temperature: 32.7 – 33.6°C; relative humidity: 51.8 – 63.2%;
Statistical method:	One-way analysis of variance
Endpoints:	honeybee mortality after 10 days of exposure

7.2. Definitive test

Average consumption of a 50% sucrose solution in the control group was 24.48 mg/bee/day. Average consumption in the group treated with the test item at the concentration of 666.7 mg/kg (20 µg/30 mg) was 20.33 mg/bee/day. Average consumption of a 50% sucrose solution containing the reference item at the concentration of 0.8 mg/kg (0.024 µg/30 mg) was 13.66 mg/bee/day (Table 9).

On the basis of average consumption of a 50% sucrose solution in the study groups, it may be concluded that each bee treated with the test item at the nominal concentration of 666.7 mg/kg (i.e. 20 µg/30 mg) ingested 13.6 µg/day. In the group treated with the reference item, at the concentration of 0.8 mg/kg (i.e. 0.024 µg/30 mg) each bee ingested 0.011 µg/day (Table 11). All doses consumed by the bees were calculated taking into account the mean evaporation value (Table 10 and Figure 1).

Mortality in the control was 8.0% after 10 days of exposure. The percentage of mortality of the honeybees exposed to the test item, at the concentration of 666.7 mg/kg (dietary

dose 13.6 µg/bee/day) at exposure termination (after 10 days), was -6.5% (after Abbott's correction [7], Table 8).

There were no statistically significant differences in mortality between group treated with the test item at the dose of 666.7 mg/kg (dietary dose 13.6 µg/bee/day) and the control group (one-way analysis of variance, $p(F) > \alpha$).

On the basis of the obtained results the LC_{50} is higher than 666.7 mg/kg, and the LDD_{50} value is higher than 13.6 µg/bee/day.

The mortality of the bees treated with the reference item at the concentration of 0.8 mg/kg (dietary dose of 0.011 µg/bee/day) at the exposure termination (on day 10) was 92.9% (after Abbot's correction, Table 8). The results obtained in the reference item group showed that the insects were sensitive to dimethoate.

In the definitive test no sublethal effects were observed in the group treated with the test item and reference item with one exception (Table 12).

7.2.1. Results of chemical determinations

The concentrations of fludioxonil were chemically determined using the validated high performance liquid chromatographic method with DAD detection. Fresh samples of the test item concentration and the control were chemically analyzed at test initiation and at the end of the maximum storage period (i.e. after 4 days). At exposure initiation, in the fresh sample of the test item of 666.7 mg/kg, the determined concentration of fludioxonil was 104.4% of nominal concentration. The results confirm that the test item concentration was prepared correctly.

After 4 days of the storage period, in the sample of the test item of 666.7 mg/kg, the determined concentration of fludioxonil was 105.3% of nominal concentration. Based on the results of chemical analyses, the concentration of fludioxonil was stable under storage conditions.

Details regarding the stability test during the definitive experiment are presented in Table 7.

Representative chromatograms for the analyzed samples are presented in Appendix No. 5.

8. TEST VALIDITY CRITERIA

The following validity criteria were met during the test:

- At the end of the experiment average mortality of the control groups was 8.0% (criterion: it must not exceed 15%) [1].
- After 10 days of exposure corrected mortality of the honeybees exposed to the reference item at the concentration of 0.8 mg/kg (0.011 µg/bee/day) was 92.9%, after Abbot's correction (criterion: it must be ≥ 50% on day 10 of exposure).

9. DEVIATIONS IN THE STUDY

The test was performed according to the OECD Guideline No. 245: 'Honey bee (*Apis mellifera* L.) Chronic oral toxicity test (10-day feeding)', other references given in section 10, the SOP's listed in section 11 of the report and the study plan.

Comments of zRMS:	<p>The study was performed in line with OECD 239 with no deviations.</p> <p>All validity criteria were met.</p> <p>The study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>The endpoint values for FLUDIO 025 GF at the end of the assessment (D22):</p> <ul style="list-style-type: none">- ED50 value is higher than 100.0 µg test item/larva,- EC50 value is higher than 649.4 mg/kg,- NOED value is higher than or equal to 100.0 µg test item/larva,- NOEC value is higher than or equal to 649.4 mg/kg.
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Reference: KCP 10.3.1.1
Repor: Honeybees (<i>Apis mellifera</i> L.), Honeybees (<i>Apis mellifera</i> L.), Larval Toxicity Test, Repeated Exposure B-01-22
Guideline(s): OECD Guideline No. 239 (2021)
Deviations: There were no deviations from the documents mentioned above.
GLP: Yes
Acceptability: Yes

Duplication (if vertebrate study) No

SUMMARY

The larval toxicity test of FLUDIO 025 GF was conducted to determine the toxicity of the test item to honey-bee larvae (*Apis mellifera* L.) after repeated exposure of the test item using a laboratory method and demonstrate that the median effective concentration/dose, i.e. EC50/ED50 is higher than the test item concentration used for exposure (limit test). One cumulative dose of the test item was used, i.e. 100.0 µg/larva. It was selected on the basis of the preliminary non-GLP range-finding test.

From day 3 (D3) to day 6 (D6) of the experiment, each larva (3 replicates; 12 larvae/replicate) was fed with treated diet in the volume of 20, 30, 40 or 50 µL, respectively (total volume of treated diet was 140 µL). During the experiment, the larvae were kept in grafting cells placed into 48-well plates.

The plates were kept in a desiccator, in an incubator.

A toxic standard, i.e. dimethoate, was used to verify the sensitivity of the larvae and the precision of the test procedure.

Mortality of the larvae was recorded daily from day 4 (D4) – to day 8 (D8) and at day 10 (D10). On day 15 (D15) mortality of pupae was recorded. The test was ended on day 22 when the emergence of adults was evaluated.

Materials and methods	FLUDIO 025 GF
Test item:	content: 25.0 g/L of fludioxonil (CAS No. 131341-86-1) batch no.: SNS-F-06-22 production date: 10.2021 expiry date: 10.2023
Biological test system:	the honeybee, <i>Apis mellifera</i> L.; strain: carnica; source: an apiary at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna; age: one-day-old larvae
Experimental design:	– the test item: number of cumulative dose: 1 and a control; number of replicates: 3; number of larvae: 12/replicate – the reference item: number of cumulative doses: 1; number of replicates: 3; number of larvae: 12/replicate
Reference item dose:	7.39 µg dimethoate/larva
Test duration:	22 days
Test conditions:	temperature: 34.0 – 34.9°C; relative air humidity: D1 – D8: 91.3 – 98.0% D8 – D15: 75.7 – 85.0% D15 – D22: 52.5 – 79.4%
Endpoints:	mortality of larvae and pupae, adult emergence on day 22 (D22)
Statistical method:	Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity, One-way analysis of variance.

Results:

Mortality of the control group on day 8 (D8) of the test was 0.0% (criterion: $\leq 15\%$). The percentage of mortality of the honeybee larvae, exposed to the test item, FLUDIO 025 GF at the cumulative dose of 100.0 μg test item/larva at D8 was 11.1%. The percentage of larval mortality on D8 in the reference item group was 94.4%.

Pupal mortality of the control group on day 15 (D15) of the test was 8.3%. The percentage of mortality of the honeybee pupae corrected using Abbott's formula [7], exposed to the test item, FLUDIO 025 GF at the cumulative dose of 100.0 μg /larva at D15 was 9.1%. The percentage of pupal mortality, corrected using Abbott's formula [7], on D15 in the reference item group was 100.0%.

Cumulative mortality (larval and pupal) of the control group on day 22 (D22) of the test was 13.9%. The percentage of mortality of the honeybee pupae corrected using Abbott's formula [7], exposed to the test item, FLUDIO 025 GF at the cumulative dose of 100.0 μg /larva at D22 was 6.5%. The percentage of pupal mortality, corrected using Abbott's formula [7], on D15 in the reference item group was 100.0%.

The emergence of adults (emergence rate) at the end of the test (on D22) in the control group was 86.1%. In the groups treated with the test item at the cumulative dose of 100.0 μg test item/larva the adult emergence rates were: 80.6%, respectively.

The endpoint values for FLUDIO 025 GF at the end of the assessment (D22):

- ED₅₀ value is higher than 100.0 μg test item/larva,
- EC₅₀ value is higher than 649.4 mg/kg,
- NOED value is higher than or equal to 100.0 μg test item/larva,
- NOEC value is higher than or equal to 649.4 mg/kg.

The effects of FLUDIO 025 GF on mortality of honey bee larvae are summarized below:

Dose [µg test item/larva]	Concen- tration [mg test item/kg food]	Number of tested larvae [no.]	Total mortality (larval and pupal) on day 22 (D22)				
			Number [no.]	[%]	Corr ^a [%]	Number of emerged adults [No.]	Emergence rate [%]
Test item: FLUDIO 025 GF							
0.0 (Control)		36	5	13.9	–	31	86.1
100.0	649.4	36	7	19.4	6.5	29	80.6
ED ₅₀ [µg test item/larva]			> 100.0				
EC ₅₀ [mg/kg]			> 649.4				
NOED [µg test item/larva]			≥ 100.0				
NOEC [mg/kg]			≥ 649.4				
Reference item: Technical dimethoate mortality on day 8 (D8)							
7.39	48.0	36	36	100.0	100.0	not determined	

*: Mortality corrected according to the Abbott formula [7]

7. TEST VALIDITY CRITERIA

The following validity criteria were met:

- Cumulative larval mortality in the control group was 0.0% at day 8 (D8) (criterion: ≤ 15%).
- Abbott corrected mortality of the larvae treated with the reference item at day 8 (D8) (dimethoate) was 94.4% (criterion: ≥ 50%).
- Emergence rate in the control group on D22 was 86.1% (criterion: ≥ 70%).

8. DEVIATIONS IN THE STUDY

The study was performed according to the OECD Guidance Document No. 239 (2021): 'Honeybees, larval toxicity test, repeated exposure', other references given in section 9, SOP's listed in section 10 of the report, and the study plan. There were no deviations from the documents mentioned above.

A 2.3.1.3	KCP 10.3.1.3	Effects on honey bee development and other honey bee life stages
A 2.3.1.4	KCP 10.3.1.4	Sub-lethal effects

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

A 2.4 KCP 10.3.2 Effects on arthropods other than bees

Study 1

Comments of zRMS:	<p>The study was performed in line ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Grimm C., et al., 2000 with a minor deviation: – short time deviation from recommended range of humidity, with no impact on study results. It this deviation is considered to have no impact on the outcome of the study since all validity criteria were met:</p> <p>Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>NOER was established to be higher or equal to 4.5 L of product/ha.</p>
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Report	GLP laboratory study to determine the effect of a plant protection product FLUDIO 025 GF on the entomophagous rove beetle <i>Aleochara bilineata</i> ; Nácarová J., 2023, Study code: 21/350
Guideline(s):	ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Grimm C., et al., 2000)
Deviations:	Yes – short time deviation from recommended range of humidity, with no impact on study results
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Aim of the study:

A GLP laboratory study was required to determine the potential toxic effects of FLUDIO 025 GF on the entomophagous rove beetle *Aleochara bilineata* in terms of reproductive performance.

Material and methods:

Test organism	<i>Aleochara bilineata</i> 1-3 days old
Test design	9 weeks of exposure – 4 weeks of mortality assessment and 5 week of hatching assessment; 10 pairs (10 male and 10 female) per container, 4 replicates per treatment.
Nominal test item concentrations	4.5, 1.5, 0.5, 0.16, 0.05 L/ha

Test conditions:	Temperature 19.1 – 21.1°C, humidity 67 – 98%, average light: 1388 lux, 16h light : 8h dark.
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Validity criteria:

The average number of beetles emerging from the fly pupae in the water treated control was 981 per replicate. For the test to be considered valid, the average number should be > 400. Reproductive capacity in reference item was 127 emerged beetles per treatment (87% reduction relative to control). For the test to be considered valid, it should be > 50% relative to the control. The test fulfils both criteria and is thus valid.

Results:

Results of mortality assessments:

Table 1. Overview of the rove beetle mortality after exposure to each treatment (UTC: water control, T5-T1: test item treatments - FLUDIO 025 GF, REF: Dimethoate 400 EC). Mortality corrected using Abbott correction (Abbott 1925).

Treatment	Substance	Application rate (L of product/ha)	% Mortality	% Abbott's corrected mortality
UTC	water only	n/a	6.25	-
T5	FLUDIO 025 GF	0.05	10.00	4.00
T4		0.16	13.75	8.00
T3		0.5	18.75	13.33
T2		1.5	11.25	5.33
T1		4.5	16.25	10.67
Tox. Ref	Dimethoate 400 EC	1.1	98.75	98.67

Results of hatching assessment:

Table 2. Overview of the number of emerged rove beetles in each treatment (UTC: water control, T5-T1: test item treatments - FLUDIO 025 GF, REF: Dimethoate 400 EC).

Treatment	Substance	Application rate (L of product/ha)	Mean number of hatched beetles ± SD	% reduction compared to UTC
UTC	water only	n/a	981 ± 246	-
T5	FLUDIO 025 GF	0.05	911 ± 117	7.1
T4		0.16	835 ± 136	14.8
T3		0.5	805 ± 36	17.9
T2		1.5	803 ± 174	18.1
T1		4.5	891 ± 56	9.2
Tox. Ref	Dimethoate 400 EC	1.1	127 ± 26	87.1

Conclusion:

FLUDIO 025 GF has no negative impact on beetle mortality in concentrations up to 4.5 L/ha. The number of emerging beetles was not influenced by the highest tested rate (4.5 L of product/ha). NOER was established to be higher or equal to 4.5 L of product/ha.

Study 2

Comments of zRMS:	<p>The study was performed in line ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Grimm C., et al., 2000) with – short time deviation from recommended range of humidity, with no impact on study results.</p> <p>It this deviation is considered to have no impact on the outcome of the study since all validity criteria were met: Mortality in the water control was 3.3 % after 2 weeks - maximum acceptable mortality is 6.7 % (2 spiders). Mortality in the toxic reference after 2 weeks was 25 spiders (83.3%) - should be 65±35 %.</p> <p>The study is considered acceptable with the following endpoints relevant for the risk assessment: Test item FLUDIO 025 GF didn't show any influence on mortality nor behaviour of spiders in concentrations up to 4.5 L/ha</p>
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Report	GLP laboratory study to determine the effect of a plant protection product FLUDIO 025 GF on spiders of the genus Pardosa (Arenea, Lycosidae); Diáková K., 2022, Study code: 21/349
Guideline(s):	ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Candolfi. M.P, et al., 2000), (Heimbach, U 2000)
Deviations:	Yes – short time deviation from recommended range of humidity, with no impact on study results
GLP:	Yes
Acceptability:	Yes

Duplication (if vertebrate study)	No
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Aim of the study:

A GLP laboratory study was required to assess the side effects of FLUDIO 025 GF on *Pardosa* sp.

Material and methods:

Test organism	<i>Pardosa</i> spider
Test design	2 weeks of exposure 30 spiders (15 male and 15 female) per treatment, spiders were kept individually,
Nominal test item concentrations	Seeds were coated with test items in concentrations 4.5, 1.5, 0.5, 0.16, 0.05 L/ha, and 250 kg seeds per ha were incorporated into the sand (5 seeds per replicate).
Test conditions:	Temperature 18.5 – 21.4°C, humidity 55 – 96%, light: 503 - 552 lux, 16h light : 8h dark.

Validity criteria:

Mortality in the water control was 3.3 % after 2 weeks - maximum acceptable mortality is 6.7 % (2 spiders). Mortality in the toxic reference after 2 weeks was 25 spiders (83.3%) - should be 65±35 %.

Results:

Results of mortality assessments:

Table 2. Overview of the *Pardosa* sp. mortality after exposure to treatments at the end of the test (UTC: untreated control, T1-T5: test item treatments - FLUDIO 025 GF, Tox. standard – Dimethoate 400 EC).

Treatment	Application rate	Number of dead spiders		Number of dead spiders	Mortality (%)	Abbott's corrected mortality (%)
		Female	Male			
UTC	n/a	1	0	1	3.3	0
T5	0.05 L/ha	0	0	0	0	0
T4	0.16 L/ha	0	0	0	0	0
T3	0.5 L/ha	1	1	2	6.7	3.4
T2	1.5 L/ha	0	0	0	0	0
T1	4.5 L/ha	1	0	1	3.3	0
Tox. standard	600 g a.i./ha	11	14	25	83.3	n/a

Table 3. Mean number of prey item (*Drosophila spp.*) taken per spider at each assessment and cumulative food uptake per spider for all treatments (UTC: untreated control, T1-T5: test item treatments – FLUDIO 025 GF, Tox. standard – Dimethoate 400 EC). The total number of fruit flies provided to each spider during the whole test period was 30.

Treatment	Mean no. of prey item taken per spider at each assessment						Mean no. of prey item consumed per spider/all days
	1DAA	2DAA	3DAA	4DAA	8DAA	11DAA	
UTC	4.4	3.7	3.0	2.6	4.6	3.9	3.7
T5	4.2	3.3	3.1	2.9	4.3	3.6	3.1
T4	4.5	3.6	3.4	2.8	4.1	3.6	3.7
T3	4.0	3.3	2.6	2.5	4.5	4.0	3.5
T2	4.4	3.5	2.7	2.5	4.2	3.6	3.5
T1	4.1	3.2	3.0	2.2	4.2	3.7	3.3
Tox. standard	3.9	2.1	1.7	2.0	4.3	2.7	2.8

Conclusion:

Test item FLUDIO 025 GF didn't show any influence on mortality nor behaviour of spiders in concentrations up to 4.5 L/ha

A 2.5 KCP 10.4 Effects on non-target soil meso- and macrofauna

A 2.5.1 KCP 10.4.1 Earthworms

A 2.5.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

Comments of zRMS:	<p>The study was performed in line with OECD 222 with no deviations.</p> <p>The results are considered valid because the following criteria were satisfied in the controls:</p> <ul style="list-style-type: none"> – each replicate produced from 115 to 208 juveniles (168.9 mean) at the end of the exposure period (criterion: ≥ 30 juveniles by the end of the experiment), – the coefficient of variation of reproduction was 19.5% (criterion: $\leq 30\%$), – adult mortality over the initial 4 weeks of the experiment was 7.5% (criterion: $\leq 10\%$). <p>The study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>NOEC is equal to or above 1000.0 mg/kg dry weight of the artificial soil (i.e. equal to or above 23.5 mg of fludioxonil/kg dry weight of the artificial soil).</p>
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Report	FLUDIO 025 GF: Earthworm reproduction test (<i>Eisenia andrei</i>), Wróbel, A., Study code: G-23-21
Guideline(s):	OECD Guideline for the Testing of Chemicals No. 222
Deviations:	No

GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Aim of the study

The aims of the study were to assess the impact of the test item on reproduction of the earthworm, *Eisenia andrei* and to determine the EC10, EC20, EC50, and NOEC.

Materials and methods

Test organism	<i>Eisenia andrei</i>
Test design	56 days of exposure, 4 replicates per test item concentration, 8 replicates of control, 10 earthworms per replicate
Nominal test item concentrations	1000, 560, 320, 180, 100, 56, 32, 18 mg/kg soil plus the control
Test conditions	temperature: 20.8 – 22.0°C; pH at the beginning of the experiment: 6.04 – 6.13; pH at the end of the experiment: 5.75 – 5.83; soil moisture content at the beginning of the experiment: 25.0 – 26.7% (52.9 – 56.5% of the maximum water holding capacity); soil moisture content at the end of the experiment: 23.2 – 24.5% (49.1 – 51.8% of the maximum water holding capacity); light-dark cycle: 16h : 8h; light intensity at the beginning of the experiment: 505.3 – 580.5 lux light intensity at the end of the experiment: 517.8 – 592.4 lux

Validity criteria

- each replicate produced 168.9 juveniles (mean) at the end of the experiment - (criterion: ≥ 30 juveniles by the end of the experiment),
- the coefficient of variation of reproduction was 19.5% (criterion: $\leq 30\%$),
- adult mortality over the initial 4 weeks of the experiment was 7.5% (criterion: $\leq 10\%$).

Analytical measurements

Results of determination of fludioxonil:

Nominal test item concentration [mg/kg d.w.]	Mean concentration of fludioxonil determined (n=3) in samples collected					
	at 0 day (26.01.2022) [mg/kg d.w.]	Recovery %± RSD	at 28 days (23.02.2022) [mg/kg d.w.]	Recovery %± RSD	at 56 days (23.03.2022) [mg/kg d.w.]	Recovery %± RSD
Control	< LoD	---	< LoD	---	< LoD	---
1000	24.826	105.6±0.8	23.515	100.1±0.7	23.430	99.7±1.1

Results

At concentrations ranging from 18.0 to 1000.0 mg of the test item/kg dry weight of artificial soil, after 4 weeks of exposure to the test item, mortality of the adult earthworms was between 0.0 and 7.5%.

After 4 weeks of the experiment, at the concentrations between 18.0 and 1000.0 mg of the test item/kg dry weight of the artificial soil, the changes in appearance and behaviour of the adult earthworms were not observed.

After 4 weeks of the exposure period of the test item at the concentrations ranging from 18.0 to 1000.0 mg/kg dry weight of artificial soil, the body weight increase was between 24.8 and 34.6%. As for the control group, the body weight increase was equal to 22.6%.

After the application of the test item at the concentrations ranging from 18.0 to 1000.0 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 166.0 and 185.5 per replicate. The mean number of juveniles in the control group was equal to 168.9 per replicate.

After 8 weeks of the experiment, it was concluded that FLUDIO 025 GF had no statistically significant impact on reproduction of the earthworms at the concentrations ranging from 18.0 to 1000.0 mg/kg dry weight of artificial soil.

The concentration of the test item causing a 10% reduction in the number of juveniles produced within the exposure period (**EC10**) is **above 1000.0 mg/kg dry weight of the artificial soil** (i.e. above 23.5 mg of fludioxonil/kg dry weight of the artificial soil).

The concentration of the test item causing a 20% reduction in the number of juveniles produced within the exposure period (**EC20**) is **above 1000.0 mg/kg dry weight of the artificial soil** (i.e. above 23.5 mg of fludioxonil/kg dry weight of the artificial soil).

The concentration of the test item causing a 50% reduction in the number of juveniles produced within the exposure period (**EC50**) is **above 1000.0 mg/kg dry weight of the artificial soil** (i.e. above 23.5 mg of fludioxonil/kg dry weight of the artificial soil).

The highest concentration at which the test item is observed to have no statistically significant effects on reproduction (**NOEC**) is **equal to or above 1000.0 mg/kg dry weight of the artificial soil** (i.e. equal to or above 23.5 mg of fludioxonil/kg dry weight of the artificial soil).

The lowest concentration at which the test item is observed to have a statistically significant effect on reproduction (**LOEC**) is **above 1000.0 mg/kg dry weight of the artificial soil** (i.e. above 23.5 mg of fludioxonil/kg dry weight of the artificial soil).

Conclusion

The endpoint values determined on the basis of the nominal test item concentrations are given below:

Parameter	Value [mg test item/kg dry weight of artificial soil]	Value [mg of fludioxonil/kg dry weight of artificial soil]
EC ₁₀	> 1000.0	>23.5
EC ₂₀	> 1000.0	>23.5
EC ₅₀	> 1000.0	>23.5
NOEC (reproduction)	≥ 1000.0	≥ 23.5
LOEC (reproduction)	> 1000.0	>23.5
LC ₅₀	> 1000.0	>23.5
NOEC (survival)	≥ 1000.0	≥ 23.5
LOEC (survival)	> 1000.0	>23.5

The LC₅₀ (mortality)/48 h values is higher than 1000 mg/kg soil.

The NOEC (mortality)/48 h values is higher than or equal to 1000 mg/kg soil.

The EC₅₀ (reproduction)/48 h values is higher than 1000 mg/kg soil.

The NOEC (reproduction)/48 h values is higher than or equal to 1000 mg/kg soil.

A 2.5.1.2 KCP 10.4.1.2 Earthworms - field studies

A 2.5.2 KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

Study 1

Comments of zRMS:	<p>The study was performed in line with OECD 232 with no deviations.</p> <p>The results are considered valid because the following criteria were satisfied in the controls:</p> <ul style="list-style-type: none"> - mean adult mortality: 6.3% (criterion: ≤ 20%), - the mean number of juveniles per vessel at the end of the test: 1022.3 (criterion: ≥100 juveniles at the end of the test), - the coefficient of variation calculated for the number of juveniles: 11.4 (criterion: ≤ 30%). <p>The study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>NOEC is equal to or above 1000.0 mg/kg dry weight of the artificial soil (i.e. equal to or above 23.5 mg of fludioxonil/kg dry weight of the artificial soil).</p>
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Report	FLUDIO 025 GF Collembolan (<i>Folsomia candida</i>) Reproduction Test, Gierbuszewska A., 2022, Study code: G-24-21
Guideline(s):	OECD Guideline No. 232 (2026)
Deviations:	Yes (The deviations have no effect on the obtained results)
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Aim of the study

The aims of the study were to assess the impact of the test item on reproduction of the collembolan, *Folsomia candida* and to determine the EC10, EC20, EC50, and NOEC.

Materials and methods

Test organism	<i>Folsomia candida</i>
Test design	28 days of exposure, 4 replicates per test item concentration, 8 replicates of control, 10 collembolans per replicate
Nominal test item concentrations	1000, 560, 320, 180, 100, 56, 32, 18 mg/kg soil plus the control
Test conditions	temperature: 21.0 – 22.0°C; pH at the beginning of the experiment: 5.67 – 5.73; pH at the end of the experiment: 5.61 – 5.69; soil moisture content at the beginning of the experiment: 17.8 – 18.3% (46.9 – 48.3% of the maximum water holding capacity); soil moisture content at the end of the experiment: 16.6 – 17.9% (43.8 – 47.2% of the maximum water holding capacity); light-dark cycle: 16h : 8h; light intensity at the beginning of the experiment: 743.1 – 760.7 lux light intensity at the end of the experiment: 716.5 – 760.2 lux

Validity criteria

- mean adult mortality: 6.3% (criterion: $\leq 20\%$),
- the mean number of juveniles per vessel at the end of the test: 1022.3 (criterion: ≥ 100 juveniles at the end of the test),
- the coefficient of variation calculated for the number of juveniles: 11.4 (criterion: $\leq 30\%$).

Deviations

- culturing of collembolans takes place in plastic containers containing an artificial substrate consisting of plaster and charcoal in ratio 9:1 and not 10:1 or 8:1 as is mentioned in OECD Guideline No. 232 (2016) (3.3),
- at the end of the test the soil moisture content was determined by drying small sample of the artificial soil in 105°C instead of weighing the test vessels as it is mentioned in OECD Guideline No. 232 (2016) (3.6.6),
- physiological or pathological symptoms or distinct changes in behavior were not described (3.6.7).

Analytical measurements

Table 4. Data of test samples for fludioxonil (n=3) 0 day, 14 days and 28 days.

Concentration of test item [mg/kg]	Concentration of fludioxonil [mg/kg d.w.]	Lab Sample Amount [g]	Final Volume [mL]	Measured Concentration [µg/mL]	Analysed Analyte Concentration (II.1) [mg/kg]	Dry matter content [%]	Concentration of fludioxonil (II.2) [mg/kg d.w.]
Collembolan control Day 0	--	10	20	--	--	84.7	--
	--	10	20	--	--		--
	--	10	20	--	--		--
Collembolan 1000 mg/kg d.w. Day 0	23.5	10	20	10.5906	21.181	84.5	25.066
	23.5	10	20	10.1922	20.384		24.123
	23.5	10	20	10.6572	21.314		25.224
Collembolan control day 14	--	10	20	--	--	84.8	--
	--	10	20	--	--		--
	--	10	20	--	--		--
Collembolan 1000 mg/kg d.w. Day 14	23.5	10	20	9.9670	19.934	85.6	23.287
	23.5	10	20	10.3657	20.731		24.218
	23.5	10	20	10.1519	20.304		23.720
Collembolan control Day 28	--	10	20	--	--	84.6	--
	--	10	20	--	--		--
	--	10	20	--	--		--
Collembolan 1000 mg/kg d.w. Day 28	23.5	10	20	10.3219	20.644	85.3	24.202
	23.5	10	20	10.3146	20.629		24.184
	23.5	10	20	9.0414	18.083		21.199

The range of linearity of the analytical graph is from 0.2 µg/mL to 20.0 µg/mL

--not detected i.e. below LoD 0.4 mg/kg

Results

Table 7. Number of juvenile collembolans (*Folsomia candida*) after 28 days of the experiment.

Concentration [mg/kg dry weight of the artificial soil]	Replicate	Number of juveniles	Mean ±SD	Comparison to the control [%]	Coefficient of variation [%]
control	1	979	1022.3 ± 116.9	-	11.4
	2	931			
	3	979			
	4	918			
	5	1014			
	6	1197			
	7	1213			
	8	947			
18	1	1215	1317.3 ± 188.7	128.86	14.3
	2	1351			
	3	1136			
	4	1567			
32	1	1064	1087.3 ± 99.5	106.36	9.2
	2	961			
	3	1130			
	4	1194			
56	1	1211	1025.3 ± 177.1	100.29	17.3
	2	816			
	3	1125			
	4	949			
100	1	1530	1126.0 ± 293.5	110.15	26.1
	2	1155			
	3	926			
	4	893			
180	1	1077	1184.5 ± 104.4	115.87	8.8
	2	1115			
	3	1255			
	4	1291			
320	1	1160	948.0 ± 216.6	92.74	22.8
	2	1070			
	3	893			
	4	669			
560	1	1363	1197.0 ± 115.7	117.09	9.7
	2	1124			
	3	1112			
	4	1189			
1000	1	830	964.5 ± 182.9	94.35	19.0
	2	873			
	3	922			
	4	1233			

No statistically significant differences between the control and the treatment group were noticed (Williams Multiple Sequential t-test Procedure, significance level = 0.05, one-sided smaller)

After 28 days of exposure FLUDIO 025 GF at the rate of 1000 mg/kg dw no effects on mortality and reproduction were observed

Based on the obtained mortality and reproduction results LR₅₀ and ER₅₀ could not be estimated. It can be assumed that the LR₅₀ and ER₅₀ are higher than 1000 mg/kg dw of FLUDIO 025 GF and NOEC for mortality and reproduction is higher than or equal to 1000 mg/kg dw.

Conclusions

Endpoint values are presented in the table below:

Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg of fludioxonil/kg dry weight of the artificial soil]
EC ₁₀	> 1000	> 23.54
EC ₂₀	> 1000	> 23.54
EC ₅₀	> 1000	> 23.54
NOEC	≥ 1000	≥ 23.54

On the basis of the obtained results it can be concluded that FLUDIO 025 GF at the rate of 1000 mg/kg soil has no adverse effect on the mortality and reproduction of the collembolans.

Study 2

Comments of zRMS:	<p>The study was performed in line with OECD 226 with following deviations:</p> <ul style="list-style-type: none"> - according to the OECD Guideline No. 226 (2016) the water content of the artificial soil should be maintained throughout the test by weighing and if needed re-watering the vessels periodically. In the study to maintain proper moisture content, a small sample of soil was drying at 105°C and re-weighing at the beginning, after 7 days of the test and at the end of the test - due to the use of the temperature extraction method, there was no need for euthanasia of the extracted organisms since the mites are fixed in a 70% ethanol solution - Due to the use of the temperature extraction method, it was not possible to record the symptoms with behavioral and morphology changes of the extracted predatory mites. <p>The deviations have no effect on the obtained results</p> <p>The results are considered valid because the following criteria were satisfied in the controls:</p> <ul style="list-style-type: none"> - mean adult mortality: 6.3% (criterion: ≤ 20%), - the mean number of juveniles per vessel at the end of the test: 1022.3 (criterion: ≥100 juveniles at the end of the test), - the coefficient of variation calculated for the number of juveniles: 11.4 (criterion: ≤ 30%). <p>The study is considered acceptable with the following endpoint relevant for the risk assessment: NOEC is equal to or above 1000.0 mg/kg dry weight of the artificial soil (i.e. equal to or above 23.5 mg of fludioxonil/kg dry weight of the artificial soil).</p>
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Report	FLUDIO 025 GF predatory mite (<i>Hypoaspis (Geolaelaps) aculeifer</i>) reproduction test in soil, Wróbel A., 2022, Study code: G-25-21
Guideline(s):	OECD Guideline No. 226 (2016)

Deviations:	Yes (The deviations have no effect on the obtained results)
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Aim of the study

The aims of the study were to assess the effects of the test item on the reproductive output of the predatory mite, *Hypoaspis aculeifer* and to determine the EC10, EC20, EC50, and NOEC.

Materials and methods

Test organism	<i>Hypoaspis aculeifer</i>
Test design	14 days of exposure, 4 replicates per test item concentration, 8 replicates of control, 10 mites per replicate
Nominal test item concentrations	1000, 560, 320, 180, 100, 56, 32, 18 mg/kg soil plus the control
Test conditions	temperature: 21.2 – 21.9°C; pH at the beginning of the experiment: 5.65 – 5.72; pH at the end of the experiment: 5.61 – 5.80; soil moisture content at the beginning of the experiment: 17.8 – 18.7% (46.9 – 49.3% of the maximum water holding capacity); soil moisture content at the end of the experiment: 16.6 – 17.5% (43.8 – 46.1% of the maximum water holding capacity); light-dark cycle: 16h : 8h; light intensity at the beginning of the experiment: 444.2 – 526.4 lux light intensity at the end of the experiment: 512.7 – 623.4 lux

Validity criteria

- mean adult mortality: 6.3% (criterion: $\leq 20\%$),
- the mean number of juveniles per vessel at the end of the test: 94.4 (criterion: ≥ 50 juveniles at the end of the test),
- the coefficient of variation calculated for the number of juveniles: 15.6 (criterion: $\leq 30\%$).

Deviations

1. According to the OECD Guideline No. 226 (2016) the water content of the artificial soil should be maintained throughout the test by weighing and if needed re-watering the vessels periodically. In the study to maintain proper moisture content, a small sample of soil was drying at 105°C and re-weighing at the beginning, after 7 days of the test and at the end of the test.
2. Due to the use of the temperature extraction method, there was no need for euthanasia of the extracted organisms since the mites are fixed in a 70% ethanol solution.
3. Due to the use of the temperature extraction method, it was not possible to record the symptoms with behavioral and morphology changes of the extracted predatory mites.

Analytical measurements

Table 4. Data of test samples for fludioxonil (n=3) 0 day, 7 days and 14 days.

Concentration of test item [mg/kg]	Concentration of fludioxonil [mg/kg d.w.]	Lab Sample Amount [g]	Final Volume [mL]	Measured Concentration [µg/mL]	Analysed Analyte Concentration (II.1) [mg/kg]	Dry matter content [%]	Concentration of fludioxonil (II.2) [mg/kg d.w.]
Hypoaspis control Day 0	--	10	20	--	--	84.5	--
	--	10	20	--	--		--
	--	10	20	--	--		--
Hypoaspis 1000 mg/kg d.w. Day 0	23.5	10	20	10.3952	20.790	84.4	24.633
	23.5	10	20	10.5690	21.138		25.045
	23.5	10	20	10.5763	21.153		25.063
Hypoaspis control day 7	--	10	20	--	--	84.9	--
	--	10	20	--	--		--
	--	10	20	--	--		--
Hypoaspis 1000 mg/kg d.w. Day 7	23.5	10	20	10.0992	20.198	84.7	23.847
	23.5	10	20	9.8392	19.678		23.233
	23.5	10	20	10.0198	20.040		23.660
Hypoaspis control Day 14	--	10	20	--	--	85.2	--
	--	10	20	--	--		--
	--	10	20	--	--		--
Hypoaspis 1000 mg/kg d.w. Day 14	23.5	10	20	10.2467	20.493	84.9	24.138
	23.5	10	20	10.2247	20.449		24.086
	23.5	10	20	9.0932	18.186		21.420

The range of linearity of the analytical graph is from 0.2 µg/mL to 20.0 µg/mL

--not detected i.e. below LoD 0.4 mg/kg

Results

Table 8. Number of juvenile mites (*Hypoasis aculeifer*) after 14 days of the experiment.

Concentration [mg/kg dry weight of soil]	Replicate	Number of juvenile mites	Mean ±SD	Comparison to the control [%]	CV* [%]
0.0 (control)	1	105	94.4 ± 14.7	-	15.6
	2	74			
	3	98			
	4	85			
	5	101			
	6	76			
	7	100			
	8	116			
18	1	89	91.5 ± 3.7	97.0	4.0
	2	93			
	3	88			
	4	96			
32	1	85	92.3 ± 10.5	97.7	11.3
	2	98			
	3	82			
	4	104			
56	1	90	91.5 ± 6.2	97.0	6.8
	2	84			
	3	99			
	4	93			
100	1	85	91.5 ± 11.8	97.0	12.9
	2	109			
	3	88			
	4	84			
180	1	93	95.0 ± 14.6	100.7	15.4
	2	116			
	3	88			
	4	83			
320	1	105	93.8 ± 12.2	99.3	13.0
	2	87			
	3	80			
	4	103			
560	1	84	91.3 ± 7.9	96.7	8.6
	2	92			
	3	102			
	4	87			
1000	1	112	103.8 ± 13.9	109.9	13.4
	2	119			
	3	91			
	4	93			

* - coefficient of variation

No statistically significant difference between the control and the treatment group (Dunnett's Multiple t-test Procedure, significance level = 0.05, one-sided smaller)

After 14 days of exposure FLUDIO 025 GF at the rate of 1000 mg/kg dw no effects on mortality and reproduction were observed

Based on the obtained mortality and reproduction results LR₅₀ and ER₅₀ could not be estimated. It can be assumed that the LR₅₀ and ER₅₀ are higher than 1000 mg/kg dw of FLUDIO 025 GF and NOEC for mortality and reproduction is higher than or equal to 1000 mg/kg dw.

Conclusions

Table 9. Endpoint values - the impact of the test item on reproduction of the predatory mites (*Hypoaspis aculeifer*).

Endpoint	Value [mg of the test item/kg dry weight of the artificial soil]	Value [mg of fludioxonil/kg dry weight of the artificial soil]
EC ₁₀	> 1000.0	> 23.5
EC ₂₀	> 1000.0	> 23.5
EC ₅₀	> 1000.0	> 23.5
NOEC (reproduction)	≥ 1000.0	≥ 23.5

On the basis of the obtained results it can be concluded that FLUDIO 025 GF at the rate of 1000 mg/kg soil has no adverse effect on the mortality and reproduction of the predatory mites.

A 2.5.2.1 KCP 10.4.2.1 Species level testing

A 2.5.2.2 KCP 10.4.2.2 Higher tier testing

A 2.6 KCP 10.5 Effects on soil nitrogen transformation

Comments of zRMS:	<p>The study was performed with OECD 216 and EU Method C.21.</p> <p>All validity criteria were met:</p> <ul style="list-style-type: none"> - the variation between replicate control samples was ≤ 15 % (observed max. 6.2 %). <p>Deviation:</p> <p>according the Guideline, the soil extraction should be conducted at 150 rpm for 60 min. However, in this study, the extraction was performed at 90 rpm and time duration between 18 to 24 hours. The modification resulted from the optimization of the nitrate extraction FLUDIO 025 GF did not affect the results of the study</p> <p>The study is considered acceptable.</p> <p>The effects of the test item on soil nitrogen formation rates were < 25 % at the end of the study period (28 days).</p>
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Report	FLUDIO 025 GF: Soil microorganisms: Nitrogen transformation test, Gierbuszewska, A., Study code: G-26-21
Guideline(s):	OECD Guideline for the Testing of Chemicals No. 216 (2000) and EU Method C.21

Deviations:	Yes (The deviation has no impact on the results)
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Aim of the study

The aim of this study was to detect long-term adverse effects of **FLUDIO 025 GF** on the process of nitrogen transformation in aerobic surface soils.

Materials and methods

Test design	On control group and two treated group, three replicates per each group, 500g of soil per replicate, 28 days of exposure
Nominal test item concentrations	0.71 and 3.55 mg/kg soil
Test conditions	Temperature: 20.2 – 22.0°C, soil moisture: 44.2% - 47.7% of the maximum water holding capacity, incubation in darkness

Validation criteria

The coefficients of variation (CV) in the control group were 1.8, 2.9, 5.9 and 0.9%, after 0, 7, 14 and 28 days of incubation. The validity criterion was met, because the variation between replicate control samples is less than $\pm 15\%$.

Deviations

According to the Guideline, the soil extraction should be conducted at 150 rpm for 60 min. However, in this study, the extraction was performed at 90 rpm and time duration between 18 to 24 hours. The modification resulted from the optimization of the nitrate extraction which showed that the extraction was more effective when the shaking rate was lower and the extraction lasted longer. This deviation did not affect the results of the study.

Results

Table 8. Concentration of the nitrate ions on day 28 of incubation.

Concentration	Control			PEC			5 x PEC		
Replicate	I	II	III	I	II	III	I	II	III
Reading* [mg/L]	37.901	38.201	38.621	42.421	42.531	43.571	39.821	42.321	42.291
Nitrate ion concentration [mg/kg of dry soil]	189.51	191.04	193.11	212.11	212.66	217.86	199.11	211.61	211.46
Mean nitrate ion concentration [mg/kg of dry soil] \pm SD	191.21 \pm 1.81			214.21 ⁺ \pm 3.17			207.39 ⁺ \pm 7.17		
CV	0.9			1.5			3.5		

* - values adjusted for the value of the blank sample

* - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure, significance level = 0.05, two-sided)

The difference in the nitrate formation rate between the control soil and the one treated with the test item at the concentration corresponding to the PEC: 0.71 mg of the test item / kg dry weight of soil, and 5 x PEC: 3.55 mg of the test item / kg dry weight of soil did not exceed 25% on 28 day of analysis.

Conclusion

Table 10. Deviations from the control based on nitrate formation rate for selected time intervals [%].

Time interval [d]	PEC	5 x PEC
0 – 7	27.6	19.5
0 – 14	23.3	22.3
0 – 28	-22.9	-16.1

Values obtained using ToxRat 2.10. computer software.

On the basis of the results, it was concluded that FLUDIO 025 GF at the concentration corresponding to the PEC: 0.71 mg of the test item / kg dry weight of soil, and 5 x PEC: 3.55 mg of the test item / kg dry weight of soil did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.

A 2.7 KCP 10.6 Effects on terrestrial non-target higher plants

A 2.7.1 KCP 10.6.1 Summary of screening data

A 2.7.2 KCP 10.6.2 Testing on non-target plants

A 2.7.3 KCP 10.6.3 Extended laboratory studies on non-target plants

A 2.8 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

A 2.9 KCP 10.8 Monitoring data