REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: RNB 072 A

Product name(s): **MATLAM**

Chemical active substance:

Florasulam, 50 g/L

Central Zone

Zonal Rapporteur Member State: POLAND

CORE ASSESSMENT

(authorization)

Applicant: XXXX

Submission date: June 2024

Evaluation date: February 2025

MS Finalisation date: May 2025

Version history

When	What
June 2024	dRR version 1 submitted by applicant
December 2024	Update by the applicant
February 2025	zRMS finalized dRR evaluation

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9 Ecotoxicology (KCP 10)

Review Comments:

This is the application for registration plant protection product according to Article 33 of Regulation 1107/2009 based on protected data for compositionally comparable formulation - Floras 50 SC. The LoA to all studies considered necessary for the fulfilment of the risk assessment was provided. MATLAM (RNB 072 A) is a suspension concentrate containing 50 g/L of florasulam and is used as a herbicide in cereals.

This Part B document only reviews data and additional information that has not previously been considered within the EU review process.

Since this document is based on the information provided by the applicant, all review comments, additions and corrections have been made using commenting boxes or highlighted in grey.

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use-No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			PHI (days)	Remarks: e.g. g safener/ synergist per ha	**Conclusion**						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	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 [illegible]	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1.	PL	**Winter wheat Winter spelt, Winter barley, Winter triticale, Winter rye**	F	dicotyledonous weeds (TTTDS)	Broadcast spray	BBCH 12-33 (spring application)	a) 1 b) 1	NA	a) 0.1 b) 0.1	a) 5.0 b) 5.0	200-400	60		A	A	A	A	A	A	R
2.	PL	**Spring barley Spring wheat Spring triticale, Spring oat**	F	dicotyledonous weeds (TTTDS)	Broadcast spray	BBCH 12-33 (spring application)	a) 1 b) 1	NA	a) 0.1 b) 0.1	a) 5.0 b) 5.0	200-400	55		A	A	A	A	A	A	A
Interzonal uses (use as seed treatment, in greenhouses (or other closed places of plant production), as post-harvest treatment or for treatment of empty storage rooms)																				
3																				
4																				
Minor uses according to Article 51 (zonal uses)																				
5	PL	Winter wheat durum	F	dicotyledonous weeds (TTTDS)	Broadcast spray	BBCH 12-33 (spring application)	a) 1 b) 1	NA	a) 0.1 b) 0.1	a) 5.0 b) 5.0	200-400	60		A	A	A	A	A	A	R
6	PL	Winter oat	F	dicotyledonous weeds (TTTDS)	Broadcast spray	BBCH 12-33 (spring application)	a) 1 b) 1	NA	a) 0.1 b) 0.1	a) 5.0 b) 5.0	200-400	60		A	A	A	A	A	A	R

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
7	PL	Spring wheat durum	F	dicotyledonous weeds (TTTDS)	Broadcast spray	BBCH 12-33 (spring application)	a) 1 b) 1	NA	a) 0.1 b) 0.1	a) 5.0 b) 5.0	200-400	55		A	A	A	A	A	A	R

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

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

Explanation for column 15 – 21 “Conclusion”

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

Remarks table:

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

9.1.1 Overall conclusions

An estimation of risk indicate acceptable risk for each organisms of each range of assessed issues, taking into consideration adequate mitigation measures.

9.1.1.1 Effects on birds (KCP 10.1.1), According to the screening assessments, all the TERa and TERlt values for Florasulam are greater than the Annex VI trigger of 10 and 5, respectively, indicating that MATLAM presents no unacceptable acute and long-term risk to birds according to the intended uses.

Review Comments:

The acute and chronic risks of MATLAM (RNB 072 A) to birds were assessed from toxicity exposure ratios between toxicity endpoints, estimated from study with active ingredient and maximum residues occurring on food items.

All TER values exceed the relevant triggers indicating that MATLAM (RNB 072 A) does not pose an unacceptable risk to birds following applications according to recommended use pattern.

Evaluation of exposing to birds through the drinking water demonstrated the acceptable risk. The potential risk of secondary poisoning is not triggered.

9.1.1.2 Effects on terrestrial vertebrates other than birds (KCP 10.1.2), According to the screening assessments for cereals, all the TERa and TERlt values for active substance are greater than the Annex VI trigger of 10 and 5, respectively, indicating that MATLAM presents no unacceptable acute and long-term risk to mammals according to the intended uses.

Review Comments:

The acute and chronic risks of MATLAM (RNB 072 A) to mammals were assessed from toxicity exposure ratios between toxicity endpoints, estimated from study with active ingredient and maximum residues occurring on food items.

All TER values exceed the relevant triggers indicating that MATLAM (RNB 072 A) does not pose an unacceptable risk to mammals following applications according to recommended use pattern.

Evaluation of exposing to mammals through the drinking water demonstrated the acceptable risk. The potential risk of secondary poisoning is not triggered.

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

Birds

According to the screening assessments, all the TER_a and TER_{lt} values for Florasulam are greater than the Annex VI trigger of 10 and 5, respectively, indicating that MATLAM presents no unacceptable acute and long-term risk to birds according to the intended uses.

Mammals
According to the screening assessments for cereals, all the TER_a and TER_{lt} values for active substance are greater than the Annex VI trigger of 10 and 5, respectively, indicating that MATLAM presents no unacceptable acute and long-term risk to mammals according to the intended uses.

9.1.1.4 Effects on aquatic organisms (KCP 10.2)

Florasulam
For the active substance Florasulam, calculated PEC/RAC ratios for spring and winter cereals did indicate an acceptable risk in all FOCUS Steps 3 scenarios relevant for Poland.

Metabolites of Florasulam
Regarding the metabolites, calculated PEC/RAC ratios did indicate an acceptable risk in all FOCUS Step 2 scenarios.

9.1.1.5 Effects on bees (KCP 10.3.1)

Use of MATLAM indicate low risk for bees.

9.1.1.6

9.1.1.7 Review Comments:

The evaluation of the acute risk for bees was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002). The submitted risk assessment, based on laboratory studies, has been accepted. It can therefore be concluded that there will be negligible acute risk associated with the exposure of *Apis mellifera* to MATLAM.

The data requirements in accordance with Commission Regulation (EU) No 284/2013 for the chronic toxicity to adult honeybees and honeybee larvae are fulfilled. Nevertheless, it should be noted that for larvae the single exposure test was submitted.

9.1.1.8 Effects on arthropods other than bees (KCP 10.3.2)

Use of MATLAM indicate low risk for non-target arthropods other than bees.

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

Use of MATLAM indicate low risk for soil meso- and macrofauna and soil microbial activity.

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

No potential risk to non-target plants located outside the treated area after application of MATLAM according to the GAP table is expected when the following risk mitigation measures are considered:

SPe 3: *To protect non-target plants respect an unsprayed buffer zone of 5m to non-agricultural land OR an unsprayed buffer zone of 1m to non-agricultural land with 75% drift reducing nozzles.*

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

No studies submitted.

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).

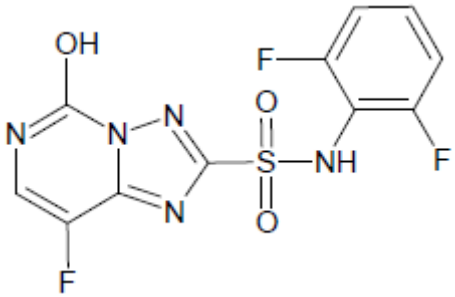
Table 9.1-2: Critical use pattern of MATLAM grouped according to criterion

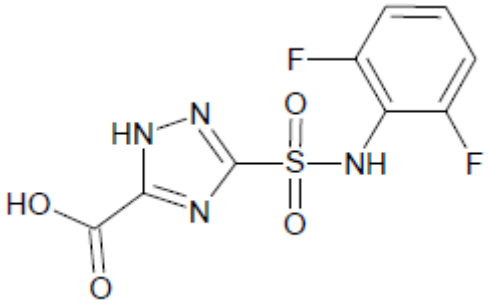
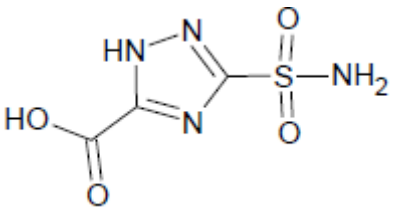
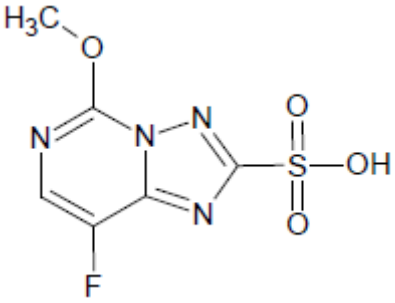
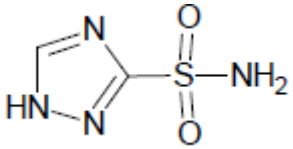
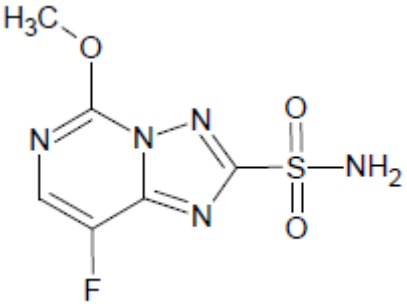
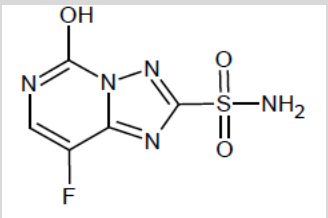
Grouping according to criterion			
Group	**Intended uses**	**Relevant use parameters for grouping**	**Relevant parameter or value for sorting**
Cereals	Uses No. 1-4	Application rate BBCH	0.1 L/ha; 5 g florasulam/ha BBCH 12-39 SPRING APPLICATION

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 MATLAM is indicated in the table.

Table 9.1-3 Metabolites of Florasulam

Metabolite	**Molar mass**	**Chemical structure**	**Maximum observed occurence in compartements**	**Exposue assessment required due to**
5-OH florasulam; N-(2,6-difluorophenyl)-8-fluoro-5-oxo-5,6-dihydro[1,2,4]triazolo[1,5-c]pyrimidine-2-sulfonamide	345.3		71.6 % 99.0 %	Soil Water/Sediment

Metabolite	Molar mass	Chemical structure	Maximum observed occurence in compartements	Exposue assessment required due to
DFP-ASTCA; 3-[(2,6-difluorophenyl)sulfamoyl]-1H-1,2,4-triazole-5-carboxylic acid	304.2		17.8 % 8.9 %	Soil Water/Sediment
ASTCA; 3-sulfamoyl-1H-1,2,4-triazole-5-carboxylic acid	192.1		40 % 53.8 %	Soil Water/Sediment
TPSA; 8-fluoro-5-methoxy[1,2,4]triazolo[1,5-c]pyrimidine-2-sulfonic acid	248.2		0.0001 % ~~58.0~~ 58.3 %	~~Soil~~ Water/Sediment
TSA; 1H-1,2,4-triazole-3-sulfonamide	148.1		15.9 % 0.0001 %	Soil Water/Sediment
ASTP; 8-fluoro-5-methoxy[1,2,4]triazolo[1,5-c]pyrimidine-2-sulfonamide	247.2		0.0001 % ~~21.0~~ 21.9 %	~~Soil~~ Water/Sediment
5-OH-ASTP	233.2		Soil: NR Water/sediment: 28.9%	Water/Sediment

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

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

Effects on birds of formulation were not evaluated.

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

Species	Substance	Time scale	Results [mg/kg bw]	Reference
Japanese quails *Coturnix coturnix japonica*	Florasulam	Oral 1 d Acute	**LD_{50} = 1046 mg/kg bw**	EFSA Journal 2015; 13(1):3984
Japanese quails *Coturnix coturnix japonica*	Florasulam	Dietary 8 d Short-term	LDD_{50} > 938 mg/kg bw/d	EFSA Journal 2015; 13(1):3984
Mallard duck *Anas platyrhynchos*	Florasulam	Dietary 8 d Short-term	LDD_{50} > 950 mg/kg bw/d	EFSA Journal 2015; 13(1):3984
Mallard Duck *Anas platyrhynchos*	Florasulam	Dietary Reproductive toxicity	NOEC = 1500 mg/kg feed NOEL = 150 mg/kg bw (factor 0.1)	EFSA Journal 2015; 13(1):3984
Bobwhite quail *Colinus viriginianus*	Florasulam	Dietary Reproductive toxicity	NOEC = 1500 mg/kg feed NOEL = 150 mg/kg bw (factor 0.1)	EFSA Journal 2015; 13(1):3984

9.2.1.1 Justification for new endpoints

For acute risk assessment, the LD_{50} of 1046 mg/kg bw was used. The LD_{50}/10 is **104.6 mg/kg bw**. Since the LD_{50}/10 is lower than the lowest NOEL (1500 mg/kg diet multiplied by a factor 0.1), the LD_{50}/10 was used for reproductive assessment.

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).

To achieve a concise risk assessment, the risk envelope approach is applied (see 9.1.2).

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

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

Table 9.2-2: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of MATLAM in Cereals regarding Florasulam data.

Intended use		**Cereals**			
Active substance/product		Florasulam			
Application rate (kg/ha)		1 × 0.005			
Acute toxicity (mg/kg bw)		1046			
TER criterion		10			
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals	Small omnivorous bird	158.8	1	0.79	1324.05
Reprod. toxicity (mg/kg bw/d)		104.6			
TER criterion		5			
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals	Small omnivorous bird	64.8	1.0 x 0.53	0.17	615.29

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

9.2.2.2 Higher-tier risk assessment

Not relevant.

9.2.2.3 Drinking water exposure

Leaf scenario

The leaf scenario is not relevant for the intended uses.

Puddle scenario

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

Florasulam has Koc below 500 L/kg.

Active substance	**Time scale**	**Toxicity endpoint**	**Effective application rate [g a.s./ha]**	**Ratio (AR_{eff}/endpoint)**
florasulam	Acute	1046 mg a.s./kg bw	5.0	0.005
	Chronic	104.6 mg a.s./kg bw/d		0.05

There’s no risk from puddle scenario.

9.2.2.4 Effects of secondary poisoning

According to EFSA Guidance Document on Risk Assessment for Birds and Mammals, 2009, substances with a log POW lower than 3 haven't potential for bioaccumulation. Florasulam has a log P_{OW} value below 3, and not indicating a potential risk of secondary poisoning, therefore a risk assessment is not required.

Risk assessment for earthworm-eating birds via secondary poisoning

Not relevant.

Risk assessment for fish-eating birds via secondary poisoning

Not relevant.

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.2.4 Overall conclusions

According to the screening assessments, all the TER_a and TER_{lt} values for Florasulam are greater than the Annex VI trigger of 10 and 5, respectively, indicating that MATLAM presents no unacceptable acute and long-term risk to birds according to the intended uses.

Review Comments:

The acute and chronic risks of MATLAM (RNB 072 A) to birds were assessed from toxicity exposure ratios between toxicity endpoints, estimated from study with active ingredient and maximum residues occurring on food items.

All TER values exceed the relevant triggers indicating that MATLAM (RNB 072 A) does not pose an unacceptable risk to birds following applications according to recommended use pattern.

Evaluation of exposing to birds through the drinking water demonstrated the acceptable risk. The potential risk of secondary poisoning is not triggered.

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

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

Species	Substance	Time scale	Results [mg/kg bw/day]	Reference
Mouse	Florasulam	Oral 1 d Acute	**LD_{50} > 5000 mg/kg bw**	EFSA Journal 2015; 13(1):3984
Rat	Florasulam	Long term	**NOAEL > 100 mg/kg bw/d**	EFSA Journal 2015; 13(1):3984

9.3.1.1 Justification for new endpoints

Not relevant as there is no deviation to the EU agreed 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).

To achieve a concise risk assessment, the risk envelope approach is applied (see 9.1.2).

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

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

Table 9.3-2: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of MATLAM in Cereals regarding Florasulam data.

Intended use	Cereals				
Active substance/product	Florasulam				
Application rate (kg/ha)	1 × 0.005				
Acute toxicity (mg/kg bw)	5000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals	Small herbivorous mammal	118.4	1.0	0.59	8474.58
Reprod. toxicity (mg/kg bw/d)	100				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals	Small herbivorous mammal	48.3	1.0 x 0.53	0.13	769.23

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

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).

Puddle scenario

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

Florasulam has Koc below 500 L/kg.

Active substance	Time scale	Toxicity endpoint	Effective application rate [g a.s./ha]	Ratio (AR_{eff}/endpoint)
florasulam	Acute	5000 mg a.s./kg bw	5.0	0.001
	Chronic	100 mg a.s./kg bw/d		0.05

There's no risk from puddle scenario.

9.3.2.4 Effects of secondary poisoning

According to EFSA Guidance Document on Risk Assessment for Birds and Mammals, 2009, substances with a log POW lower than 3 haven't potential for bioaccumulation. Florasulam has a log P_{OW} value below 3, and not indicating a potential risk of secondary poisoning, therefore a risk assessment is not required.

Risk assessment for earthworm-eating mammals via secondary poisoning

Not relevant.

Risk assessment for fish-eating mammals via secondary poisoning

Not relevant.

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

According to the screening assessments for cereals, all the TER_a and TER_{lt} values for active substance are greater than the Annex VI trigger of 10 and 5, respectively, indicating that MATLAM presents no unacceptable acute and long-term risk to mammals according to the intended uses.

Review Comments:

The acute and chronic risks of MATLAM (RNB 072 A) to mammals were assessed from toxicity exposure ratios between toxicity endpoints, estimated from study with active ingredient and maximum residues occurring on food items.

All TER values exceed the relevant triggers indicating that MATLAM (RNB 072 A) does not pose an unacceptable risk to mammals following applications according to recommended use pattern.

Evaluation of exposing to mammals through the drinking water demonstrated the acceptable risk. The potential risk of secondary poisoning is not triggered.

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

There is no additional data.

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 Floras 50 SC, active substance and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents, as well as in Appendix 2 of this document.

Effects on aquatic organisms of MATLAM were not evaluated in EU. 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 – Florasulam and its relevant metabolites

Species	Substance	Exposure System	Results [mg/L]	Reference
Fish				
Oncorhynchus mykiss, Lepomis macrochirus	Florasulam	96 h, s	**LC_{50} > 100 mg a.s./L $_{nom}$**	EFSA Journal 2015; 13(1):3984
Menidia beryllina	Florasulam	96 h, s	LC_{50} > 122 mg a.s./L $_{m}$	EFSA Journal 2015; 13(1):3984
Oncorhynchus mykiss	EF-1343	96 h, s	LC_{50} > 100 (>5.57 mg a.s./L $_{mm}$)	EFSA Journal 2015; 13(1):3984
Oncorhynchus mykiss	5-OH-florasulam	96 h, s	**LC_{50} > 91 mg/L $_{nom}$**	EFSA Journal 2015; 13(1):3984

Species	Substance	Exposure System	Results [mg/L]	Reference
Oncorhynchus mykiss	Florasulam	28-d, f	NOEC = 119 mg a.s./L $_{m}$	EFSA Journal 2015; 13(1):3984
Pimephales promelas	Florasulam	33-d, f (juveniles)	**NOEC = 2.9 mg a.s./L $_{mm}$**	EFSA Journal 2015; 13(1):3984
Aquatic invertebrate				
Daphnia magna	Florasulam	48 h, s	**EC_{50} > 292 mg a.s./L $_{m}$**	EFSA Journal 2015; 13(1):3984
Daphnia magna	EF-1343	48 h, s	EC_{50} > 100 (>5.50 mg a.s./L $_{mm}$)	EFSA Journal 2015; 13(1):3984
Palaemonetes pugio	Florasulam	96 h, s	EC_{50} > 120 mg a.s./L $_{nom}$	EFSA Journal 2015; 13(1):3984
Crassostrea virginica	Florasulam	96 h, s (shell deposition)	EC_{50} > 125 mg a.s./L $_{nom}$	EFSA Journal 2015; 13(1):3984
Daphnia magna	5-OH-florasulam	48 h, s	**EC_{50} > 96.7 mg/L $_{mm}$**	EFSA Journal 2015; 13(1):3984
Daphnia magna	DFP-ASTCA	48 h, s	**EC_{50} > 0.030 mg/L $_{nom}$**	EFSA Journal 2015; 13(1):3984
Daphnia magna	ASTCA	48 h, s	**EC_{50} > 0.030 mg/L $_{nom}$**	EFSA Journal 2015; 13(1):3984
Daphnia magna	TSA	48 h, s	**EC_{50} > 0.030 mg/L $_{nom}$**	EFSA Journal 2015; 13(1):3984
Daphnia magna	Florasulam	21 d, ss	**NOEC = 23.4mg a.s./L $_{nom}$**	EFSA Journal 2015; 13(1):3984
Sediment dwelling organisms				
Chironomus riparius	Florasulam	28 d, ss	**NOEC = 10 mg a.s./L $_{nom}$**	EFSA Journal 2015; 13(1):3984
Algae				
Pseudokirchneriella subcapitata	Florasulam	72 h, s	**E_rC_{50} = 0.00894 mg a.s./L $_{mm}$**	EFSA Journal 2015; 13(1):3984
Navicula pelliculosa	Florasulam	120 h, s	E_bC_{50} = 1.38 mg a.s./L $_{m}$	EFSA Journal 2015; 13(1):3984
Anabaena flos-aquae	Florasulam	96 h, s	E_bC_{50} = 0.363 mg a.s./L $_{mm}$	EFSA Journal 2015; 13(1):3984
Skeletonema costatum	Florasulam	120 h, s	E_bC_{50} = 43.1 mg a.s./L $_{mm}$	EFSA Journal 2015; 13(1):3984
Pseudokirchneriella subcapitata	EF-1343	72 h, s	E_bC_{50} = 0.0611 (0.00345 mg a.s./L $_{mm}$)	EFSA Journal 2015; 13(1):3984
Pseudokirchneriella subcapitata	5-OH-florasulam	72 h, s	E_bC_{50} = 21.32 mg/L $_{mm}$ **E_rC_{50} = 21.57 mg/L $_{mm}$**	EFSA Journal 2015; 13(1):3984

Species	Substance	Exposure System	Results [mg/L]	Reference
Pseudokirchneriella subcapitata	DFP-ASTCA	72 h, s	**E_yC_{50} = 96 mg/L $_{nom}$**	EFSA Journal 2015; 13(1):3984
Pseudokirchneriella subcapitata	ASTCA	72 h & 96 h, s	**E_rC_{50} & E_bC_{50} > 9.2 mg/L $_{mm}$**	EFSA Journal 2015; 13(1):3984
Pseudokirchneriella subcapitata	TPSA	72 h & 96 h, s	**E_yC_{50} & E_rC_{50} > 100 mg/L $_{nom}$**	EFSA Journal 2015; 13(1):3984
Pseudokirchneriella subcapitata	TSA	72 h, s	**E_yC_{50} & E_rC_{50} > 94 mg/L $_{mm}$**	EFSA Journal 2015; 13(1):3984
Pseudokirchneriella subcapitata	5-OH-ASTP	72 h & 96 h, s	**E_yC_{50} & E_rC_{50} > 100 mg/L $_{nom}$**	EFSA Journal 2015; 13(1):3984
Pseudokirchneriella subcapitata	ASTP	72 h & 96 h, s	**E_yC_{50} & E_rC_{50} > 100 mg/L $_{nom}$**	EFSA Journal 2015; 13(1):3984
Higher plant				
Lemna gibba	Florasulam	14 d, ss	**EC_{50} = 0.00118 mg a.s./L $_{im}$** NOEC = 0.00063 mg a.s./L $_{im}$	EFSA Journal 2015; 13(1):3984
Lemna gibba	EF-1343	14 d, ss	EC_{50} = 0.0466 (0.002 mg a.s./L $_{mm}$)	EFSA Journal 2015; 13(1):3984
Lemna gibba	5-OH-florasulam	7 d, ss	**EC_{50} = 0.0378 mg a.s./L $_{imm}$**	EFSA Journal 2015; 13(1):3984
Lemna gibba	DFP-ASTCA	7 d, ss	**E_yC_{50} & E_rC_{50} > 100 mg/L $_{nom}$**	EFSA Journal 2015; 13(1):3984
Lemna gibba	ASTCA	7 d & 14 d, ss	**EC_{50} > ~~100~~ 10.2 mg/L $_{nom}$**	EFSA Journal 2015; 13(1):3984
Lemna gibba	TPSA	7 d, ss	**E_yC_{50} & E_rC_{50} > 100 mg/L $_{nom}$**	EFSA Journal 2015; 13(1):3984
Lemna gibba	TSA	7 d, ss	**E_yC_{50} & E_rC_{50} > 100 mg/L $_{nom}$**	EFSA Journal 2015; 13(1):3984
Lemna gibba	5-OH-ASTP	7 d, ss	**E_yC_{50} & E_rC_{50} > 100 mg/L $_{nom}$**	EFSA Journal 2015; 13(1):3984
Lemna gibba	ASTP	7 d, ss	**E_yC_{50} = 88 mg/L $_{mm}$ (frond no.)**	EFSA Journal 2015; 13(1):3984

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

Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – formulation Floras 50 SC.

Species	Substance	Exposure System	Results [mg/L]	Reference
Daphnia magna	Floras 50 SC	48 h	EC_{50} > 100 mg f.p./L (4.84 mg a.i./L)*	KCP 10.2.1-1 Hodorek G, 2022 Report W-18-22

Anabaena flos-aquae	Floras 50 SC	72 h	E_rC_{50} = 10.01 mg f.p./L (0.48 mg a.i./L)* NOE_rC = 1.1 mg f.p./L E_yC_{50} = 2.75 mg f.p./L (0.13 mg a.i./L)* NOE_yC = 0.37 mg f.p./L	KCP 10.2.1-2 Hodorek G, 2022 Report W-20-22
Lemna gibba	Floras 50 SC	7 d	Frond: E_rC_{50} = 0.062 mg f.p./L (0.00299 mg a.i./L)* E_yC_{50} = 0.030 mg f.p./L (0.00145 mg a.i./L)* Dry weight: E_rC_{50} >20 mg f.p./L (0.97 mg a.i./L)* E_yC_{50} = 0.053 mg f.p./L (0.00256 mg a.i./L)* NOEC = 0.0064 mg f.p./L	KCP 10.2.1-3 Hodorek G, 2022 Report W-19-22

*- basis on density = 1.04 g/mL and content of florasulam = 50.3 g/L w/v

Review Comments:

It should be noted that the formulation toxicity test for algae was done for *Anabaena flos-aquae*, not for the most sensitive *Pseudokirchneriella subcapitata.* Nevertheless, from the studies for Floras 50 SC, submitted for evaluation, it appears that the product is of similar toxicity to florasulam, or a representative formulation. Therefore, additional data are not required.

9.5.1.1 Justification for new endpoints

Please refer to Conclusion on the peer review of the pesticide risk assessment of the active substances:
- Florasulam (EFSA Journal 2015; 13(1):3984) and Draft Assessment Report for Florasulam,

9.5.2 Risk assessment

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

The relevant global maximum FOCUS Step 1, 2, 3 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the tables below.

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

Risk assessment for Florasulam

Table 9.5-3: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Florasulam for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of MATLAM in winter cereals (spring application)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. Dwell. prolonged**	**Aquatic Plants**
Test species		***Oncorhynchus mykiss***	***Pimephales promelas***	***Dapnia magna***	***Dapnia magna***	***Pseudokirchneriella subcapitata***	***Chironomus riparius***	***Lemna gibba***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	NOEC	EC_{50}
(µg/L)		100000	2900	292000	23400	8.94	10000	1.18
AF		100	10	100	10	10	10	10
RAC (µg/L)		1000	290	2920	2340	0.894	1000	0.118
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**	**PEC/RAC**						
Step 1								
	1.69	0.002	0.006	0.001	0.001	1.890	0.002	**14.322**
Step 2								
N-Europe March-May	0.09	<0.001	<0.001	<0.001	<0.001	0.101	<0.001	0.763
S-Europe March-May	0.15	<0.001	0.001	<0.001	<0.001	0.168	<0.001	**1.271**
Step 3								
D1/ ditch	0.353	<0.001	0.001	<0.001	<0.001	0.395	<0.001	**2.992**
D1/ stream	0.221	<0.001	0.001	<0.001	<0.001	0.247	<0.001	**1.873**
D2/ ditch	0.671	0.001	0.002	<0.001	<0.001	0.751	0.001	**5.686**
D2/ stream	0.469	<0.001	0.002	<0.001	<0.001	0.525	<0.001	**3.975**

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. Dwell. prolonged**	**Aquatic Plants**
Test species		***Oncorhynchus mykiss***	***Pimephales promelas***	***Dapnia magna***	***Dapnia magna***	***Pseudokirchneriella subcapitata***	***Chironomus riparius***	***Lemna gibba***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	NOEC	EC_{50}
(µg/L)		100000	2900	292000	23400	8.94	10000	1.18
AF		100	10	100	10	10	10	10
RAC (µg/L)		1000	290	2920	2340	0.894	1000	0.118
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**	**PEC/RAC**						
D3/ ditch	0.032	<0.001	<0.001	<0.001	<0.001	0.036	<0.001	0.271
D4/ pond	0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	0.008
D4/ stream	0.025	<0.001	<0.001	<0.001	<0.001	0.028	<0.001	0.212
D5/ pond	0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	0.008
D5/stream	0.025	<0.001	<0.001	<0.001	<0.001	0.028	<0.001	0.212
D6/ditch	0.032	<0.001	<0.001	<0.001	<0.001	0.036	<0.001	0.271
R1/pond	0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	0.008
R1/stream	0.025	<0.001	<0.001	<0.001	<0.001	0.028	<0.001	0.212
R3/stream	0.029	<0.001	<0.001	<0.001	<0.001	0.032	<0.001	0.246
R4/stream	0.021	<0.001	<0.001	<0.001	<0.001	0.023	<0.001	0.178

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 Florasulam for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of MATLAM in spring cereals

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. Dwell. prolonged**	**Aquatic Plants**
Test species		***Oncorhynchus mykiss***	***Pimephales promelas***	***Dapnia magna***	***Dapnia magna***	***Pseudokirchneriella subcapitata***	***Chironomus riparius***	***Lemna gibba***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	NOEC	EC_{50}
(µg/L)		100000	2900	292000	23400	8.94	10000	1.18
AF		100	10	100	10	10	10	10
RAC (µg/L)		1000	290	2920	2340	0.894	1000	0.118
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**	**PEC/RAC**						
Step 1								
	1.69	0.002	0.006	0.001	0.001	1.890	0.002	**14.322**
Step 2								
N-Europe March-May	0.09	<0.001	<0.001	<0.001	<0.001	0.101	<0.001	0.763
S-Europe March-May	0.15	<0.001	0.001	<0.001	<0.001	0.168	<0.001	**1.271**
Step 3								
D1/ ditch	0.045	<0.001	<0.001	<0.001	<0.001	0.050	<0.001	0.381
D1/ stream	0.030	<0.001	<0.001	<0.001	<0.001	0.034	<0.001	0.254
D3/ ditch	0.032	<0.001	<0.001	<0.001	<0.001	0.036	<0.001	0.271
D4/ pond	0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	0.008
D4/ stream	0.024	<0.001	<0.001	<0.001	<0.001	0.027	<0.001	0.203
D5/ pond	0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	0.008
D5/stream	0.025	<0.001	<0.001	<0.001	<0.001	0.028	<0.001	0.212

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. Dwell. prolonged**	**Aquatic Plants**
Test species		***Oncorhynchus mykiss***	***Pimephales promelas***	***Dapnia magna***	***Dapnia magna***	***Pseudokirchneriella subcapitata***	***Chironomus riparius***	***Lemna gibba***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	NOEC	EC_{50}
(µg/L)		100000	2900	292000	23400	8.94	10000	1.18
AF		100	10	100	10	10	10	10
RAC (µg/L)		1000	290	2920	2340	0.894	1000	0.118
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**	**PEC/RAC**						
R1/pond	0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	0.008
R1/stream	0.021	<0.001	<0.001	<0.001	<0.001	0.023	<0.001	0.178
R4/stream	0.021	<0.001	<0.001	<0.001	<0.001	0.023	<0.001	0.178

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 winter cereals, calculated PEC/RAC ratios did not indicate an acceptable risk for some scenarios (aquatic plants RAC=0.118 µg/L). Therefore, further assessment is necessary. PEC/RAC ratios were calculated based on FOCUS Step 4 PECsw considering reduced exposure of surface water bodies.

FOCUS Step 4

Table 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Florasulam for each organism group based on FOCUS Steps 4 calculations for the use of MATLAM in winter cereals

PEC_{sw} (µg/L)	**Scenario**	**STEP 4**	
Nozzle reduction	Vegetative strip (m)	None	None
	No spray buffer (m)	5	10
None	D1/ditch	0.353	0.353
50 %		0.353	-
None	D1/stream	0.221	0.221
50 %		0.221	-
None	D2/ditch	0.671	0.671
50 %		0.671	-
None	D2/stream	0.469	0.469
50 %		0.469	-
RAC (µg/L) = 0.118		**PEC/RAC ratio**	
None	D1/ditch	**2.992**	**2.992**
50 %		**2.992**	-
None	D1/stream	**1.873**	**1.873**
50 %		**1.873**	-
None	D2/ditch	**5.686**	**5.686**
50 %		**5.686**	-
None	D2/stream	**3.975**	**3.975**
50 %		**3.975**	-

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

The scenarios D1 and D2 are not relevant for ZRMS Poland, therefore the obtained results of the PECsw are considered sufficient.

Risk assessment for metabolites of Florasulam

Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for 5-OH Florasulam for each organism group based on FOCUS Steps 1, 2 calculations for the use of MATLAM in winter and spring cereals

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Higher plants**
Test species		***O. mykiss***	***D. magna***	***P. subcapitata***	***L. gibba***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}	EC_{50}
(µg/L)		91000	96700	21570	37.8
AF		100	100	10	10
RAC (µg/L)		910	967	2157	3.78
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**	**PEC/RAC**			
Step 1					
	2.72	0.003	0.003	0.001	0.720
~~**Step 2**~~					
~~N-Europe~~	~~0.28~~	~~<0.001~~	~~<0.001~~	~~<0.001~~	~~0.074~~
~~S-Europe~~	~~0.52~~	~~0.001~~	~~0.001~~	~~<0.001~~	~~0.138~~

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 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for DFP-ASTCA for each organism group based on FOCUS Steps 1, 2 calculations for the use of MATLAM in winter and spring cereals

Group		**Inverteb. acute**	**Algae**	**Higher plants**
Test species		***D. magna***	***P. subcapitata***	***L. gibba***
Endpoint		EC_{50}	E_yC_{50}	EC_{50}
(µg/L)		30	96000	100000
AF		100	10	10
RAC (µg/L)		0.30	9600	10000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**	**PEC/RAC**		
Step 1				
	0.34	**1.133**	<0.001	<0.001
Step 2				
N-Europe	0.05	0.167	<0.001	<0.001
S-Europe	0.09	0.300	<0.001	<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 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for ASTCA for each organism group based on FOCUS Steps 1, 2 calculations for the use of MATLAM in winter and spring cereals

Group		**Inverteb. acute**	**Algae**	**Higher plants**
Test species		***D. magna***	***P. subcapitata***	***L. gibba***
Endpoint		EC_{50}	E_rC_{50}	EC_{50}
(µg/L)		30	9200	100000
AF		100	10	10
RAC (µg/L)		0.30	920	10000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**	**PEC/RAC**		
Step 1				
	0.75	**2.500**	0.001	<0.001
Step 2				
N-Europe	0.09	0.300	<0.001	<0.001
S-Europe	0.16	0.533	<0.001	<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
* as no toxicity values were available for metabolites, a factor 10 was applied on the toxicity value of parent compound

Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for TPSA for each organism group based on FOCUS Steps 1, 2 calculations for the use of MATLAM in winter and spring cereals

Group		**Algae**	**Higher plants**
Test species		***P. subcapitata***	***L. gibba***
Endpoint		E_rC_{50}	EC_{50}
(µg/L)		100000	100000
AF		10	10
RAC (µg/L)		10000	10000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**	**PEC/RAC**	
Step 1			
	0.65	<0.001	<0.001
~~**Step 2**~~			
~~N-Europe~~	~~0.04~~	~~<0.001~~	~~<0.001~~
~~S-Europe~~	~~0.06~~	~~<0.001~~	~~<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 9.5-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for TSA for each organism group based on FOCUS Steps 1, 2 calculations for the use of MATLAM in winter and spring cereals

Group		**Inverteb. acute**	**Algae**	**Higher plants**
Test species		***D. magna***	***P. subcapitata***	***L. gibba***
Endpoint		EC_{50}	E_rC_{50}	EC_{50}
(µg/L)		30	94000	100000
AF		100	10	10
RAC (µg/L)		0.30	9400	10000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**	**PEC/RAC**		
Step 1				
	0.11	0.367	<0.001	<0.001
~~**Step 2**~~				
~~N Europe~~	~~0.02~~	~~0.067~~	~~<0.001~~	~~<0.001~~
~~S Europe~~	~~0.04~~	~~0.133~~	~~<0.001~~	~~<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 9.5-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for 5-OH ASTP for each organism group based on FOCUS Steps 1, 2 calculations for the use of MATLAM in winter and spring cereals

Group		**Algae**	**Higher plants**
Test species		***P. subcapitata***	***L. gibba***
Endpoint		E_rC_{50}	E_rC_{50}
(µg/L)		100000	100000
AF		10	10
RAC (µg/L)		10000	10000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**	**PEC/RAC**	
Step 1			
	0.29	<0.001	<0.001
~~**Step 2**~~			
~~N Europe~~	~~0.02~~	~~<0.001~~	~~<0.001~~
~~S Europe~~	~~0.03~~	~~<0.001~~	~~<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 9.5-12: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for ASTP for each organism group based on FOCUS Steps 1, 2 calculations for the use of MATLAM in winter and spring cereals

Group		**Algae**	**Higher plants**
Test species		***P. subcapitata***	***L. gibba***
Endpoint (µg/L)		E_rC_{50} 100000	E_rC_{50} 88000
AF		10	10
RAC (µg/L)		10000	8800
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**	**PEC/RAC**	
Step 1			
	0.23	<0.001	<0.001
Step 2			
N-Europe	0.01	<0.001	<0.001
S-Europe	0.02	<0.001	<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

9.5.3 Overall conclusions

Florasulam
For the active substance Florasulam, calculated PEC/RAC ratios for spring and winter cereals (spring application) did indicate an acceptable risk in all FOCUS Steps 3 scenarios relevant for Poland.

Metabolites of Florasulam
Regarding the metabolites, calculated PEC/RAC ratios did indicate an acceptable risk in all FOCUS Step 2 scenarios.

Review Comments:

The relevant predicted environmental concentrations in water (PEC_{sw}) for risk assessments covering the proposed use pattern are taken from Part B Section 8 (Environmental Fate). The risk assessment was based on the worst case $PEC_{sw/sed}$ values and the results of laboratory toxicity testing.

The PEC/RAC calculations were performed with FOCUS STEP 1-2 values for florasulam and relevant metabolites and FOCUS STEP 3 for active substance. For the formulation additional calculations were not required. The risk of MATLAM (RNB 072 A) is covered by the risk assessment for the active substance.

The calculated PEC/RAC ratios indicate an acceptable risk for all groups of aquatic organisms without the need for any mitigation measures (considering CEU zone relevant scenarios).

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 active substance and formulation Floras 50 SC. Full details of these studies are provided in the respective EU DAR and related documents as well as in Appendix 2 of this document (new studies).

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

Species	Substance	Exposure System	Results	Reference
Apis mellifera	Florasulam	Oral	**LD_{50} > 100 µg/bee**	EFSA Journal 2015; 13(1):3984
Apis mellifera	Florasulam	Contact	**LD_{50} > 100 µg/bee**	EFSA Journal 2015; 13(1):3984
Apis mellifera	Floras 50 SC	Oral acute	LD_{50} > 200 µg f.p./bee (>9.67 µg a.i./bee)	KCP 10.3.1.1.1-1 Dybek M., 2023 Report B-127-22
Bombus spp	Floras 50 SC	Oral acute	LD_{50} > 100 µg f.p./bumblebee (>4.84 µg a.i./bumblebee)	KCP 10.3.1.1.1-2 Dybek M., 2023 Report B-128-22
Apis mellifera	Floras 50 SC	Contact acute	LD_{50} > 200 µg f.p./bee (>9.67 µg a.i./bee)	KCP 10.3.1.1.2-1 Dybek M., 2023 Report B-129-22
Bombus spp	Floras 50 SC	Contact acute	LD_{50} > 100 µg f.p./bumblebee (>4.84 µg a.i./bumblebee)	KCP 10.3.1.1.2-2 Dybek M., 2023 Report B-130-22
Apis mellifera	Floras 50 SC	Chronic oral	LC_{50} > 666.7 mg f.p./kg LDD_{50} > 12.4 µg f.p./bee/day	KCP 10.3.1.2 Dybek M., 2023 Report B-126-22
Apis mellifera	Floras 50 SC	Larval development, single exposure	LD_{50} > 100 µg f.p./bee (>4.84 µg a.i./bee)	KCP 10.3.1.3 Dybek M., 2023 Report B-125-22

9.6.1.1 Justification for new endpoints

Not relevant as there is no deviation to the EU agreed endpoints. New studies were conducted for Floras 50 SC and were also considered for the risk assessment.

9.6.2 Risk assessment

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

9.6.2.1 Hazard quotients for bees

Table 9.6-2: First-tier assessment of the risk for bees due to the use of MATLAM regarding Florasulam.

Intended use **Active substance** **Application rate (g/ha)**	 Florasulam 1 × 5.0		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	100	5.0	0.05
Contact toxicity	100		0.05
Product **Application rate (g/ha)**	Floras 50 SC 1 × ~~644~~ 104		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	200	104*	0.52
Contact toxicity	200		0.52

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.
*- basis on density = 1.04 g/mL

Risk assessment according to the EFSA 2013

The risk assessment below was performed for illustrative purposes. It is considered as supplementary only, since the EFSA 2013. Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera, Bombus spp*. and solitary bees) (EFSA J 2013;11(7):3295) has not been implemented. Wherever relevant, the provisions of the more recent EFSA 2023. Revised guidance on the risk assessment of plant protection products on bees (*Apis mellifera, Bombus spp*. and solitary bees) (EFSA J 2023;21(5):7989) have been taken into account.

Table 9.6-3 Screening assessment of the acute and chronic risk for bees and acute risk for bumblebees due to the use of MATLAM in cereals (EFSA Journal 2013; 11(7): 3295)

Intended use **Active substance** **Application rate (L/ha)**	**Cereals** Florasulam - 5 g/ha 0.1			
Contact route of exposure				
	"calculation factor" (linked with dust)	**HQ**	**Trigger**	**Risk indicator**
Honey bee	1	1.5	42	OK
Bumblebees	1	1.0	7	OK
Oral route of exposure (pollen and nectar)				
	"calculation factor" (Ef x SV)	**ETR**	**Trigger**	**Risk indicator**
Honey bee acute	7.6	0.00	0.2	OK
Honey bee chronic	7.6	**0.064**	0.03	**!**
Honey bee larvae	4.4	0.00	0.2	OK
Bumblebees	11.2	0.01	0.036	OK

Accumulative effects	
Has the substance a potential for accumlative toxicity (see section 8.1.1.3 and pertinent part of Appendix O in the GD)?	*No*

Exposure to contaminated water

Note: the EFSA 2023 considers that "*whilst water foraging is potentially a relevant exposure route, we currently lack the relevant information and technical ability to effectively formulate an effective risk assessment, therefore we have elected to remove the water scenario from the updated risk assessment*". Therefore, the risk assessment is presented for the formal reasons only.

Guttation

-

Surface water

	water consumption (µL)	**ETR**	**Trigger**	**Risk indicator**
acute	11.4	0.00	0.2	OK
chronic	11.4	0.000	0.03	OK
larvae	111	0.00	0.2	OK

Puddle water

	water consumption (µL)	**ETR**	**Trigger**	**Risk indicator**
acute	11.4	0.00	0.2	OK
chronic	11.4	0.000	0.03	OK
larvae	111	0.00	0.2	OK

Results of the screening step indicated that further risk assessment at tier 1 is required for chronic risk to adult bees via oral (pollen and nectar) exposure

Table 9.6-4 First-tier assessment of the chronic risk for bees due to the use of MATLAM in cereals

Intended use	**Cereals**
Active substance	Florasulam – 5 g/ha
Application rate (L/ha)	0.1

Category	**Endpoint**	**BBCH**	**Scenario**	**ETR**	**trigger**
Chronic	LDD_{50} >12.4 µg f.p./bee/day	10 - 29	treated crop	0.006	0.03
			weeds	0.018	
			field margin	0.000	
			adjacent crop	0.000	
			next crop	0.003	
Chronic	LDD_{50} >12.4 µg f.p./bee/day	30 - 39	treated crop	0.006	0.03
			weeds	0.009	
			field margin	0.000	
			adjacent crop	0.000	
			next crop	0.003	

It can be concluded, that application of product under the real field conditions do not pose unacceptable risk to bees.

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

Not relevant.

9.6.3 Effects on bumble bees

Additional studies with formulated product were performed.

9.6.4 Effects on solitary bees

Tests are not required as the test substance is of low toxicity to honey bees.

9.6.5 Overall conclusions

Use of MATLAM indicate low risk for bees.

Review Comments:

The evaluation of the acute risk for bees was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002). The submitted risk assessment, based on laboratory studies, has been accepted. It can therefore be concluded that there will be negligible acute risk associated with the exposure of *Apis mellifera* to MATLAM.

The data requirements in accordance with Commission Regulation (EU) No 284/2013 for the chronic toxicity to adult honeybees and honeybee larvae are fulfilled. Nevertheless, it should be noted that for larvae the single exposure test was submitted.

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

Studies on the toxicity to non-target arthropods have been carried out with active substances and formulation Floras 50 SC. Full details of these studies are provided in the respective EU DAR and related documents.

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

Species	Substance	Exposure System	Results [g/ha]	Reference
Typhlodromus pyri (protonymphs)	EF-1343	Laboratory test glass plates (2D)	LR_{50} > 15 g a.s./ha	EFSA Journal 2015; 13(1):3984
Aphidius rhopalosiphi (adults)	EF-1343	Laboratory test glass plates (2D)	LR_{50} > 15 g a.s./ha	EFSA Journal 2015; 13(1):3984

Species	Substance	Exposure System	Results [g/ha]	Reference
Poecilus cupreus	EF-1343	Laboratory test glass plates (2D)	Mortality: 0 % at 0 g a.s./ha 0 % at 7.5 g a.s./ha 0 % at 15 g a.s./ha Sublethal effects: 1.07 % at 0 g a.s./ha 1.12 % at 7.5 g a.s./ha 1.33 % at 15 g a.s./ha	EFSA Journal 2015; 13(1):3984
Chrysoperla carnea	EF-1343	Laboratory test glass plates (2D)	Mortality: 14.7 % at 0 g a.s./ha 6.9 % at 7.5 g a.s./ha 21.4 % at 15 g a.s./ha Sublethal effects: 19.8 % at 0 g a.s./ha 4.4 % at 7.5 g a.s./ha 0 % at 15 g a.s./ha	EFSA Journal 2015; 13(1):3984
Episyrphus balteatus	EF-1343	Extended laboratory study	Mortality: 4 % at 0 g a.s./ha 2 % at 5 g/ a.s./ha 2 % at 7.5 g a.s./ha Sublethal effects: 29.6 % at 0 g a.s./ha 32.0 % at 5 g a.s./ha 25.4 % at 7.5 g a.s./ha	EFSA Journal 2015; 13(1):3984
Aphidius rhopalosiphi (adults)	Floras 50 SC	Laboratory test glass plates (2D)	LR_{50} > 0.1 L/ha **ER_{50} ~~= 0.052~~ >0.025 L/ha**	KCP 10.3.2.1-1 Dybek M., 2023 Report B-123-22
Typhlodromus pyri (protonymphs)	Floras 50 SC	Laboratory test glass plates (2D)	LR_{50} > 0.1 L/ha **ER_{50} > 0.1 L/ha**	KCP 10.3.2.1-2 Dybek M., 2023 Report B-122-22

9.7.1.1 Justification for new endpoints

Not relevant as there is no deviation to the EU agreed endpoints. New studies were conducted for Floras 50 SC and were also considered for the risk assessment.

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: Assessment of the in-field risk for non-target arthropods due to the use of MATLAM

Intended use	Cereals		
Active substance/product	MATLAM		
Application rate (g/ha)	1 × 0.1 L/ha		
MAF	1		
Test species Tier I	**LR_{50} (lab.)/ER50 (L/ha)**	**$PER_{in\text{-}field}$ (L/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	> 0.1	0.1	<1.00
Aphidius rhopalosiphi	~~0.052~~ >0.1/>0.025		~~1.92~~ 1.0/**<4.0**

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient; DALT: Days after last treatment.
Criteria values shown in bold breach the relevant trigger.
* If an LR_{50} or ER_{50} from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Review Comments:

The HQ value for *Aphidius rhopalosiphi* calculated based on ER_{50} is above 2. However, the ESCORT 2 guideline clearly states that the primary toxicity endpoint of Tier 1 studies should be mortality. Only for products where effects on reproduction are expected (e.g. IGRs), assessment of oviposition should be also evaluated.

Given that Matlam is a herbicide, containing a single active substance, florasulam, with proven low toxicity to bees and aquatic invertebrates, the acceptable risk for *T. pyri* based on Tier 1 study (LR_{50}/ER_{50}) and low toxicity for other arthropods (see LoEP), extended laboratory test for *Aphidius rhopalosiphi* is not required.

Acceptable in-field risk for arthropods was found based on mortality endpoints (LR_{50} values). In zRMS opinion, no unacceptable effects are expected in in-field habitats within one year.

9.7.2.2 Risk assessment for off-field exposure

Table 9.7-3: Assessment of the off-field risk for non-target arthropods due to the use of MATLAM

Intended use	Cereals				
Active substance/product	MATLAM				
Application rate (g/ha)	1 × 0.1 L/ha				
MAF	1				
vdf	10/5				
Test species Tier I	**LR_{50} (lab.)/ER50 (L/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (L/ha)**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	> 0.1	2.77	0.000277 / 0.000554	10	<0.0277 / <0.0554
Aphidius rhopalosiphi	~~0.052~~> 0.1 / >0.025				~~0.0533~~ LR_{50}: <0.0277 / <0.0554 ER_{50}: <0.111 / <0.222

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

* If an LR_{50} or ER_{50} from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

9.7.2.3 Additional higher-tier risk assessment

Not relevant.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

Use of MATLAM indicate low risk for non-target arthropods other than bees.

Review Comments:

Based on the results of the conducted risk assessment, it can be concluded that low risk for non-target arthropods is expected from the use of MATLAM according to the proposed use pattern. No unacceptable effects on non-target arthropods are expected in in-field and off-field habitats.

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

9.8.1 Toxicity data

Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with active substance (plus certain metabolite) and Floras 50 SC. Full details of these studies are provided in the respective EU DAR and related documents as well as in Appendix 2 of this document (new studies).

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

Species	Substance	Exposure System	Results	Reference
~~*Eisenia foetida*~~	~~Florasulam~~	~~14 d, acute~~	~~LC_{50} > 1320 mg/kg dw~~	~~EFSA Journal 2015; 13(1):3984~~
Eisenia foetida	Florasulam	Chronic	NOEC = 0.203 mg/kg dw	EFSA Journal 2015; 13(1):3984
Eisenia andrei	Floras 50 SC	Chronic, 56 d	NOEC = 180 mg f.p./kg dw soil (8.71 mg a.i./kg dw soil) **EC_{10} = 106.0 mg/kg dw soil (5.13 mg a.i. /kg dw soil)**	KCP 10.4.1.1 Pieczka P., 2022 Report G-10-22
~~*Eisenia foetida*~~	~~5-OH Florasulam~~ ~~DFP-ASTCA~~ ~~ASTCA~~ ~~TSA~~	~~14 d, acute~~	~~**LC_{50} > 1120 mg/kg dw**~~ ~~**LC_{50} > 0.1 mg/kg dw**~~ ~~**LC_{50} > 100 mg/kg dw**~~ ~~**LC_{50} > 0.1 mg/kg dw**~~	~~EFSA Journal 2015; 13(1):3984~~

Species	Substance	Exposure System	Results	Reference
Eisenia foetida	5-OH Florasulam DFP-ASTCA ASTCA TSA	Chronic	**NOEC = 0.14 mg/kg dw** **NOEC = 0.0304 mg/kg dw** **NOEC = 1.0 mg/kg dw** **NOEC = 10.0 mg/kg dw**	EFSA Journal 2015; 13(1):3984
Folsomia candida	5-OH Florasulam DFP-ASTCA ASTCA TSA	Chronic	**NOEC = 2.5 mg/kg dw** **NOEC = 10 mg/kg dw** **NOEC = 12.5 mg/kg dw** **NOEC = 50.0 mg/kg dw**	EFSA Journal 2015; 13(1):3984
Hypoaspis aculeifer	5-OH Florasulam DFP-ASTCA ASTCA TSA	Chronic	**NOEC = 1.25 mg/kg dw** **NOEC = 10 mg/kg dw** **NOEC = 100 mg/kg dw** **NOEC = 50.0 mg/kg dw**	EFSA Journal 2015; 13(1):3984

9.8.1.1 Justification for new endpoints

Not relevant as there is no deviation to the EU agreed endpoints. New studies were conducted for Floras 50 SC and were also considered for the risk assessment.

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 PECsoil for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2.

Table 9.8-2: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of MATLAM

Intended use	Cereals		
Chronic effects on earthworms			
Product/active substance	**NOEC/EC_{10} (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Florasulam	0.203	0.007	29.00
MATLAM	106	0.139	762.59
5-OH Florasulam DFP-ASTCA ASTCA TSA	0.14 0.0304 1.0 10.0	0.005 0.001 ~~0.001~~ 0.002 <0.001	28.00 30.40 ~~1000.00~~ 500 10000.00

Chronic effects on other non-target soil organisms			
Product/active substance	**NOEC/EC$_{10}$** (mg/kg dw)	**PEC$_{soil}$** (mg/kg dw)	**TER$_{lt}$** (criterion TER ≥ 5)
Folsomia candida			
5-OH Florasulam	2.5	0.005	500
DFP-ASTCA	10.0	0.001	10000
ASTCA	12.5	~~0.001~~ 0.002	~~12500~~ 6250
TSA	50.0	<0.001	50000
Hypoaspis aculeifer			
5-OH Florasulam	1.25	0.005	250
DFP-ASTCA	10.0	0.001	10000
ASTCA	100.0	~~0.001~~ 0.002	~~100000~~ 50000
TSA	50.0	<0.001	50000

TER values shown in bold fall below the relevant trigger.

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

Use of MATLAM indicate low risk for earthworms and other soil macro-organisms.

Review Comments:

The long-term risks of MATLAM to soil meso- and macro-organisms were assessed from toxicity exposure ratios between toxicity endpoints and maximum PEC$_{soil}$. The relevant predicted environmental concentration in soil (PEC$_{soil}$) for risk assessment covering the proposed use pattern was taken from Part B Section 8 (Environmental Fate).

Safe use of MATLAM was confirmed based on TER$_{LT}$ calculations for formulation (earthworms), florasulam and its relevant metabolites.

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 Florasulam and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on soil microorganisms of MATLAM were not evaluated as part of the EU assessment of Florasulam. 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	Florasulam	100 days	Treatment causing <25% deviation from control: 0.05 mg/kg dry soil	EFSA Journal 2015; 13(1):3984
N-mineralisation	5-OH-florasulam	100 days	Treatment causing <25% deviation from control: 0.036 mg/kg dry soil	
N-mineralisation	DFP-ASTCA	100 days	Treatment causing <25% deviation from control: 0.00760 mg/kg dry soil	
N-mineralisation	ASTCA	100 days	Treatment causing <25% deviation from control: 1.0 mg/kg dry soil	
N-mineralisation	TSA	100 days	Treatment causing <25% deviation from control: 0.05 mg/kg dry soil	
N-mineralisation	Floras 50 SC	28 d, aerobic soil type	Treatement causing <25% deviation from control: 0.520 mg f.p./kg dw soil (0.025 mg florasulam/kg dw soil)	KCP 10.5 Pieczka P., 2022 Report G-11-22

9.9.1.1 Justification for new endpoints

Not relevant as there is no deviation to the EU agreed endpoints. New study were conducted for Floras 50 SC and was also considered for the risk assessment.

9.9.2 Risk assessment

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

The relevant PECsoil for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.8).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of MATLAM

Intended use	Cereals		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
Florasulam	0.05	0.007	Yes
5-OH-florasulam	0.036	0.005	Yes
DFP-ASTCA	0.0076	0.001	Yes
ASTCA	1.0	~~0.001~~ 0.002	Yes
TSA	0.05	<0.001	Yes
Floras 50 SC	0.52	0.139	Yes

9.9.3 Overall conclusions

Use of MATLAM indicate low risk for soil microbial activity.

Review Comments:

The use of MATLAM at the proposed rates poses no unacceptable risk to soil micro-organisms.

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

9.10.1 Toxicity data

Studies on the toxicity to non-target terrestrial plants have been carried out with Florasulam. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target terrestrial plants of MATLAM were not evaluated as part of the EU assessment of Florasulam.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

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

Species	Substance	Exposure System	Results	Reference
Cabbage *(Brassica oleracea var. capitata)* Flax *(Linum usitatissimum)* Carrot *(Daucus carota)*, Onion *(Allium cepa)*, Oats *(Avena sativa)*, Perennial ryegrass *(Lolium perenne)*	Floras 50 SC	Seedling emergence	**ER_{50} = 4.6 ml f.p./ha** (cabbage, shoot length)	KCP 10.6.2-1 Pieczka P., 2022 Report G-13-22
Cabbage *(Brassica oleracea var. capitata)* Flax *(Linum usitatissimum)* Carrot *(Daucus carota)*, Onion *(Allium cepa)*, Oats *(Avena sativa)*, Perennial ryegrass *(Lolium perenne)*	Floras 50 SC	Vegetative vigour	ER_{50} = 6.4 ml f.p./ha (carrot, plant dry weight)	KCP 10.6.2-2 Pieczka P., 2022 Report G-12-22

9.10.1.1 Justification for new endpoints

Risk assessment was conducted basis on Floras 50 SC studies results.

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Not relevant.

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

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

Table 9.10-2: Assessment of the risk for non-target plants due to the use of MATLAM

Intended use	Cereals		
Active substance/product	MATLAM		
Application rate [ml/ha]	100		
MAF	1		
ER_{50} (ml/ha)	**Drift rate**	**$PER_{off\text{-}field}$ (ml/ha)**	**TER Criterion: TER ≥ 5**
4.6	2.77	2.77	**1.66**

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

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

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

Table 9.10-3: Risk assessment for non-target terrestrial plants due to the use of MATLAM in all crops considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Cereals			
Active substance/product		MATLAM			
Application rate (ml/ha)		1 × 100			
MAF		1			
Buffer strip (m)	**Drift rate (%)**	**$PER_{off\text{-}field}$ (g/ha)**	**$PER_{off\text{-}field}$ 50 % drift red. (g/ha)**	**$PER_{off\text{-}field}$ 75 % drift red. (g/ha)**	**$PER_{off\text{-}field}$ 90 % drift red. (g/ha)**
1	2.77	2.77	1.39	0.69	-
5	0.57	0.57	-	-	-

Toxicity value ER_{50} = 4.6 ml f.p./ha	**TER** **criterion: TER ≥ 5**			
1	**1.66**	**3.31**	6.67	-
5	8.07	-	-	-

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

9.10.3 Overall conclusions

No potential risk to non-target plants located outside the treated area after application of MATLAM according to the GAP table is expected when the following risk mitigation measures are considered.

SPe 3: *To protect non-target plants respect an unsprayed buffer zone of 5m to non-agricultural land OR an unsprayed buffer zone of 1m to non-agricultural land with 75% drift reducing nozzles.*

Review Comments:

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002).

Based on the risk assessment it can be concluded that the proposed use of MATLAM poses no unacceptable risk to non-target plants, if applied according to the recommended use pattern. Particular precautions to reduce the environmental concentrations resulting from MATLAM applications are required.

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

No additional data.

9.12 Monitoring data (KCP 10.8)

No additional data.

9.13 Classification and Labelling

CLASSIFICATION	
Hazard class(es), categories:	Aquatic acute 1 Aquatic chronic 1
LABELLING	
Codes for hazard pictogram(s):	GHS09
Signal word:	Warning
Hazard statement(s):	H400: Very toxic to aquatic life H410: Very toxic to aquatic life with long lasting effects
Precautionary statement(s):	~~P273 – Avoid release to the environment~~ P391 – Collect spillage P501 – Dispose of contents/container in accordance to national regulations

Review Comments:

According to the criteria given in Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008, the following classification and labelling with regard to ecotoxicological data is proposed for:

1. Active substance:

For florasulam, classified as H400 and H410, no M factors are currently set in Regulation (EC) No 1272/2008. Based on available data and classification given in LoEP (EFSA Journal 2015; 13 (1):3984), the zRMS propose:

- Aquatic acute 1 with M factor of 100 : based on *Lemna gibba* E_rC_{50} of 0.00118 mg/L
- Aquatic chronic 1 with M factor of 100: based on *Lemna gibba* NOE_rC of 0.00063 mg/L

2. Formulation:

- Aquatic acute 1: based on *Lemna gibba* E_rC_{50} of 0.062 mg/L (Hoderek G., 2022 Report W-19-22)
- Aquatic chronic 1: based on *Lemna gibba* NOE_rC of 0.0064 mg/L (Hoderek G., 2022 Report W-19-22) and considering that florasulam is not rapidly degradable

Therefore, the classification proposed by the Applicant is correct.

Labelling:

Hazard Statement (CLP): H410, very toxic to aquatic life with long lasting effects.
Precautionary statements: P391, P501.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.1-1	Hodorek G.	2022	Floras 50 SC. *Daphnia magna*, Acute Immobilization Test IPO Pszczyna W-18-22 GLP/No Published	N	Elvita Sp. z o.o.
KCP 10.2.1-2	Hodorek G.	2022	Floras 50 SC. *Anabaena flos-aquae* UTEX B 1444 Growth inhibition test. IPO Pszczyna W-20-22 GLP/No Published	N	Elvita Sp. z o.o.
KCP 10.2.1-3	Hodorek G.	2022	Floras 50 SC. *Lemna gibba* CPCC 310, Growth inhibition test IPO Pszczyna W-19-22 GLP/No Published	N	Elvita Sp. z o.o.
KCP 10.3.1.1.1-1	Dybek M.	2023	Floras 50 SC. Honeybees (*Apis mellifera* L.), Acute Oral Toxicity Test IPO Pszczyna B-127-22 GLP/No Published	N	Elvita Sp. z o.o.
KCP 10.3.1.1.1-2	Dybek M.	2023	Floras 50 SC. Bumblebees (*Bombus spp*.), Acute Oral Toxicity Test IPO Pszczyna B-128-22 GLP/No Published	N	Elvita Sp. z o.o.
KCP 10.3.1.1.2-1	Dybek M.	2023	Floras 50 SC. Honeybees (*Apis mellifera* L.), Acute Contact Toxicity Test IPO Pszczyna B-129-22 GLP/No Published	N	Elvita 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 10.3.1.1.2-2	Dybek M.	2023	Floras 50 SC. Bumblebees (*Bombus spp*.), Acute Contact Toxicity Test IPO Pszczyna B-130-22 GLP/No Published	N	Elvita Sp. z o.o.
KCP 10.3.1.2	Dybek M.	2023	Floras 50 SC. Honeybees (*Apis mellifera* L.), Chronic Oral Toxicity Test. IPO Pszczyna B-126-22 GLP/No Published	N	Elvita Sp. z o.o.
KCP 10.3.1.3	Dybek M.	2023	Floras 50 SC. Honeybees (*Apis mellifera* L.), Larval Toxicity Test, Single Exposure IPO Pszczyna B-125-22 GLP/No Published	N	Elvita Sp. z o.o.
KCP 10.3.2.2-1	Dybek M.	2023	A laboratory test for evaluating the effects of Floras 50 SC on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani - Perez) IPO Pszczyna B-123-22 GLP/No Published	N	Elvita Sp. z o.o.
KCP 10.3.2.2-2	Dybek M.	2023	A laboratory test for evaluating the effects of Floras 50 SC on the predatory mite, *Typhlodromus pyri* (Sch.) IPO Pszczyna B-122-22 GLP/No Published	N	Elvita Sp. z o.o.
KCP 10.4.1.1	Pieczka P.	2022	Floras 50 SC. Earthworm Reproduction Test (*Eisenia andrei*). IPO Pszczyna G-10-22 GLP/No Published	N	Elvita Sp. z o.o.
KCP 10.5	Pieczka P.	2022	Floras 50 SC. Soil Microorganisms: Nitrogen Transformation Test IPO Pszczyna G-11-22 GLP/No Published	N	Elvita 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 10.6.2-1	Pieczka P.	2022	Floras 50 SC.Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test IPO Pszczyna G-13-22 GLP/No Published	N	Elvita Sp. z o.o.
KCP 10.6.2-2	Pieczka P.	2022	Floras 50 SC. Terrestrial Plant Test: Vegetative Vigour Test IPO Pszczyna G-12-22 GLP/No Published	N	Elvita Sp. z o.o.

Appendix 2 Detailed evaluation of the new studies

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

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

A 2.2 KCP 10.2 Effects on aquatic organisms

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

Comments of zRMS:	The study was conducted to OECD guideline 202 and according to the principles of GLP. No deviations to the guideline were noted. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.

Reference Report	KCP 10.2.1-1 Floras 50 SC. *Daphnia magna*, Acute immobilization test. Hodorek G., 2022, W-18-22
Guideline(s):	Yes (OECD 202)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	NA

Materials and methods

Materials

Test item:

Name:	Florasulam 50 g/L SC
Batch No.:	RFEAR0501
Production Date:	05.04.20222
Expiry Date:	04.2024
A.i. content:	florasulam 50.3 g/L
Density at 20°C	1.04 g/cm^3

Test system:

Species:	*Daphnia magna* Straus
Culturing:	Was cultured in glass beakers with a capacity of 150 mL (one parent per vessel) at room temperature with daily cycle: 16 h light : 8 h dark. The culture was maintained in the Elendt M7 medium. *Daphnia magna* were fed with a suspension of algae, mixture of two species *Raphidocelis subcapitata : Desmodesmus subspicatus* (in 2:1 ratio) originating from separate cultures cultivated in the Laboratory of Aquatic Toxicology. Group B vitamins and micronutrients necessary for proper growth were supplied with a lyophilized suspension of *Spirulina* sp.
Age:	< 24 h old at exposure initiation
Source:	Neonates collected from laboratory culture cultivated at the Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicology, Laboratory of Aquatic Toxicology.

Experimental conditions:

Test medium:	Elendt M7 medium
Temperature:	18.5 – 20.2°C
pH (control):	7.09 -7.19
DO (control):	9.0 – 9.2 mg/L
Light and photoperiod:	fluorescent light source; light-dark cycle: 16h : 8h
Feeding:	no feeding during test

Experimental period: 48 hours

Test design: Static test (48 h of exposure); 4 replicates per each test item concentration and the control; 5 Daphnia magna in each replicate.

Nominal test item concentrations: 100 mg/L plus the control.

Test design and treatment:

The *Daphnia magna* was exposed to the test item concentrations of 100 mg/L plus the control for 48 hours in a static test design.
The Elendt M7 medium was used as a diluent necessary to prepare the test item concentration. The medium was aerated prior to exposure initiation. The *Daphnia magna* were exposed in glass beakers of 150 mL capacity, containing 100 mL of either the test item concentration or the control. The beakers were covered with transparent lids in order to minimize evaporation and to prevent accidental contamination. Each test item concentration and the control were split up into four replicates. Five individuals of *Daphnia magna* were introduced into each replicate.
During exposure, the *Daphnia magna* were not fed. Tests were conducted with a daily cycle 16 h light : 8 h dark using fluorescent lighting. The

temperature was continuously recorded using an electronic device with a sensor in an additional test vessel containing the test medium. The pH values and the dissolved oxygen concentrations were measured in every test item concentration and the control at exposure initiation before splitting up into replicates and at exposure termination in pooled replicates.
The effect of the test item on immobilisation of *Daphnia magna* was assessed after 24 and 48 h of exposure. The *Daphnia magna* were considered immobile if they showed no ability to swim within 15 seconds after gentle swirling of the test vessel.
The test with reference material, potassium was performed between September 14, 2022 and September 16, 2022. During exposure, the temperature was in the range of 19.0 – 19.6°C, and the dissolved oxygen concentration was higher than 3 mg/L. Five concentrations of the reference material of 0.32, 0.56, 1.0, 1.8, and 3.2 mg/L and a control were used. There were four replicates of every concentration and the control.
The median concentration causing 50% immobilisation of *Daphnia magna* after 24 h of exposure, i.e. the EC50/24 h value is 1.01 mg of reference substance/L and the median concentration causing 50% immobilisation of *Daphnia magna* after 48 h of exposure, i.e. the EC50/48 h value is 0.87 mg of reference substance/L (95% confidence interval 0.75 – 1.00). The LOEC/24 and 48 h is 1.00 mg of reference substance/L whilst the NOEC/24 h and 48 h is 0.56 mg of reference substance/L.
The results confirmed sensitivity of *Daphnia magna* used in the definitive test.

Statistics:

No statistical analysis was needed.

Validity criteria:

In the definitive test, the following validity criteria specified in the OECD Guideline No. 202 (2004) were met:
- the immobilisation 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 8.8 – 9.3 mg/L (criterion: not less than 3 mg/L).

Chemical verification of nominal concentration:

The concentrations of florasulam were chemically determined using validated high performance liquid chromatographic method with DAD detection. The validated analytical methods were performed according to SANTE/2020/12830, Rev. 1.
Test item concentration of 100 mg/L and the control, collected at exposure initiation and exposure termination were analysed.
Florasulam method validation.
The following liquid chromatography parameters were used for analysis of florasulam:
- column - Synergi 4μm Fusion-RP 80Å 150x4.6
- mobile phase - acetonitrile and 0.05% solution of orthophosphoric acid in deionized water (60:40, v/v),
- wave length 260 nm,
- flow rate 0.7 mL/min.,
- injected volume 20 μL,
- oven temperature 35°C.

Linearity: Working solutions of florasulam at the concentrations of 0.05, 0.1, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0 and 100.0 μg/mL were injected successively to the chromatographic column and the chromatograms were

recorded. The standard curves (peak area versus quantity of the standard) are linear with coefficient (r^2) of 0.9999427 and 0.9999318. The range of linearity of the analytical graphs are from 0.05 mg/L to 5.0 mg/L and from 1.0 to 20.0 mg/L.
Selectivity and specificity: The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experiments conditions. Therefore, the specificity of the method was demonstrated. Furthermore, any interference are directly apparent and would be observed in the chromatograms of the matrix control sample.
Precision: RSD for fortification level = LOQ was 5.9% and for level = 10xLOQ it was 0.5%
Accuracy: mean recovery for fortification level = LOQ it was 102.5% and for level = 10xLOQ it was 97.5%
Matrix effect: Assessment of matrix effects was performed by comparing the standard preparing in solvent to standard preparing in control matrix at appropriate concentration and the matrix effect was equal to 4.1%.
The Limit of Detection (LOD): was 0.1 mg/L.
The Limit of Quantification (LOQ): was 0.2 mg/L

In the sample collected at exposure initiation, the determined florasulam concentration was 100.2% of nominal concentration. Therefore, the test item concentration was prepared correctly.
In the sample collected at exposure termination, the determined florasulam concentration was 99.9% of nominal concentration. Therefore, the florasulam concentration was stable under test conditions.

Results:

The endpoint values were determined based on the nominal test item concentrations.
At exposure termination in the test item concentration of 100 mg/L and the control, no immobilisation of *Daphnia magna* was observed. No abnormal behaviour of *Daphnia magna* was observed during exposure.
The EC_{50}/48 h is higher than 100 mg/L;
The LOEC/48 h is higher than 100 mg/L;
The NOEC/48 h is higher than or equal to 100 mg/L.

Table 1. Test results.

Nominal test item concentration [mg/L]	Number of *Daphnia magna*	Number of immobilised *Daphnia magna*								Total of immobilised *Daphnia magna* [%]	
		24 h				48 h					
		Replicates									
		A	B	C	D	A	B	C	D	24 h	48 h
Control	20	0	0	0	0	0	0	0	0	0	0
100	20	0	0	0	0	0	0	0	0	0	0

Table 2. Endpoint values based on nominal test item concentration

Endpoint value [mg/L]	Test item	
	Time of exposure	
	24 h	48 h
EC_{50}	>100	>100
LOEC	>100	>100
NOEC	≥100	≥100

Comments of zRMS:	The study was conducted to OECD guideline 201 and according to the principles of GLP. No relevant deviations to the guideline were noted. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment

Reference KCP 10.2.1-2
Report Floras 50 SC. *Anabaena flos-aquae* UTEX B 1444 Growth inhibition test. Hodorek G., 2022, W-20-22

Guideline(s): Yes (OECD 201)

Deviations: No

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) NA

Materials and methods

Test item:

Name:	Florasulam 50 g/L SC
Batch No.:	RFEAR0501
Production Date:	05.04.20222
Expiry Date:	30.04.2024
A.i. content:	florasulam 50.3 g/L
Density at 20°C	1.04 g/cm^3

Test system:

Species:	The freshwater cyanobacteria, Anabaena flos-aquae (Lyng.) Bréb UTEX B 1444
Culturing:	The cyanobacteria were transferred to a fresh liquid medium, contained in Erlenmeyer flasks with a capacity of 250 mL, and incubated at temperature between 21 – 24°C under constant illumination. The

		cyanobacterial culture was renewed (transferred to a fresh medium) twice a week under sterile conditions. The pre-culture with a cell density of 1 x 10^6 cells/mL was renewed three days before the definitive exposure initiation. The pre-culture was used for inoculation of all test item concentrations and the control.
	Source:	Cultivated at the Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicology, Laboratory of Aquatic Toxicology. The culture was obtained from the Culture Collection of Algae at the University of Texas at Austin, USA.
Test design:		72 hours of exposure; three replicates per each test item concentration; six replicates per the control; initial cyanobacterial cell density: 1 x 10^4 cells/mL.
Nominal test item concentration:		10, 3.13, 1.1, 0.37 and 0.12 mg/L plus the control.
Experimental conditions:		
	Test medium:	AAP medium
	Temperature:	22.6 – 22.9°C
	pH (control):	7.38 - 8.05
	Light and photoperiod:	constant illumination; cool white light was used, mean light intensity 4070 – 4140 lux
Experimental period:	72 hours	

Test design and treatment:

The definitive test was performed using the following test item concentrations: 10, 3.3, 1.1, 0.37, 0.12 mg/L (with a spacing factor of 3.0) plus the control.

The cell density in the three-day-old cyanobacterial pre-culture was determined by counting the number of cells in the Bürker chamber under a microscope. It was 1.372 x 10^6 cells/mL. The pre-culture was used to inoculate each test item concentration and the control in order to obtain the initial cyanobacteria cells density of 1 x 10^4 cells per mL.

The Erlenmeyer flasks with a capacity of 250 mL containing 100 mL of either test item concentration or the control plugged with air permeable stoppers were used as test vessels. All test item concentrations were split up into three replicates, whereas the control into six replicates.

The replicates were arranged at random and continuously mechanically shaken at 90 rounds per minute to maintain stable conditions during the test.

The pH values were measured at exposure initiation, before splitting up into replicates, and at exposure termination, in pooled replicates. The number of cyanobacterial cells was determined with a direct method, which involves counting the number of cells in the Bürker chamber under a microscope.

To maintain constant conditions throughout exposure for 72 h, a thermostatic chamber was used. The temperature was continuously measured using a sensor submerged in an additional test vessel containing 100 mL of the AAP medium. The test were performed under constant illumination; fluorescent

lighting was used (cool white light). In the preliminary test (non-GLP), the light intensity was measured at exposure initiation and at exposure termination. In the definitive test, the light intensity was measured using lux meter with a 2π receptor at exposure initiation and every 24 h.
The test with reference material, 3,5-dichlorophenol was conducted. Five concentrations of the reference material in the range of 0.32 – 3.2 mg/L were used. There were three replicates of each concentration of reference material and six replicates of the control. Obtained results were: E_rC_{50} 3.57 (2.99 – 4.61) after 72 h; E_yC_{50} 1.06 (0.92 – 1.24) after 72 h. The results are within the range given in the literature reference.

Test validity criteria:

In the definitive test, the following validity criteria specified in OECD Guidelines No. 201 (2006) were met:
- the biomass in the control increased by a factor of 31.5 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 4.7% (criterion: it must not exceed 10%).
- the mean coefficient of variation for the section-by-section growth rate in the control culture was 30.8% (criterion: it must not exceed 35%).

Statistics:

Probit method calculations and analyses by: Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Williams Multiple Sequential t-test Procedure. To make calculations and to conduct statistical analyses, the ToxRat Professional commercial software was used.

Chemical verification of nominal concentration:

The concentrations of florasulam were chemically determined using validated high performance liquid chromatographic method with DAD detection in each treatment at exposure initiation and at exposure termination. The validated analytical methods were performed according to SANTE/2020/12830, Rev. 1.

Florasulam method validation.
The following liquid chromatography parameters were used for analysis of florasulam:
- column - Synergi 4μm Fusion-RP 80Å 150x4.6
- mobile phase - acetonitrile and 0.05% solution of orthophosphoric acid in deionized water (60:40, v/v),
- wave length 260 nm,
- flow rate 0.7 mL/min.,
- injected volume 20 μL,
- oven temperature 35°C.

Linearity: Working solutions of florasulam at the concentrations of 0.05, 0.1, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0 and 100.0 μg/mL were injected successively to the chromatographic column and the chromatograms were recorded. The standard curves (peak area versus quantity of the standard) are linear with coefficient (r^2) of 0.9999427 and 0.9999318. The range of linearity of the analytical graphs are from 0.05 mg/L to 5.0 mg/L and from 1.0 to 20.0 mg/L.
Selectivity and specificity: The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experiments conditions. Therefore, the specificity of

the method was demonstrated. Furthermore, any interference are directly apparent and would be observed in the chromatograms of the matrix control sample.
Precision: RSD for fortification level = LOQ was 4.3% and for level = 10xLOQ it was 0.4%
Accuracy: mean recovery for fortification level = LOQ it was 98.6% and for level = 10xLOQ it was 93.9%
Matrix effect: Assessment of matrix effects was performed by comparing the standard preparing in solvent to standard preparing in control matrix at appropriate concentration and the matrix effect was equal to 4.1%.
The Limit of Detection (LOD): was 0.0005 mg/L.
The Limit of Quantification (LOQ): was 0.001 mg/L

At exposure initiation, the determined concentrations of florasulam were in the range of 92.7 – 100.6% of the nominal concentration. The results confirm that the test item concentrations were prepared correctly.
At exposure termination, the determined concentrations of florasulam were in the range of 93.9 – 112.6% of the nominal concentration. Therefore, the concentrations of florasulam were stable under test conditions.

Results:

The endpoint values based on the nominal test item concentrations are given below:
The ErC50/72 h value is 10.01 mg/L (95% confidence interval: 8.25 – 12.96).
The LOEC/72 h value for growth rate is 3.3 mg/L.
The NOEC/72 h value for growth rate is 1.1 mg/L.
The EyC50/72 h value is 2.75 mg/L (95% confidence interval: 2.20 – 3.44).
The LOEC/72 h value for yield is 1.1 mg/L.
The NOEC/72 h value for yield is 0.37 mg/L.

Table 1. Inhibition of growth rate and yield

Nominal test item concentration [mg/L]	% inhibition after 72 h of exposure (growth rate)	% inhibition after 72 h of exposure (yield)
Control	-	-
0.12	0.1	0.9
0.37	-4.5	-16.0
1.1	6.2	20.2
3.3	25.0	59.4
10.0	49.3	84.0

Table 2. Endpoint values for growth rate based on the nominal test item concentrations

Endpoint value [mg/L]	Test item		
	Time of exposure:		
	24 h	48 h	72 h
E_rC_{50}	1.06 (0.64 – 1.77)	14.06 (7.80 – 45.43)	10.01 (8.25 – 12.96)
E_rC_{20}	0.43 (0.12 – 0.69)	1.327 (0.55 – 2.20)	2.77 (2.05 – 3.42)
E_rC_{10}	0.26 (0.04 – 0.48)	0.39 (0.08 – 0.82)	1.41 (0.88 – 1.93)
LOEC	1.1	1.1	3.3
NOEC	0.37	0.37	1.1

(–) – 95% confidence interval; n.d. – not determined

Table 3. Endpoint values for yield based on the nominal test item concentrations

Endpoint value [mg/L]	Test item		
	Time of exposure:		
	24 h	48 h	72 h
E_yC_{50}	0.78 (0.58 – 1.00)	2.03 (1.22 – 3.60)	2.75 (2.20 – 3.44)
E_yC_{20}	0.47 (0.27 – 0.62)	0.37 (0.11 – 0.69)	1.06 (0.69 – 1.40)
E_yC_{10}	0.37 (0.17 – 0.51)	0.15 (0.03 – 0.35)	0.65 (0.36 – 0.93)
LOEC	1.1	1.1	1.1
NOEC	0.37	0.37	0.37

(–) – 95% confidence interval; n.d. – not determined

Comments of zRMS:	The study was conducted to OECD guideline 221 and according to the principles of GLP. No deviations to the guideline were noted. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.

Reference KCP 10.2.1-3
Report Floras 50 SC. *Lemna gibba* CPCC 310, Growth inhibition test. Hodorek G., 2022, W-19-22

Guideline(s): Yes (OECD 221)

Deviations: No

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) NA

Materials and methods

Test item:

Name:	Florasulam 50 g/L SC
Batch No.:	RFEAR0501
Production Date:	05.04.20222
Expiry Date:	30.04.2024
A.i. content:	florasulam 50.3 g/L
Density at 20°C	1.04 g/cm^3

Test system:

Species:	*Lemna gibba* CPCC 310
Culturing:	Duckweed *Lemna gibba* was cultured in 20X AAP medium in glass beakers with a capacity of 600 mL with transparent lids and incubated in 22 – 26°C with constant illumination.
Source:	The freshwater aquatic plant, *Lemna gibba*

CPCC 310 was cultivated at the Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicology, Laboratory of Aquatic Toxicology; the plants were obtained from the Canadian Phycological Culture Centre (CPCC), Department of Biology, University of Waterloo, Ontario, Canada.

Experimental conditions:

Test medium:	20X AAP nutrient solution
Temperature:	22.9 – 23.2ºC.
pH (control):	7.49 -8.48
Light and photoperiod:	constant illumination, mean light intensity: 7690 – 7774 lux
Test vessels:	glass beakers with a capacity of 600 mL containing 400 mL of treatments
Initial frond number:	9, i.e. 3 plants per 3 fronds

Test design: **S**tatic system; 7 days of exposure; three replicates for each test item concentration and six replicates for control.

Nominal test item concentration: 20, 4, 0.8, 0.16, 0.032, 0.0064 mg/L plus the control.

Experimental period: 7 days

Test design and treatment:

Basis on results obtained from preliminary test (non-GLP) in the definitive test, the *Lemna gibba* was exposed to the following test item concentrations of
20, 4, 0.8, 0.16, 0.032 and 0.0064 mg/L plus the control (with a separation factor of 5.0) in static test design . The exposure was for 7 days.

The 20X APP medium was used as a diluent necessary to prepare the test item concentrations. The medium was aerated prior to exposure initiation. The test was performed in glass beakers with a capacity of 600 mL containing 400 mL of either the test item concentration or the control. Each test item concentration was split up into three replicates, whereas the control into six. Into each replicate 3 colonies containing 3 fronds per colony were introduced. Transparent lids were used to minimize evaporation and accidental contamination, allowing necessary air exchange. The test vessels were arranged at random and repositioned daily during the test.
The tests were conducted with constant illumination, using fluorescent light source. The light intensity was measured at exposure initiation, twice during exposure and at exposure termination.
The temperature was continuously recorded in an additional test vessel with 20X AAP medium. In the tests, the pH values were measured in fresh test item concentrations and the control before splitting up into replicates at exposure initiation and at each renewal. The pH values were also measured in spent test item concentrations and the control at each renewal and at exposure termination in pooled replicates.
The total number of fronds in each test vessel was counted twice during

exposure and at exposure termination. The observations of plant development, i.e. size of fronds, necrosis, chlorosis, colony break-up, gibbosity, changes in the appearance of roots were performed at the same time. The dry weight of the plants was determined after exposure termination.

The test with reference material, 3,5-dichlorophenol, was performed under semi-static conditions with three renewals during the 7 day exposure. The exposure was between 05/07/2022 – 12/07/2022. Five concentrations of the reference material ranging from 0.32 to 32 mg/L were used. Each concentration of reference material was split up into three replicates, whereas the control into six replicates.
The median concentration causing 50% inhibition of the mean specific growth rate of *Lemna gibba* determined on the basis of the frond number ErC50/7 d value is 12.21 mg/L (95% confidence interval: 10.84 – 14.00) of reference substance.
The median concentration causing 50% inhibition of yield of *Lemna gibba* determined on the basis of the frond number EyC50/7 d value is 8.02 (95% confidence interval: 5.93 – 10.39) mg/L of reference substance.
The median concentration causing 50% inhibition of the mean specific growth rate of *Lemna gibba* determined on the basis of the dry weight ErC50/7 d value is 11.12 mg/L L (95 % confidence interval: 10.00 – 13.30) of reference substance. The median concentration causing 50% inhibition of yield of *Lemna gibba* determined on the basis of the dry weight EyC50/7 d value is 6.14 mg/L (95 % confidence interval: 4.69 – 7.99) of reference substance. The results confirmed sensitivity of *Lemna gibba* used in the definitive test.

Statistics:

Probit method calculations and analysis by Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Williams Multiple Sequential t-test Procedure, Step-down Jonckheere-Terpstra Test Procedure, Multiple Sequentially-rejective Welsh-t-test After Bonferroni-Holm.

Chemical verification of nominal concentration:

The concentrations of florasulam were chemically determined using validated high performance liquid chromatographic method with DAD detection. The validated analytical methods were performed according to SANTE/2020/12830, Rev. 1.
Test item concentrations of 0.032, 0.16, 0.8, 4.0 and 20.0 mg/L and the control, collected at exposure initiation, at exposure termination were analysed.
Florasulam method validation.
The following liquid chromatography parameters were used for analysis of florasulam:
- column - Synergi 4μm Fusion-RP 80Å 150x4.6
- mobile phase - acetonitrile and 0.05% solution of orthophosphoric acid in deionized water (60:40, v/v),
- wave length 260 nm,
- flow rate 0.7 mL/min.,
- injected volume 20 μL,
- oven temperature 35°C.

Linearity: Working solutions of florasulam at the concentrations of 0.05, 0.1, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0 and 100.0 μg/mL were injected successively to the chromatographic column and the chromatograms were

recorded. The standard curves (peak area versus quantity of the standard) are linear with coefficient (r^2) of 0.9999427 and 0.9999318. The range of linearity of the analytical graphs are from 0.05 mg/L to 5.0 mg/L and from 1.0 to 20.0 mg/L.
Selectivity and specificity: The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experiments conditions. Therefore, the specificity of the method was demonstrated. Furthermore, any interference are directly apparent and would be observed in the chromatograms of the matrix control sample.
Precision: RSD for fortification level = LOQ was 4.3% and for level = 10xLOQ it was 0.4%
Accuracy: mean recovery for fortification level = LOQ it was 98.6% and for level = 10xLOQ it was 93.9%
Matrix effect: Assessment of matrix effects was performed by comparing the standard preparing in solvent to standard preparing in control matrix at appropriate concentration and the matrix effect was equal to 4.1%.
The Limit of Detection (LOD): was 0.0005 mg/L.
The Limit of Quantification (LOQ): was 0.001 mg/L

At exposure initiation, the determined concentrations of florasulam, were in the range of 89.2 – 99.2% of the nominal concentration. The results confirm that the test item concentrations were prepared correctly.
At exposure termination, the determined concentrations of florasulam, were in the range of 81.4 – 101.3% of the nominal concentration. Therefore, the concentrations of florasulam were stable under test conditions.

Test validity criteria:

In the definitive test, the following validity criteria specified in the OECD Guideline No. 221 (2006) were met:
- the doubling time of frond number in the control was 2.2 days, criterion: less than 2.5 days (the factor of frond number in the control between 0 and 7 day was 9.0),
- the average specific growth rate in the control between day 0 and day 7 was 0.314 d^{-1} (minimum requirement: higher than 0.275 d^{-1}).

Results:

The endpoint values based on the nominal test item concentrations:

Endpoints based on the frond number:
The ErC50/7 d value is 0.062 mg/L (95% confidence interval 0.028 – 0.141).
The ErC20/7 d value is 0.020 mg/L (95% confidence interval 0.010 – 0.039).
The ErC10/7 d value is 0.011 mg/L (95% confidence interval 0.005 – 0.022).
The EyC50/7 d value is 0.030 mg/L (95% confidence interval 0.017 – 0.053).
The EyC20/7 d value is 0.012 mg/L (95% confidence interval 0.007 – 0.019).
The EyC10/7 d value is 0.007 mg/L (95% confidence interval 0.004 – 0.012).
For growth rate and yield, the NOEC/7 d value is 0.0064 mg/L, whereas LOEC/7 d value is 0.032 mg/L.

Endpoints based on the dry weight:
The ErC50/7 d value is higher than 20 mg/L.

The ErC20/7 d value is 0.006 mg/L (95% confidence interval: n. d. – 11.275).
The ErC10/7 d value is not determined.
For growth rate, the NOEC/7 d value is 0.0064 mg/L, whereas LOEC/7 d value is 0.032 mg/L.
The EyC50/7 d value is 0.053 mg/L (95% confidence interval: 0.0002 – 12.657).
The EyC20/7 d value is 0.001 mg/L (95% confidence interval: n. d. – 0.044).
The EyC10/7 d value is not determined.
For yield, the NOEC/7 d value is lower than 0.0064 mg/L, whereas LOEC/7 d value is lower than or equal to 0.0064 mg/L.

Endpoint values of growth rate based on the nominal test item concentrations [mg/L], definitive test:

Endpoint values	**Frond number**			**Dry weight**
	0-2 d	**0-5 d**	**0-7 d**	**0-7 d**
E_rC_{50}	0.053 (0.014 – 0.207)	0.044 (0.019 – 0.101)	0.062 (0.028 – 0.141)	>20
E_rC_{20}	0.016 (0.005 – 0.049)	0.016 (0.008 – 0.032)	0.020 (0.010 – 0.039)	0.006 (n.d. – 11.275)
E_rC_{10}	0.009 (0.003 – 0.028)	0.009 (0.005 – 0.019)	0.011 (0.005 – 0.022)	n.d.
LOEC	0.032	0.032	0.032	0.032
NOEC	0.0064	0.0064	0.0064	0.0064

(-) 95% confidence interval, n.d. – not determine

Endpoint values of yield based on the nominal test item concentrations [mg/L], definitive test:

Endpoint values	**Frond number**			**Dry weight**
	0-2 d	**0-5**	**0-7 d**	**0-7 d**
E_yC_{50}	0.029 (0.002 – 0.389)	0.031 (n.d.)	0.030 (0.017 – 0.053)	0.053 (0.0002 – 12.657)
E_yC_{20}	0.017 (0.002 – 0.136)	0.029 (n.d.)	0.012 (0.007 – 0.019)	0.001 (n.d. – 0.044)
E_yC_{10}	0.012 (0.001 – 0.106)	0.028 (n.d.)	0.007 (0.004 – 0.012)	n.d.
LOEC	0.032	0.032	0.032	≤0.0064
NOEC	0.0064	0.0064	0.0064	<0.0064

(-) 95% confidence interval, n.d. – not determine

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:	The study was conducted to OECD guideline 213 and according to the principles of GLP. No deviations to the guideline were noted. The study is considered to be reliable and suitable for the risk assessment.

Reference Report	KCP 10.3.1.1.1-1 Floras 50 SC. Honeybees (*Apis mellifera* L.), Acute Oral Toxicity Test. Dybek M., 2023, B-127-22
Guideline(s):	Yes (OECD 213)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	NA

Materials and methods

Materials

Test item:

Name:	Florasulam 50 g/L SC
Batch No.:	RFEAR0501
Production Date:	05.04.20222
Expiry Date:	04.2024
A.i. content:	florasulam 50.3 g/L
Density at 20°C	1.04 g/cm^3

Test system:

Species:	the honeybee, *Apis mellifera* L., strain: carnica
Age:	approximately 3 weeks
Source:	an apiary at the Institute of Industrial Organic Chemistry, Branch Pszczyna

Experimental conditions:

Temperature:	25°C
Relative air humidity:	63 - 65%
Light and photoperiod:	test was conducted in the dark experimental room

Test design: test item: 5 doses and a control; 3 replicates; 10 bees/replicate
reference item: 3 doses; 3 replicates; 10 bees/replicate

Experimental period: 48 hours test item, 24 hours reference item

Test item dose: 12.5, 25.0, 50.0, 100.0 and 200.0 µg test item/bee and a control (0.0 µg/bee)

Reference item dose: 0.1, 0.2, and 0.4 µg a.i./bee and a control (0.0 µg/bee)

Test design and treatment:

The acute oral toxicity study was carried out on adult worker honeybees (strain: carnica) from an apiary at the Institute of Industrial Organic Chemistry, Branch Pszczyna. Approximately 3-week-old worker honeybees from healthy, queen-right families with known history and physiological status were collected from honeycombs directly before the treatment. These colonies had not been treated with any chemicals, such as antibiotics or anti-varroa for four weeks preceding the study.
Before the experiment, the honeycomb with the honeybees was transferred from the apiary to an experimental room. The honeybees were removed from the comb and starved for up to two hours before the initiation of the food administration.
In the definitive test, five doses of the test item i.e.: 12.5, 25.0, 50.0, 100.0 and 200.0 µg/bee were used (with a separation factor of 2) plus the control.
Three reference item series at the doses of: 0.1, 0.2 and 0.4 µg/bee (with a separation factor of 2) conducted simultaneously with the treatment series.
In the definitive test, there were three replicates of each dose of the test item, reference item and the control containing 10 bees.
The bees divided into groups of 10 were placed in plastic cages. The size of the test cages (5 x 7 x 4.5 cm) was appropriate to the number of bees, i.e. provided adequate space. There was an opening on the side of each cage which provided free access to the sucrose solution with the test item (or reference item). The bees from every group consumed the whole volume of either the treated or the untreated sucrose solution, within 4 hours, from common micropipettes. The amount of the treated or the untreated sucrose solution taken by one bee from each group from one micropipette was approximately 1/10 (i.e. 10 µl) of the whole amount of the consumed sucrose solution. When the treated solution was consumed, the pipettes with the test item were replaced with 5 mL syringes containing the 50% sucrose solution alone (w/v).
In the definitive test, the insects were observed for mortality and other sublethal toxicity effects (uncoordinated movement, increased activity, intensive cleaning or any signs of paralysis) 4 hours after the beginning of the treatment and then every 24 hours after the beginning of the treatment. The test finished after 48 hours. The amount of the sugar solution consumed by the bees was determined every 24 hours.

Statistics:

Mortality were statistically analysed using the ToxRat Professional 3.3.0 statistical software.
The median lethal dose of dimethoate (LD_{50} oral) after 24 hours determined with the probit analysis using linear max. likelihood regression.

Test validity criteria:

The following validity criteria were met during the test:
- the average mortality for the control was 6.7% at the end of the experiment (criterion: it must not exceed 10%).
- the LD_{50}/24 h of the reference item (dimethoate) was 0.117 µg a.i./bee (criterion: 0.10 – 0.35 µg a.i./bee).

Results:

Mortality of the groups treated with the test item at the doses of 12.5, 25.0, 50.0, 100.0 and 200.0 µg/bee after 48 hours of exposure, after Abbot correction, were (-7.1), (-7.1), 0.0, (-3.6) and 0.0%, respectively.
The median lethal doses LD50/24 h and LD50/48 h are higher than the highest test item dose used in the test, i.e. 200.0 µg/honeybee, i.e. > 9.67 µg/florasulam.

No abnormal behavioural effects were observed during the test.

Table 1. Honeybee mortality and the LD50 after 48 hours of exposure – test item

Dose Test item [µg/bee]	Number of tested bees [no.]	Mortality after 48 h			LD_{50} after 48 h	
		Total				
		[no.]	[%]	Corr. [%][a]	Test item [µg/bee]	Active ingredient [µg/bee]
0.0 (control)	30	2	6.7	-	**>200.0**	**>9.67**
12.5	30	0	0.0	-7.1		
25	30	0	0.0	-7.1		
50	30	2	6.7	0.0		
100	30	1	3.3	-3.6		
200	30	2	6.7	0.0		

[a]: mortality corrected according to the formula of Abbott

Comments of zRMS:	The study was conducted to OECD guideline 247 and according to the principles of GLP. No significant deviations to the guideline were noted. The study is considered to be valid.

Reference KCP 10.3.1.1.1-2
Report Floras 50 SC. Bumblebees (*Bombus spp*.), Acute Oral Toxicity Test. Dybek M., 2023, B-128-22

Guideline(s): Yes (OECD 247)

Deviations: Yes
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 deviations had no impact on the quality, integrity and final results of the study.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) NA

Materials

Test item:

Name:	Florasulam 50 g/L SC
Batch No.:	RFEAR0501
Production Date:	05.04.20222
Expiry Date:	04.2024
A.i. content:	florasulam 50.3 g/L
Density at 20°C	1.04 g/cm^3

Test system:

Species:	bumblebee, *Bombus* spp
Age:	adult worker bumblebees
Source:	Koppert Polska sp. z o.o.

Experimental conditions:

Temperature:	25°C
Relative air humidity:	59 - 60%
Light and photoperiod:	test was conducted in the dark experimental room

Test design: control: number of replicates – 50, 1 insect/replicate
test item: 1 dose; 50 replicates; 1 insect/replicate
reference item: 1 dose; 30 replicates; 1 insect/replicate

Experimental period: 48 hours

Test item dose: 100.0 µg test item/bumblebee

Reference item dose: 4.0 µg/bumblebee

Test design and treatment:

The adult bumblebees were collected from the hives under a red light and individually placed in plastic isolators of known weight. There was 1 bumblebee in each isolator. Very small or very large individuals were excluded by visual inspection. After that, they were acclimatized to the test conditions for about 24 hours before starting the experiment. Food, i.e. 50% sucrose solution was provided. To determine the weight of each bumblebee, the isolator was weighed.
For a maximum of 4 hours before starting the exposure the bumblebees were starved. The bumblebees were exposed to the test or reference item distributed in the 50% sucrose solution. The treated diet was provided in calibrated pipettes. Glass pipettes facilitate ongoing monitoring consumption of the treated diet and determination of the exact diet consumption. Each pipette contained 40 µL of the sucrose solution with the test item at a suitable dose.
Once consumed (not later than after 4 hours), the pipettes were removed and replaced with syringes containing a sucrose solution alone. After the treatment, the bumblebees were kept individually in isolators. The isolators were well-ventilated and provide enough space. After the treatment, the insects had continuous access to food, i.e. a 50% sucrose solution (w/v) in 2-mL syringes. Fresh food was added if necessary.
In the preliminary and definitive test, the insects were observed for mortality and sublethal effects 4, 24 and 48 hours after the treatment. The test finished after 48 hours.

Chemical determinations:

The aim of the analytical part of the definitive test was to determine the concentration of florasulam using a validated high performance liquid chromatographic method with DAD detection. Fresh samples of the test item concentration of 2.5 g/L (i.e. 100 µg/40 µL) and control at exposure initiation were chemically analysed.
The validated analytical method was performed according to SANTE/2020/12830, Rev. 1.

<u>Florasulam method validation.</u>
The following liquid chromatography parameters were used for analysis of florasulam:
- column - Synergi 4µm Fusion-RP 80Å 150x4.6
- mobile phase - acetonitrile and 0.05% solution of orthophosphoric acid in deionized water (60:40, v/v),

- wave length 260 nm,
- flow rate 0.7 mL/min.,
- injected volume 1 µL,
- oven temperature 35°C.

Linearity: Working solutions of florasulam at the concentrations of 1.0, 5.0, 10.0, 50.0 and 100.0 µg/mL were injected successively to the chromatographic column and the chromatograms were recorded. The standard curve (peak area versus quantity of the standard) is linear with coefficient (r2) of 0.9999355. The range of linearity of the analytical graphs are from 10.0 mg/L to 1000.0 mg/L.

Selectivity and specificity: The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experiments conditions. Therefore, the specificity of the method was demonstrated. Furthermore, any interference are directly apparent and would be observed in the chromatograms of the matrix control sample.

Precision: RSD for fortification = LOQ it was 1.9% and for level = 10xLOQ it was 0.3%.

Accuracy: mean recovery for fortification level = LOQ it was 100.6% and for level = 10xLOQ it was 93.9%.

Matrix effect: Assessment of matrix effects was performed by comparing the standard preparing in solvent to standard preparing in control matrix at appropriate concentration and the matrix effect was equal to 3.5%.

The Limit of Detection (LOD): for florasulam was 10.0 mg/L.

The Limit of Quantification (LOQ): for florasulam was 20.0 mg/L.

At exposure initiation, in the fresh test item sample, the concentration of florasulam was 105.0±0.4% of the nominal concentration. The results confirm that the test item concentration was prepared correctly.

Statistics:

Statistical analysis was not needed due to the lack of mortality.

Test validity criteria:

The following validity criteria were met:

− Mortality of the control group 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%).

Results:

Mortality in the control group was 0.0% after 48 hours of exposure. The percentage of mortality after 4, 24 and 48 hours of exposure to the test item at the dose of 100.0 µg test item/bumblebee was 0.0%.

During the experiment no sublethal effects (toxic symptoms) in the group treated with the test item were observed.

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. > 100.0 µg test item/ bumblebee, i.e. > 4.84 µg florasulam/ bumblebee.

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 no affected bumblebees of exposure were observed.

Table 1. Bumblebee mortality and the LD50 after 48 hours of exposure – test item

Dose		Number of tested bees [no.]	Mortality after 48 h		LD50 after 48 h	
			Total			
Test item [µg/bee]	Active ingredients [µg/bee]		[no.]	[%]	Test item [µg/ bumblebee]	Active ingredients [µg/bee]
Control		50	0	0.0	**> 100**	**> 4.84**
100.0	4.84	50	0	0.0		
Reference item: dimethoate						
Dose [µg/bumblebee]	4.0	30	29	96.7	-	

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

Comments of zRMS:	The study was conducted to OECD guideline 214 and according to the principles of GLP. No significant deviations to the guideline were noted. The study is considered to be reliable and suitable for the risk assessment.

Reference KCP 10.3.1.1.2-1
Report Floras 50 SC. Honeybees (*Apis mellifera* L.), Acute Contact Toxicity Test. Dybek M., 2023, B-129-22

Guideline(s): Yes (OECD 214)

Deviations: YES
Anesthesia recommended by the OECD Guideline No. 214 / EU Method C.17. was replaced with mechanical immobilization. This deviation had no impact on the results.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) NA

Materials and methods

Materials

Test item:

Name:	Florasulam 50 g/L SC
Batch No.:	RFEAR0501
Production Date:	05.04.20222
Expiry Date:	04.2024
A.i. content:	florasulam 50.3 g/L
Density at 20°C	1.04 g/cm^3

Test system:

Species:	the honeybee, *Apis mellifera* L., strain: carnica
Age:	approximately 3 weeks
Source:	The apiary at the Institute of Industrial Organic Chemistry, Branch Pszczyna

Experimental conditions:

Temperature:	25°C
Relative air humidity:	63-65%
Light and photoperiod:	test was conducted in the dark experimental room

Test design: test item: 5 doses with surfactant and two controls (water and control with surfactant); 3 replicates; 10 bees/replicate
reference item: 3 doses with surfactant; 3 replicates; 10 bees/replicate

Experimental period: 48 hours test item, 24 hours reference item

Test item dose: 12.5, 25.0, 50.0, 100.0 and 200.0 µg test item/bee and a control (0.0 µg/bee)

Reference item dose: 0.1, 0.2, and 0.4 µg a.i./bee and a control (0.0 µg/bee)

Test design and treatment:

The acute contact toxicity study was carried out on adult worker honeybees (strain: carnica) from the apiary at the Institute of Industrial Organic Chemistry, Branch Pszczyna. Approximately 3-week-old worker honeybees from healthy, queen-right families with known history and physiological status were collected from honeycombs directly before the treatment. These colonies had not been treated with any chemicals, such as antibiotics or anti-varroa for four weeks preceding the study. Before the experiment, the honeycomb was transferred from the apiary to the experimental room.
In the definitive experiment, the doses of 12.5, 25.0, 50.0, 100.0 and 200.0 µg test item/bee were used. There were three replicates of each of them (10 bees/replicate). Three control series, each with ten bees, were conducted simultaneously.
Three doses of the reference item, i. e. dimethoate were used. They were 0.1, 0.2, and 0.4 µg a.i./bee). There were three control batches, each of ten bees, in addition to the test series.
The emulsions were applied using the Arnold Automatic microapplicator (240 V) produced by Burkard Manufacturing Co Ltd.
Anaesthesia recommended by the OECD Guideline No. 214 / EU Method C.17. was replaced with immobilisation. 1 µL of the liquid containing proper dose of the test or reference item was applied to the dorsal part of the thorax of each bee. After the application, the bees were allocated to cages. There were 10 bees in each cage. This procedure was repeated for all the replicates and treatments. Two control series, with ten bees each, were conducted simultaneously. Water control group was treated with 1 µL of distilled water and control with surfactant was treated with 1 uL of 1% Triton(R) X - 100 solution.
All treated honeybees were kept in plastic cages. There were 10 bees in each of them. The size of the test cages (5 x 7 x 4.5 cm) was appropriate to the number of bees, i.e. provided adequate space.
There was an opening in each cage which allowed the insects to take food, i.e. a sucrose solution from a 5-mL syringe. After the treatment, the insects had continuous access to it. Fresh food was given when the need arose.
In the definitive test, the insects from control and test item groups were observed for mortality and signs of toxicity 4, 24 and 48 hours after the application. In the reference item group insects were observed for mortality 4 and 24 hours after the application The test finished after 48 hours. Food consumption was not determined.

Test validity criteria:

The following validity criteria were met during the test:
- 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 0.221 µg a.i./bee (criterion: 0.10−0.30 µg a.i./bee).

Statistics:

The median lethal dose (contact LD_{50}) of the reference item with 95% confidence intervals was determined by regression analysis using the log-probit method.
Mortality results were statistically analyzed using the ToxRat Professional 3.3.0 statistical software.

Results:

After 48 hours of exposure the percentages of mortality of the bees treated with the test item at the doses of 12.5, 25.0, 50.0, 100.0 and 200.0 µg/honeybee with surfactant were 0.0%.
The median lethal doses (LD50/24 h and LD50/48 h contact) are higher than 200.0 µg/honeybee (i.e. 9.67 a.i. µg/honeybee).
During the definitive test no abnormal behavioural effects were observed.

Table 1. Honeybee mortality and the LD50 after 48 hours of exposure – test item

Doses Test item [µg/bee]	Number of tested bees [no.]	Mortality after 48 h Total		LD_{50} after 48 h	
		[no.]	[%]	Test item [µg/bee]	Active ingredients [µg/bee]
Control (water)	30	0	0.0	**>200.0**	**>9.67**
Control (surfactant)	30	0	0.0		
12.5	30	0	0.0		
25	30	0	0.0		
50	30	0	0.0		
100	30	0	0.0		
200	30	0	0.0		

Comments of zRMS:	The study was conducted to OECD guideline 246 and according to the principles of GLP. No significant deviations to the guideline were noted. The study is considered to be valid.

Reference KCP 10.3.1.1.2-2
Report Floras 50 SC. Bumblebees (*Bombus spp*.), Acute Contact Toxicity Test. Dybek M., 2023, B-130-22

Guideline(s): Yes (OECD 246)

Deviations: Yes
According to the OECD Guideline No. 246 the bumblebees may be anesthetized with carbon dioxide or chilled for the application of the test item. Anesthesia with carbon dioxide or chilling was replaced with mechanical immobilisation. This deviation had no impact on the quality, integrity and final results of the study.

GLP: Yes

Acceptability: Yes

Duplication NA

(if vertebrate study)

Materials

Test item:

Name:	Florasulam 50 g/L SC
Batch No.:	RFEAR0501
Production Date:	05.04.20222
Expiry Date:	04.2024
A.i. content:	florasulam 50.3 g/L
Density at 20°C	1.04 g/cm^3

Test system:

Species:	bumblebee, *Bombus* spp
Age:	adult worker bumblebees
Source:	Koppert Polska sp. z o.o.

Experimental conditions:

Temperature:	24.0 - 25°C
Relative air humidity:	58 - 61%
Light and photoperiod:	test was conducted in the dark experimental room

Test design: control with surfactant: number of replicates – 50, 1 insect/replicate
test item with surfactant: 1 dose; 50 replicates; 1 insect/replicate
reference item with surfactant: 1 dose; 30 replicates; 1 insect/replicate

Experimental period: 48 hours

Test item dose: 100.0 µg test item/bumblebee

Reference item dose: 10.0 µg/bumblebee

Test design and treatment:

For test the adult bumblebees were collected from the hives under red light and individually placed in plastic isolators of known weight. There was 1 bumblebee in each isolator. The insects were selected for the exposure in terms of their sizes. Very small or very large individuals were excluded by visual inspection. After that, they were acclimatized to the test conditions for about 24 hours before starting the experiment. Food, i.e. 50% sucrose solution was provided.

Anaesthesia with CO_2 or chilling, recommended by the OECD Guideline No. 246, was replaced with immobilization using suitable laboratory apparatus. After acclimatization period, each bumblebee was introduced into a glass probe (15 cm long and 2.5 cm wide) plugged with a plastic stopper. There was a plunger inside each probe to immobilize a bumblebee during the application of the test item. Then, 2 µL of the test item suspension, reference item solution, distilled water with 1% surfactant were applied to the dorsal part of the thorax with a microapplicator. After the application, the bumblebees were allocated back to isolators. During the whole experiment, the insects had continuous access to food, i.e. a 50% sucrose solution (w/v) in 2-mL syringes. Fresh food was added if necessary.

In the definitive test, the insects were observed for mortality and sublethal effects 4, 24 and 48 hours after the treatment. The test finished after 48 hours.

Chemical determinations:

The aim of the analytical part of the definitive test was to determine the concentration of florasulam using a validated high performance liquid chromatographic method with DAD detection. Fresh samples of the test item concentration of 50.0 g/L (i.e. 100 µg/2 µL) with surfactant and control with surfactant (distilled water with 1% Triton X-100) at exposure initiation were chemically analysed.
The validated analytical method was performed according to SANTE/2020/12830, Rev. 1.

Florasulam method validation.
The following liquid chromatography parameters were used for analysis of florasulam:
- column - Synergi 4µm Fusion-RP 80Å 150x4.6
- mobile phase - acetonitrile and 0.05% solution of orthophosphoric acid in deionized water (60:40, v/v),
- wave length 260 nm,
- flow rate 0.7 mL/min.,
- injected volume 1 µL,
- oven temperature - 35°C.

Linearity: Working solutions of florasulam at the concentrations of 1.0, 5.0, 10.0, 50.0 and 100.0 µg/mL were injected successively to the chromatographic column and the chromatograms were recorded. The standard curve (peak area versus quantity of the standard) is linear with coefficient (r2) of 0.9999355. The range of linearity of the analytical graphs are from 10.0 mg/L to 1000.0 mg/L.
Selectivity and specificity: The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experiments conditions. Therefore, the specificity of the method was demonstrated. Furthermore, any interference are directly apparent and would be observed in the chromatograms of the matrix control sample.
Precision: RSD for fortification = LOQ it was1.0% and for level = 10xLOQ it was 0.4%.
Accuracy: mean recovery for fortification level = LOQ it was 100.8% and for level = 10xLOQ it was 95.7%.
Matrix effect: Assessment of matrix effects was performed by comparing the standard preparing in solvent to standard preparing in control matrix at appropriate concentration and the matrix effect was equal to 3.1%.
The Limit of Detection (LOD): for florasulam was 10.0 mg/L.
The Limit of Quantification (LOQ): for florasulam was 20.0 mg/L.

At exposure initiation, in the fresh test item sample, the concentration of florasulam was 105.0±0.4% of the nominal concentration. The results confirm that the test item concentration was prepared correctly.

Statistics:

Statistical analysis was not needed due to the lack of mortality.

Test validity criteria:

The following validity criteria were met:
− Mortality of the control group 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 100.0% (criterion: ≥ 50%).

Results:

Mortality in control group with surfactant after 4, 24 and 48 hours of exposure were 0.0%. The percentage of mortality after 4, 24 and 48 h hours of exposure to the test item at the dose of 100.0 µg test tem/bumblebee with 1% surfactant were 0.0%. During the experiment sublethal effects (toxic symptoms) in the group treated with the test item were not observed.
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. > 100.0 µg test item/bumblebee, i.e. > 4.84 µg florasulam/ bumblebee.

Table 1. Bumblebee mortality and the LD50 after 48 hours of exposure – test item

Dose		Number of tested bees [no.]	Mortality after 48 h		LD_{50} after 48 h	
Test item [µg/bee]	Active ingredients [µg/bee]		Total			
			[no.]	[%]	Test item [µg/ bumblebee]	Active ingredients [µg/bee]
Control with 1% surfactant		50	0	0.0	**> 100.0**	**> 4.84**
100.0	4.84	50	0	0.0		
Reference item: dimethoate with 1% surfactant						
Dose [µg/bumblebee]	10.0	30	30	100.0	-	

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

Comments of zRMS:	The study was conducted to OECD guideline 245 and according to the principles of GLP. No deviations to the guideline were noted. The study is considered to be valid.

Reference	KCP 10.3.1.2
Report	Floras 50 SC. Honeybees (*Apis mellifera* L.), Chronic Oral Toxicity Test. Dybek M., 2023, B-126-22
Guideline(s):	Yes (OECD 245)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	NA

Materials and methods

Materials

Test item:

Name:	Florasulam 50 g/L SC
Batch No.:	RFEAR0501
Production Date:	05.04.20222
Expiry Date:	30.04.2024
A.i. content:	florasulam 50.3 g/L
Density at 20°C	1.04 g/cm^3

Test system:

Species:	the honeybee, *Apis mellifera* L., strain: carnica
Age:	freshly emerged worker honeybees from the same queen-right colony
Source:	bee hive maintained at IPO/Pszczyna

Experimental conditions:

Temperature:	33.9-34.9°C
Relative humidity:	50.6-65.1%
Treatments:	Control, reference substance – dimethoate 0.0225 a.i./bee/day, test item: 20 µg a.i./bee/day
Replication:	test item: 5 replicates per concentration, 10 bees/replicate; reference item: 3 replicates per concentration, 10 bees/replicate

Experimental period: 10 days

Test design and treatment:

Freshly emerged young worker bees (strain: carnica) from an apiary at the Institute of Industrial Organic Chemistry, Branch Pszczyna were used in the chronic oral toxicity study. They were from healthy, queen-right families with known history and physiological status.

One day before the experiment, brood frames were transferred from the apiary to the experimental room. Afterwards, they were placed in hatching boxes in an incubator. After hatching, the bees were acclimated to the test conditions for about one day.

The definitive test was done on one untreated control group with five replicates, one test item group: 666.7 mg/kg (i.e. 20 µg/bee/day) with five replicates, and one reference item group: 0.8 mg/kg (i.e. 0.024 µg/bee/day) with three replicates. There were 10 bees in each replicate. Additional cages with pre-weight feeders containing no bees were also set up in the definitive test to measure possible evaporation. There were 3 replicates containing diet of untreated control and placed in the environment alongside the test units.

The honeybees after about one day of adaptation were transferred from the hatching boxes to test units, i.e. cages. There were 10 insects in each of them. The test units are made of stainless steel. However, the front removable part is made of glass. There are hole on the upper wall of each cage. The hole was used to introduce the insects into the test cages. Then, it is capped with a feeder (5-mL syringe) containing a sucrose solution treated with the test item or a sucrose solution alone.

Each group of bees was fed with 2 mL of a 50% sucrose solution containing the reference item or the test item for 10 days. Feeders were used. The control insects were fed with an untreated 50% sucrose solution.

Daily food consumption in each test group was determined by weighing the feeders and dividing the amount of food by the number of surviving bees. Evaporation was subtracted from the calculated food consumption to give corrected food consumption accounting the loss by evaporation. The consumed doses by the bees (i.e. dietary dose expressed in µg/bee/day) in the groups treated with the test item and reference item were calculated directly from sucrose solution consumption.

Statistics:

Range-to-standard-deviation-ratio and Levene’s test, Chi2 rxr 2-Contingency Table

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 2.0% (criterion: it must not exceed 15%).
- After 10 days of exposure corrected mortality of the honeybees exposed to the reference item at the concentration of 0.8 mg/kg (0.012 µg/bee/day) was 100.0%.

Chemical verification of nominal concentration:

The aim of the analytical part of the definitive test was to determine the concentration of florasulam using a validated high performance liquid chromatographic method with DAD detection.
In the definitive test fresh samples of the control, the feeding solution at the concentration of 666.7 mg/kg were chemically analyzed directly after preparation and once after 4 days of the storage (i.e. at D0 and D4). The aim was to make sure that the solution of the test item was prepared properly. The validated analytical method was performed according to SANTE/2020/12830, Rev. 1.

Florasulam method validation.
The following liquid chromatography parameters were used for analysis of florasulam:
- column - Kinetex 5 µm C18(4) 150 mm×4.6 mm
- mobile phase - acetonitrile and 0.05% solution of orthophosphoric acid in deionized water (45:55, v/v),
- wave length 220 nm,
- flow rate 0.5 mL/min.,
- injected volume 30 µL,
- oven temperature 35°C.

Linearity: Working solutions of florasulam at the concentrations of 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0 µg/mL and at the concentrations of 0.2, 0.5, 1,0, 2.0, 5.0, 10.0 and 20.0 were injected successively to the chromatographic column and the chromatograms were recorded. The standard curves (peak area versus quantity of the standard) are linear with coefficient (r2) of 0.9999295 and 0.9999323. The range of linearity of the analytical graphs are from 0.05 mg/L to 5.0 mg/L.
Selectivity and specificity: The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experiments conditions. Therefore, the specificity of the method was demonstrated. Furthermore, any interference are directly apparent and would be observed in the chromatograms of the control sample.
Precision: RSD for fortification = 1.0 mg/L it was 2.0% and for level = 10.0 mg/L it was 0.5%.
Accuracy: mean recovery for fortification level = 1.0 mg/L it was 94.9% and for level = 10.0 mg/L it was 87.7%.
Matrix effect: Assessment of matrix effects was performed by comparing the standard preparing in solvent to standard preparing in control matrix at appropriate concentration and the matrix effect was equal to 2.3%.
The Limit of Detection (LOD): for florasulam was 0.5 mg/kg.
The Limit of Quantification (LOQ): for florasulam was 1.0 mg/kg.

At exposure initiation, in the fresh sample of the test item of 666.7 mg of test item/kg, the determined concentration of florasulam was 93.2% 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 florasulam was 94.0% of nominal concentration. Based on the results of chemical analyses, the concentrations of florasulam was stable under storage conditions.

Results:

The validity criterion concerning mortality was met, because mortality in the control was 2.0% 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 12.4 µg of test item/bee/day) at exposure termination (after 10 days), corrected according to the formula of Abbott, was (–2.0) %. The negative value indicates that mortality in the group treated with the test item was lower than in the control group.
On the basis of the obtained mortality results the LC50 is higher than 666.7 mg/kg, and the LDD50 value is higher than 12.4 µg of test item/bee/day, there is no statistically significant difference in mortality between group treated with the test item at the dose of 666.7 mg/kg (dietary dose 12.4 µg of test item/bee/day) and the control group (Chi2-Contingency test, p(Chi)> Alpha 0.05).
The validity criterion concerning mortality of the honeybees exposed to the reference item, dimethoate was met, because mortality was equal to 100% after 10 days of exposure. The results obtained in the reference item group showed that the insects were sensitive to dimethoate.

Table 1. The effects of Floras 50 SC on mortality of honey bees

Nominal test item dose [µg/bee/day]	Nominal test item concentration [mg/kg]	Consumed[a] dose [µg/bee/day]	Number of tested bees [no]	Total mortality			LC50 [mg f.p./kg]	LDD50 [µg f.p./bee/day]
				No.	[%]	Corrected [%]		
Floras 50 SC								
0.0 (Control)			50	1	2.0	-	> **666.7**	> **12.4**
20.0	666.7	12.4	50	0	0.0	-2.0		
Dimethoate (reference item)								
0.024	0.8	0.012	30	30	100.0	not determined		

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

Comments of zRMS:	The study was conducted to OECD guideline 237 and according to the principles of GLP. No deviations to the guideline were noted. The study is considered to be valid. Currently the larval toxicity test with repeated exposure (OECD 239) is required.

Reference KCP 10.3.1.3
Report Floras 50 SC. Honeybees (*Apis mellifera* L.), Larval Toxicity Test, Single Exposure. Dybek M., 2023, B-125-22

Guideline(s): Yes (OECD 237)

Deviations: NO

GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	NA

Materials and methods

Materials

Test item:

Name:	Florasulam 50 g/L SC
Batch No.:	RFEAR0501
Production Date:	05.04.20222
Expiry Date:	30.04.2024
A.i. content:	florasulam 50.3 g/L
Density at 20°C	1.04 g/cm^3

Test system:

Species:	the honeybee, *Apis mellifera* L., strain: carnica
Age:	synchronized first instar larvae (L1, one day old)
Source:	an apiary at the Institute of Industrial Organic Chemistry, Branch Pszczyna

Experimental conditions:

Temperature:	34-34.6°C
Relative air humidity:	90.1- 96.7%
Light and photoperiod:	test was conducted in the darkness

Course of the trial:

Test design:	– the test item: exposure: 72 hours; number of doses: 1 and a control; number of replicates: 3; number of larvae: 12/replicate – the reference item: exposure: 72 hours; number of doses: 1; number of replicates: 3; number of larvae: 12/replicate
Test item doses:	100.0 µg/larva + control
Reference item dose:	8.8 µg dimethoate/larva

Experimental period: 72 h

Test design and treatment:

Larvae were taken from three healthy, queen-right families (3 replicates) with known history and physiological status. Families had not been treated with chemical substances, such as antibiotics, anti-varroa, etc. for four weeks before the experiment.

In the definitive test, one dose of 100.0 µg/larva, plus the control and one dose of a reference item were used. There were three replicates of each dose (3 replicates/dose; 12 larvae/replicate).

On day 1 of the test combs with larvae were transferred from the apiary to the experimental room. Next, each one-day-old larva, which had not formed a C shape yet, was transferred to a grafting cell, on the surface of diet A (20 µL), using a grafting tool. Each cell was placed into a well of a 48-well plate.

When a plate was filled with larvae, it was placed into a desiccator, which had previously been placed into an incubator.
The test and the reference items were given on D4 (three days after grafting the larvae). The volume of the test solution in the diet did not exceed 10% of the final volume of the diet.
All larvae were reared in crystal polystyrene grafting cells with a diameter of 9 mm and depth of 8 mm. Each cell was placed into a well of a 48-well plate (NEST). A piece of dental roll wetted with a 15% sterilising solution of glycerol was placed at the bottom of the wells. The plates were placed into a hermetic desiccator, whereas the desiccator was placed in an incubator. The larvae were divided into treated group (3 replicates/group), one group with the reference item and one control group. The exposure lasted on test item 72 h and the test lasted 6 days
After the treatment, the larvae had access to fresh diet. Dead larvae were removed from the plates during the experiment. In the preliminary and the definitive tests, the larvae were observed for mortality every 24 hours after the administration of the treated diet. At test termination the presence of uneaten food was determined.

Statistics:

Chi2 rx2-Contingency Table, STUDENT t-test for Homogeneous Variances, One-way Analysis of Variance. The statistical analysis was performed using the ToxRat Professional 3.3.0. software

Validity of the study:

The following validity criteria were met:
- Mortality of the control group was 8.3% at the end of the test (criterion: ≤ 15%).
- Mortality of the larvae treated with the reference item (dimethoate) was 87.9% (corrected using Abbott's formula) (criterion: ≥ 50%).

Chemical verification of nominal concentration:

The aim of the analytical part of the definitive test was to determine the concentration of florasulam using a validated high performance liquid chromatographic method with DAD detection. Fresh samples of the control and the stock test item concentration of 1000.0 µg/30 µL, i.e. 33.3 mg/mL, were chemically determined. The aim was to make sure that the stock test item concentration was prepared properly. The validated analytical method was performed according to SANTE/2020/12830, Rev. 1.

Florasulam method validation.
The following liquid chromatography parameters were used for analysis of florasulam:
- column - Kinetex 5 µm C18(4) 150 mm×4.6 mm
- mobile phase - acetonitrile and 0.05% solution of orthophosphoric acid in deionized water (45:55, v/v),
- wave length 220 nm,
- flow rate 0.5 mL/min.,
- injected volume 30 µL,
- oven temperature 35°C.

Linearity: Working solutions of florasulam at the concentrations of 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0 µg/mL were injected successively to the chromatographic column and the chromatograms were recorded. The standard curve (peak area versus quantity of the standard) is linear with coefficient (r2) of 0.9999295. The range of linearity of the analytical graphs are from 0.05 mg/L to 5.0 mg/L.
Selectivity and specificity: The analytical method specificity was estimated

on the basis of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experiments conditions. Therefore, the specificity of the method was demonstrated. Furthermore, any interference are directly apparent and would be observed in the chromatograms of the control sample.
Precision: RSD for fortification = 0.2 mg/L it was 2.5% and for level = 2.0 mg/L it was 0.8%.
Accuracy: mean recovery for fortification level = 0.2 mg/L it was 109.1% and for level = 2.0 mg/L it was 103.4%.
Matrix effect: Assessment of matrix effects was performed by comparing the standard preparing in solvent to standard preparing in control matrix at appropriate concentration and the matrix effect was equal to 2.8%.
The Limit of Detection (LOD): for florasulam was 0.1 mg/L.
The Limit of Quantification (LOQ): for florasulam was 0.2 mg/L.

At exposure initiation, in the fresh test item sample, the concentration of florasulam was 102.1±0.5% of the nominal concentration. The results confirm that the test item concentration was prepared correctly.

Results:

Mortality of the control group at the end of the test was 8.3% (criterion: ≤ 15%).
The percentage of mortality of the honeybee larvae, exposed to the test item, Floras 50 SC at the dose of 100.0 µg/larva, corrected according to the formula of Abbott, at the end of the test (D7) was 0.0%.
The percentage of larval mortality on D7 in the reference item group, corrected according to the formula of Abbott, was 87.9%.
The median lethal doses after 24, 48 and 72 h of exposure, for the test item (LD50/24, LD50/48 and LD50/72 h) are higher than 100.0 µg test item/larva.

Table 1. The effects of Floras 50 SC on mortality of honey bee larvae

Dose [µg/larva]	**Number of tested larvae [no.]**	**Mortality after 72 h of exposure (D7)**			**LD50 72 h**
		Total			
		[no.]	**[%]**	**Corrected[a] [%]**	
Mezot 100 SC					
0.0 (Control)	36	3	8.3	-	> 100 µg f.p./larva) (> 4.84 µg a.i./larva)
100.0	36	3	8.3	0.0	
Dimethoate (reference item)					
8.8	36	32	88.9	87.9	not determined

[a]: Mortality corrected according to the Abbott formula

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 2.3.2 KCP 10.3.2 Effects on non-target arthropods other than bees

A 2.3.2.1 KCP 10.3.2.1 Standard laboratory testing for non-target arthropods

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment. zRMS not accepted the calculated ER_{50} value of 0.052 L/ha since already for dose 0.05 L/ha the 63.7% effects on oviposition were noted. Based on this study, it can only be concluded that ER_{50} is above 0.025 L/ha.

Reference Report	KCP 10.3.2.1-1 A laboratory test for evaluating the effects of Floras 50 SC on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez). Dybek M., 2023, B-123-22.
Guideline(s):	Yes (ESCORT 1, ESCORT 2, IOBC, BART, and EPPO)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	NA

Materials and methods

Materials

Test item:

Name:	Florasulam 50 g/L SC
Batch No.:	RFEAR0501
Production Date:	05.04.20222
Expiry Date:	04.2024
A.i. content:	florasulam 50.3 g/L
Density at 20°C	1.04 g/cm^3

Test system:

Species:	the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez)
Culturing:	the wasps, *A. rhopalosiphi* were reared on the barley, *Hordeum vulgare* L. infested with the bird cherry-oat aphid, *Rhopalosiphum padi*.

	Age:	imago (24 - 48 hours after emerging from mummies)
	Source:	laboratory culture at the Institute of Industrial Organic Chemistry, Branch Pszczyna; the culture was obtained from commercial breeder

Experimental conditions:

	Temperature:	19 – 20°C
	relative air humidity:	65 – 80%
	Light and photoperiod:	16 hours light (mortality assessment and oviposition: 4913 lx; fecundity assessment: 5198) : 8 hours dark
Experimental design:	5 study groups: control, test item groups – 0.02, 0.05, 0.1 L/ha and dimethoate at the rate of 0.2 g/ha. Mortality assessment: 4 replicate/group; 10 females/replicate Fecundity assessment: 10 replicates/group; 1 female/replicate	
Experimental period:	15 days (mortality: 48 hours, oviposition: 24 hours and fecundity: 12 days)	

Test design and treatment:

In the definitive test, three rates of test item were used. These were 0.025, 0.05 and 0.1 L/ha. The rate of the reference item, i. e. dimethoate was 0.2 g/ha.

The study was performed according to the test method described by Mead-Briggs M.A. *et al.*. The test unit for mortality assessment (exposure unit) consisted of two glass plates (12 x 12 cm) fitted with rubbers to a stainless steel frame. On the side walls, there were ten holes covered with fine-gauge mesh providing ventilation for the insects and two holes to introduce the wasps to the test units. Later these holes were sealed with cotton bungs soaked with a 1:3 v/v solution of honey in water used as a source of food.

To assess fecundity, the pots contained approximately 20 seedlings of 7-day-old barley infested with the bird cherry-oat aphid, *Rhopalosiphum padi* (> 100 aphids per pot). To provide good ventilation, the apex of each cylinder and two longitudinal openings on its two sides are covered with fine metal netting. There is a hole in the cylinder to introduce the wasps to the test units. This port is filled with a cotton wool bung soaked with a 1:3 (*v/v*) solution of honey.

All spray fluids were prepared on the treatment day. The volume corresponded to 200 L spray fluid/ha.

The glass plates were sprayed using the Potter laboratory spray tower.

To determine mortality of the wasp, *A. rhopalosiphi* in each replicate, the condition of the insects was observed 2, 24, and 48 hours after their introduction into the test units.

After 48 hours of exposure, 15 females from the control group and 15 females from the all the groups treated with test item at the rate of 0.025 0.05 and 0.1 L/ha (mortality: < 50%) were individually introduced into the fecundity units containing barley plants infested with the aphid, *R. padi*. The aim was to allow the parasitisation process (oviposition) lasting 24 hours. After the oviposition, all surviving wasps were removed from the fecundity units, whereas the parasitized aphids were left for 12 days to allow the development of mummies (a pupal stage of a wasp in the aphid body). The impact of the reference item on fecundity of the wasps was not evaluated.

Statistics:

Probit analysis using linear weighted regression, Shapiro-Wilk’s Test on Normal Distribution, Levene’s Test on Variance Homogenity (with

Residuals), Multiple Sequentially-rejective Welsh-t-test After Bonferroni-Holm

Results:

In the definitive test, mortality of the control group, after 48 hours, was 0.0%. After 48 hours of the exposure to Floras 50 SC, at the all rates the percentages mortality of *A. rhopalosiphi* was 0.0%.
Based on the obtained mortality results it can be assumed that the LR50 is higher than 0.1 L/ha. The NOERmortality is higher or equal to 0.1 L/ha of the test item.
Mortality the wasps exposed to dimethoate (after 24 hours of exposure) at the rate of 0.2 g/ha was 77.5%.
The fecundity assessment showed that the mean number of mummies per female in the control group was 21.1. As for the wasps treated with Floras 50 SC at the rates of 0.025, 0.05 and 0.1 L/ha the mean number of mummies per female were 17.1, 7.7, and 6.7 respectively. Fecundity reduction (Pr) in the group treated with the test item at the rates of 0.025, 0.05 and 0.1 L/ha were 18.9, 63.7 and 68.5%, respectively.
Based on the obtained fecundity results it can be assumed that the ER50 is 0.052 L/ha (95% confidence limits: 0.042 – 0.066) of the test item. The NOERfecundity is 0.025 L/ha of the test item.

Test validity criteria:

The following validity criteria were met during the study:
– after 48 hours mortality of the control group was 0.0% (criterion: a maximum of 13.0%),
– after 24 hours mortality of the group treated with the reference item at the rate of 0.2 g/ha was 77.5% (criterion: from 75 to 100%),
– all wasps survived the 24-hour oviposition period (criterion: only wasps that survive oviposition can be examined for fecundity),
– the mean number of mummies per female in the control group was 21.1 (criterion: a minimum of 5.0 mummies/female),
– all wasps in the control group gave offspring (criterion: a maximum of 2 females giving no offspring).

Table 1. Definitive test results

Study group [application rate]	**Parameter (endpoint)**				
	Mortality after 48 h of exposure		**Reproduction after 12 days after the oviposition**		
Test item [L/ha]	**Mortality [%]**	**LR_{50}** **Test item [L/ha]**	**Mean no. of mummies/ female**	**Fecundity reduction Pr [%]**	**ER_{50}** **Test item [L/ha]**
Control	0.0	-	21.1	-	-
0.025	0.0	**>0.1**	17.1	18.9	**0.052** (0.042 – 0.066)
0.05	0.0		7.7	63.7	
0.1	0.0		6.6	68.5	
$NOER_{mortality}$		**$\geq$ 0.1**	**$NOER_{fecundity}$**		**0.025**

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.

Reference Report	KCP 10.3.2.1-2 A laboratory test for evaluating the effects of Floras 50 SC on the predatory mite, *Typhlodromus pyri* (Sch.). Dybek M., 2023, B-122-22
Guideline(s):	Yes (ESCORT 1, ESCORT 2, IOBC, BART, and EPPO)
Deviations:	YES According to the guideline developed by the IOBC, BART, EPPO Joint Initiative, as a food source only pollen is used. However, in the experiment additional food in the form of the two-spotted spider mite (*T. urticae*) eggs, was used. Another food source prevents the mites from escaping from discs. The obtained deviation had no impact on the quality, integrity and final results of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	NA

Materials and methods

Materials

Test item:

Name:	Florasulam 50 g/L SC
Batch No.:	RFEAR0501
Production Date:	05.04.20222
Expiry Date:	04.2024
A.i. content:	florasulam 50.3 g/L
Density at 20°C	1.04 g/cm^3

Test system:

Species:	the predatory mite, *Typhlodromus pyri* Scheuten
Culturing:	the mites are reared on the bean, *Phaseolus vulgaris* L. (Fabaceae) infested with the two-spotted spider mite, *Tetranychus urticae* Koch.,
Age:	24-hour-old protonymphs
Source:	a laboratory culture at the Institute of Industrial Organic Chemistry, Branch Pszczyna; the culture was obtained from commercial breeder

Experimental conditions:

Temperature:	24.0 – 26.0°C
relative air humidity:	63.0 – 76.5%
Light and photoperiod::	627 lux 16 h light/8 h dark
Feeding:	Pine pollen (*Pinus* sp.) and *T. urticae* eggs

Experimental design: 5 study groups: control, test item groups – 0.025, 005, 0.1 L/ha and reference item at the rate of 4 g/ha.
Number of replicates: 3; number of mites in each replicate: 20

Experimental period: 14 days

Test design and treatment:

The study was performed according to the 'island method'. Each test set consisted of a glass tray filled with water and a glass bench containing 5 test units. Plastic discs (ϕ 45 mm) were floating on the water surface in glass Petri dishes ('island dishes', ϕ 54 mm) with central holes at the bottom (ϕ 6 mm). Water in the test units prevented the mites from escaping.
In the definitive test, 1 control group, 3 treated groups, and 1 reference item group were used. There were 3 replicates of each study group (20 mites/replicate).
All spray fluids were prepared on the application day at volumes that corresponded to 200 L spray emulsion/ha. Discs were prepared, placed on wet tissue paper, and sprayed using the Potter laboratory spray tower.
After calibration, the discs were sprayed with distilled water (the control group), suspension of Floras 50 SC (the treated groups), and a water solution of dimethoate at the rate of 4 g/ha (the reference item group).
When the spray residues dried, the discs were transferred from tissue paper to the test units. Then, 20 mite protonymphs were transferred onto each disc using a fine brush and a stereomicroscope. Pine pollen (*Pinus* sp.) and *T. urticae* eggs were served as food. During the whole experiment, the mites had continuous access to water, and food deficits were supplemented when the need arose. The mites were observed for mortality (dead and escaped individuals) after 7 days of the treatment. Mites were considered dead if they were shriveled or remained motionless after being touched with a fine brush. Escapees were those mites which were lost or drowned.
Reproduction of the surviving mites from the control group and the groups treated with Floras 50 SC at the rates of 0.025, 0.05 and 0.1 L/ha was assessed since mortality of these groups was < 50.0%. After 7 days of exposure, the surviving mites were sexed, and the sex ratio was determined. The numbers of males, females, eggs, and larvae hatched from eggs were recorded on days 8, 11, and 14 of exposure. On each occasion, dead mites, eggs, and larvae were removed, and food was supplemented if necessary. Eggs that were laid until the 7th day were removed from the test units and not counted.

Statistics:

probit analysis using linear max. likelihood regression, Qualitative Trend Analysis by Contrasts (Monotonicity of Rate/Response), Chi2 2x2 Table Test with Bonferroni Correction, Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogenity (with Residuals), Williams Multiple Sequential t-test Procedure

Results:

In the definitive test, mortality of the control group after 7 days of exposure was 3.3%. After 7 days of exposure to Floras 50 SC at the rates of 0.025, 0.05 and 0.1 L/ha, the *T. pyri*, percentages of mortality, corrected according to the Abbott formula, were equal to 3.5, 1.7 and 6.9%, respectively.
On the basis of the obtained results the LR50 value is higher than 0.1 L/ha. The NOERmortality value is higher or equal to 0.1 L/ha.
After 7 days of exposure to dimethoate at the rate of 4.0 g/ha, the Abbott corrected mortality was 100.0%.
The mean reproduction rate (Rr) in the control group was 7.6 eggs/female. The mean Rr after 14 days of exposure to Floras 50 SC at rates of 0.025, 0.05 and 0.1 L/ha were 7.3, 7.9 and 5.8 eggs/female, respectively. The percentages of reproduction reduction (Pr) caused by the test item at the rates of 0.025, 0.05 and 0.1 L/ha were 4.3, -4.4 and 23.6%, respectively.
On the basis of the obtained reproduction results, the ER50 value is higher than 0.1 L/ha and the NOERreproduction value is equal to 0.05 L/ha.

Test validity criteria:

The following validity criteria were met during the study:
- mortality of the control group was 3.3% on day 7 of exposure (criterion: a maximum of 20%),
- corrected mortality of the mites exposed to the reference item at the rate of 4 g/ha was 100.0% on day 7 of exposure (criterion: from 50 to 100%),
- the mean number of eggs per female in the control group was 7.6 (required: ≥ 4 eggs per female).

Table 1. Definitive test results.

Study group [application rate]	**Parameter (endpoint)**					
	Mortality			**Reproduction**		
Test item [L/ha]	**Mortality [%]**	**Corr.[a] [%]**	**LR_{50}** **Test item [L/ha]**	**Mean number of eggs/ female (Rr) [no.]**	**Reproduction reduction Pr [%]**	**ER_{50}** **Test item [L/ha]**
Control	3.3	-	-	7.6	-	-
0.025	6.7	3.5	**>0.1**	7.3	4.3	**>0.1**
0.05	5.0	1.7		7.9	(-4.4)*	
0.1	10.0	6.9		5.8	23.6	
$NOER_{mortality}$			≥0.1	**$NOER_{fecundity}$**		0.05

[a]: mortality corrected according to the Abbott formula

A 2.3.2.2 KCP 10.3.2.2 Extended laboratory testing, aged residue with non-target arthropods

A 2.4 KCP 10.4 Effects on non-target soil meso- and macrofauna

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.

Reference KCP 10.4.1.1
Report Floras 50 SC. Earthworm Reproduction Test (*Eisenia andrei*). Pieczka P., 2022, G-10-22

Guideline(s): Yes (OECD 222)

Deviations: Yes

As it is indicated in the SOP/G/122, the amount of calcium carbonate to

adjust the pH should be in the range from 0.04 to 0.055%. In the study, the needed amount of calcium carbonate was equal to 0.150%, therefore it is a deviation from the SOP/G/122.
According to the OECD Guideline the amount of $CaCO_3$ should be less than 1.0%. The applied quantity of the calcium carbonate in the study was in line with OECD assumptions.
The deviation did not affect the results of the study.

GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	NA

Materials and methods

Materials

Test item:

Name:	Florasulam 50 g/L SC
Batch No.:	RFEAR0501
Production Date:	05.04.20222
Expiry Date:	04.2024
A.i. content:	florasulam 50.3 g/L
Density at 20°C	1.04 g/cm^3

Test system:

Species:	*Eisenia andrei*
Culturing:	In plastic boxes; culture medium: 50:50 mixture of cattle manure and straw with the pH between 7 – 8; room temperature 20±2°C
Mean body weight:	310-414 mg
Source:	synchronized culture cultivated at Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland, Department of Ecotoxicological Studies, Laboratory of Soil Toxicology
Acclimation period:	24 hours

Experimental conditions:

Test medium:	artificial soil: 10% sphagnum peat, 20% kaolin clay, 70% quartz sand
Temperature:	20.1 – 22°C
pH (day 0):	5.61 – 5.75
pH (day 56):	5.57 – 5.82
Moisture day 0:	17.7 – 19.6% (46.4 – 51.3% of the maximum water holding capacity)
Moisture day 56:	18.5 – 20.9% (48.5 – 54.7% of the maximum water holding capacity)
Light and photoperiod:	light intensity: at the beginning of the experiment: 549.4 – 628.3 lux, at the end of the experiment: 563.5 – 612.8 lux; light-dark cycle: 16h : 8h
Feeding:	air-dried finely ground cow manure; 5 g of

food/container on day 1, 7, 14, 21 and 28

Test design: number of replicates: 4 replicates/concentration + 8 replicates/control; number of earthworms: 10 earthworms/replicate

Concentrations: control, 5.6, 10, 18, 32, 56, 100, 180, 320, 560 and 1000 mg/kg dry soil

Experimental period: 56 days

Test design and treatment:

The experiment was conducted with the following concentrations of test item: 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0, 320.0, 560.0 and 1000 mg of the test item/kg of dry weight of the artificial soil. The concentrations were spaced by a factor of 1.8. There were four replicates of each test concentration and eight replicates of untreated control group.
Test item was applied into the artificial soil as an aqueous suspension.
The artificial soil was prepared before the study. It consisted of the following components: 10% sphagnum peat; 20% kaolin clay; 70% air-dried quartz sand. All components were mixed. After that, the maximum water holding capacity (WHC_{max}) and the pH (in 1M/KCl) were determined. The maximum water holding capacity of the prepared soil was 38.18%, whereas the pH was 6.02.
Before the experiment, the earthworms were acclimatised in the artificial soil for 24 hours. Then, they were washed, dried on lignin, weighed (10 earthworms per replicate), and placed on the surface of the artificial soil.
Additional containers with the artificial soil (1 for the control and 1 for each concentration) were prepared to determine the pH and the soil moisture content at the beginning and at the end of the experiment.
During the experiment, the earthworms were fed on airdried finely ground cow manure. One day after the beginning of the experiment, it was spread on the soil surface (5 g food/ container) and moistened. The food prepared in this way was provided once a week during the four-week period (5 g food/container). After 4 weeks (when the adult earthworms were removed from the soil), the juvenile earthworms were fed only once (5 g food/container).
The experiment lasted 8 weeks. After 4 weeks, the earthworms were observed for pathological changes. Also, mortality was evaluated by counting the number of living individuals and weighing them. The impact of the test item on reproduction of the earthworms was assessed after the second four-week period by counting the number of juveniles hatched from the cocoons in the test soil. In order to do it, the test containers were placed in a water bath at temperature of about 60°C. After about 20 minutes, all juveniles visible on the soil surface were collected and counted.

Statistics:

EC10, EC20, EC50, LC50 – probit analysis using linear max. likelihood regression
The NOEC was calculated using the following tests:
NOEC (reproduction):
- Shapiro-Wilk's Test on Normal Distribution,
- Levene's Test on Variance Homogeneity (with residuals),
- Williams Multiple Sequential t-test Procedure,
NOEC (survival):
- Fisher's Exact Binomial Test with Bonferroni Correction
LOEC: a value suggested by the ToxRat statistical computer software.

The EC10, EC20, EC50, LC50 and NOEC values were determined using the ToxRat Professional 2.10 statistical computer.

Test validity criteria:

The results are considered valid because the following criteria were satisfied in the controls:

- each replicate produced from 40 to 87 juveniles (64.5 mean) at the end of the exposure period (criterion: ≥ 30 juveniles by the end of the experiment),
- the coefficient of variation of reproduction was 22.3% (criterion: ≤ 30%),
- adult mortality over the initial 4 weeks of the experiment was 6.3% (criterion: ≤ 10%).

Results:

The concentration of the test item causing 50% mortality of the adult earthworms **(LC50) is above 1000.0 mg/kg dry weight of the artificial soil** (above 48.37 mg florasulam/kg dry weight of the artificial soil).
The concentration of the test item causing a 10% reduction in the number of juveniles produced within the exposure period **(EC10) is equal to 106.0 mg/kg dry weight of the artificial soil** (equal to 5.13 mg of florasulam/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 equal to 356.1 mg/kg dry weight of the artificial soil** (equal to 17.22 mg florasulam/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** (above 48.37 mg of florasulam/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 180.0 mg/kg dry weight of the artificial soil** (equal to 48.37 mg of florasulam/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 equal to 320.0 mg/kg dry weight of the artificial soil** (equal to 15.48 mg of florasulam/kg dry weight of the artificial soil).

Chemical verification of nominal concentration:

In order to verify the nominal soil concentration of the test item, the analytical measurements of the artificial soil treated with the test item at the highest concentration (i.e. 1000.0 mg/kg dry weight of the artificial soil) were performed at the beginning, during (after 4 weeks) and at the end of the test. There was 1 additional container for analysed concentration and the control group. The analytical method validation was performed according to SANTE/2020/12830, Rev. 1.

<u>Florasulam method validation.</u>

The following liquid chromatography parameters were used for analysis of florasulam:

- column - Synergi 4µm Fusion-RP 80Å 150x4.6
- mobile phase - acetonitrile and 0.05% solution of orthophosphoric acid in deionized water (60:40, v/v),
- wave length 260 nm,
- flow rate 0.7 mL/min.,
- injected volume 1 µL,
- oven temperature 35°C.

<u>Linearity</u>: Working solutions of florasulam at the concentrations of 1.0, 2.0, 5.0, 10.0, 20.0, 50.0 and 100.0 µg/mL were injected successively to the

chromatographic column and the chromatograms were recorded. The standard curve (peak area versus quantity of the standard) is linear with coefficient (r2) of 0.9997792. The range of linearity of the analytical graphs are from 1.0 µg/mL to 100.0 µg/mL which correspond to equivalent calibration range of linearity 5 – 500 mg analyte/kg.
Selectivity and specificity: The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control and fortified samples of matrix. Considering the results of the analysis, no signal of detected substances was overlapping with matrix signal of the control samples in the experimental conditions. Therefore, the specificity of the method was demonstrated.
Accuracy: in order to study the accuracy, the solution of the detected substances were added to non-treated control samples and then analysed. For florasulam recovery at concentration equal to LOQ was 82.0% and at concentration of 10xLOQ recovery was equal to 85.3%.
Precision: RSD [%] for florasulam was 13.2 (concentration = LOQ) and 12.8 (concentration = 10xLOQ).
Matrix effect: Assessment of matrix effects was performed by comparing the standard preparing in solvent to standard preparing in control matrix at appropriate concentration and the matrix effect was equal to -0.8%.
The Limit of Detection (LOD): for florasulam in soil was 5.0 mg/kg.
The Limit of Quantification (LOQ): for florasulam analysed in soil was 10.0 mg/kg.

Determined active substance recoveries in samples obtained from the highest concentrations on day 0, 28 and 56 were 94.6, 70.6 and 49.8%, respectively.

Table 1. Test results.

Concentration [mg/kg dry weight of the artificial soil]	Total mortality [%]	Mean body weight change [%]	Number of juveniles – Comparison to the control [%]
Control	6.3	3.5	-
5.6	10.0	6.9	94.2
10.0	10.0	4.7	97.7
18.0	5.0	3.2	100.4
32.0	5.0	5.0	92.6
56.0	2.5	4.7	92.6
100.0	7.5	3.9	94.6
180.0	7.5	1.4	84.5
320.0	12.5	6.7	81.0
560.0	7.5	3.7	77.1
1000.0	2.5	-4.3	66.3

Table 2. Endpoint values determined for test item.

Parameter	Value [mg test item/kg dry weight of artificial soil]	Value [mg of florasulam/kg dry weight of artificial soil]
EC_{10}	106.0 (55.8 – 157.2)	5.13 (2.70 – 7.60)
EC_{20}	356.1 (260.3 – 480.5)	17.22 (12.59 – 23.24)

EC_{50}	> 1000.0	> 48.37
NOEC (reproduction)	180.0	8.71
LOEC (reproduction)	320.0	15.48
LC_{50}	>1000.0	> 48.37
NOEC (survival)	≥1000.0	> 48.37
LOEC (survival)	>1000.0	> 48.37

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

A 2.4.2 KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

A 2.4.2.1 KCP 10.4.2.1 Species level testing

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.

Reference **Report**	KCP 10.5 Floras 50 SC. Soil Microorganisms: Nitrogen Transformation Test. Pieczka P., 2022, G-11-22
Guideline(s):	Yes (OECD 216)
Deviations:	Yes 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 for 18-24 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. These deviations did not affect the results of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	NA

Materials and methods

Materials

Test item:

Name: Florasulam 50 g/L SC

Batch No.:	RFEAR0501
Production Date:	05.04.20222
Expiry Date:	04.2024
A.i. content:	florasulam 50.3 g/L
Density at 20°C	1.04 g/cm^3

Test system:

Soil characteristics: The freshly collected agricultural soil was usedin the experiment. The soil was collected from a place belonging to the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna (49º59', 780N and 18º55', 190E). The site chosen for soil collection was covered with grass. It had not been treated with any plant protection products or organic and inorganic fertilizers for at least 5 years. Soil samples were taken from a depth of 20 cm. They were collected from different parts of the field to obtain a common laboratory sample. All the required physical and chemical parameters of the soil were determined.

Test design: Three portions of soil (3x1500 g) i.e. control group and two treated groups. Each portion was divided into three replicates. The soil was enriched with the organic substrate, i. e. lucerne at dose of 5 g/kg dw soil. Every portion of soil was divided into three replicates (3 x 500g)

Concentrations of test item: control, PEC: 0.104 mg test item/kg dw soil, 5xPEC: 0.520 mg test item/kg dw soil.

Experimental conditions:

Temperature:	18.9 – 22°C
Soil moisture:	46.9– 51.5% of the maximum water holding capacity
Light and photoperiod:	The experiment was performed in darkness.

Experimental period: 28 days

Test design and treatment:

The predicted environmental concentration (PEC) was calculated according to the formula presented below:

$$PEC = \frac{A \times (1 - f_{int})}{100 \times d \times bd}$$ [mg/kg dry weight of soil]

The following values were used: A (application rate)= 104 g/ha; number of application=1; f_{int} (interception factor)=0.25; d (depth)=5 cm; bd (bulk density)=1.5 g/cm^3.

Two concentrations of the test item were used, i.e. PEC: 0.104 mg test item/kg of soil (i.e. 0.005 mg of florasulam/ kg of soil) and 5xPEC: 0.520 mg the test item/kg of soil (i.e. 0.025 mg of florasulam/ kg of soil).

The collected soil was manually cleared of large objects, e.g. stones, parts of plants, etc. and sieved to a particle size equal to 2 mm. A batch of soil was thoroughly mixed and divided into three equal portions. Each portion was

amended with a suitable organic substrate, i.e. lucerne (N – 3.14%, C – 43.36% dry weight). The lucerne-soil ratio was 5 g of lucerne per kg of soil (dry weight).
The test item in form of aqueous suspension was applied at two concentrations equal to PEC and 5 x PEC to two portions of the soil. The control soil was mixed with deionised water alone. At the beginning of the experiment, the soil moisture content was adjusted with deionised water to obtain value about 50% of the maximum water holding capacity.
The control soil and the soil treated with the test item at the concentrations equal to PEC and 5 x PEC were incubated in three replicates.
Incubation lasted 28 days. Soil samples (one sample of each replicate of the treated and the control soils) were collected on days 0, 7, 14 and 28 days of incubation.
Determination of the quantities of nitrate formed during the test was done in a soil extracts obtained with deionised water and nitrate concentration measurement with dedicated electrode.
On the basis of nitrate ions concentration measured in a given volume of soil extract samples, concentration of nitrate ions in soil samples on 0, 7, 14 and 28 day of experiment was calculated. The values were expressed as mg of nitrate/kg dry weight of soil. On the basis of nitrate ions concentration obtained after 0, 7, 14 and 28 days of experiment, the mean nitrates formation rates (mg nitrate/kg dry weight soil/day) for selected time intervals i.e. 0 – 7, 0 – 14 and 0 – 28 days were calculated. The coefficient of variation (CV) for the control group was calculated on the basis of nitrate ions concentration after 0, 7, 14 and 28 days of the experiment. The percentage deviation from the control was calculated using the nitrate formation rate, i.e. 0 – 7, 0 – 14 and 0 – 28 days.

Validity criterion:

The coefficients of variation (CV) in the control group were 7.9, 8.9, 5.9 and 2.5%, after 0, 7, 14 and 28 days of incubation. The validity criterion was met, because the variation between replicate control samples is less than 15%.

Statistics:

In order to determine the statistically significant differences in nitrate ions concentrations after 0, 7, 14, 28 and 42 days of incubation and in nitrate formation rates for selected time intervals between control soil and soil treated with the test item, statistical analysis were performed:
- Shapiro-Wilk's test on Normal Distribution,
- Levene's Test on Variance Homogeneity (with Residuals),
- Williams Multiple Sequential t-test Procedure.

Statistical analysis was performed using ToxRat 2.10. computer software

Results:

The difference in the nitrate formation rate between the control soil and the ones treated with the test item at the concentrations corresponding to the PEC: 0.104 mg test item/kg dry weight of soil (0.005 mg of florasulam/kg dry weight of soil) and 5 x PEC: 0.520 mg test item/kg dry weight of soil (0.025 mg of florasulam/kg dry weight of soil) did not exceed 25% on 28 day of analysis.

Table 1. . Deviations from the control based on nitrate formation rate for selected time intervals [%]

Time interval [d]	PEC	5xPEC
0-7	24.8	14.4
0-14	19.7	14.7
0-28	3.4	-13.8

"-" values of nitrate formation rate higher than the one obtained for the control group

A 2.6 KCP 10.6 Effects on terrestrial non-target higher plants

A 2.6.1 KCP 10.6.1 Summary of screening data

A 2.6.2 KCP 10.6.2 Testing on non-target plants

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment. During the experiment the phytotoxic symptoms of the test item were noticed in cultivation of cabbage, flax, carrot, onion and oats. Additionally, the ER_{50} was determined for visual phytotoxicity effects, basis on the results obtained at the end exposure period. The lowest ER_{50} value is 4.6 mL/ha (cabbage, shoot length**)**

Reference **Report**	KCP 10.6.2-1 Floras 50 SC. Terrestial Plant Test: Seedling Emergence and Seedling Growth Test. Pieczka P., 2022, G-13-22
Guideline(s):	Yes (OECD 208)
Deviations:	Yes <u>Deviation from OECD Guideline No. 208 (2006):</u> According to OECD Guideline No. 208 (2006), the light intensity should be 350 ± 50µE/m2/s. However, these values are recommended for tests conducted in greenhouses. The experiment was conducted in a test room, where only artificial lighting was used. The light intensity was between 119.5 and 186.7 µE/m2/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing. <u>Deviation from the Study Plan:</u> Volume of suspensions subjected to the chemical analysis was equal to 450 mL each and not 100 mL as it was pointed in the study plan. The change of the volume of aqueous suspensions resulted from the requirements of the analytical method. These deviations did not affect the results of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	NA

Materials and methods

Materials

Test item:

Name:	Florasulam 50 g/L SC
Batch No.:	RFEAR0501
Production Date:	05.04.20222
Expiry Date:	04.2024

A.i. content:	florasulam 50.3 g/L
Density at 20°C	1.04 g/cm^3

Test system:

Soil:	Sandy loam
Source:	The soil was taken from a place belonging to the Łukasiewicz Research Network - Institute of Industrial Organic Chemistry, Branch Pszczyna (49˚ 59', 780 N; 18˚55', 190 E). The site chosen for soil collection had not been treated with any plant protection products or organic and inorganic fertilisers. The soil was collected from a depth of 20 cm.
Species:	cabbage (*Brassica oleracea* var. *capitata*), flax (*Linum usitatissimum*), carrot (*Daucus carota*), onion (*Allium cepa*), perennial ryegrass (*Lolium perenne*), oats (*Avena sativa*).

Experimental conditions:

Temperature:	16.7 – 26.8°C
Humidity:	52.8 – 84.8%
Light and photoperiod:	16 h light: 8 h dark; light intensity: 119.5 – 186.7 µE/m2 /s
Carbon dioxide concentration:	338 – 353 ppm
Experimental period:	14 days after the emergence of 50% of the. control seedlings

Test design and treatment:

Six plant species were used. These were cabbage (*Brassica oleracea var. capitata*), flax (*Linum usitatissimum*), carrot *(Daucus carota)*, oats (*Avena sativa*), onion (*Allium cepa*), perennial ryegrass (*Lolium perenne*).
The test species were selected from the list given in the OECD Guideline No. 208. Before the experiment started, seeds had been kept under dry conditions, at ambient temperature, and with no access to light. The seed viability had also been examined.
Seeds of the test plant species were sown in plastic pots (pot's diameter – 15 cm, pot's surface area – about 177 cm2) containing the soil which had been prepared previously. All seeds of each species belonged to the same size class.
There were the following numer of seeds in each pot:
- cabbage: 3 plants/pot – 21 plants/rate (7 pots/rate);
- flax: 5 plants/pot – 20 plants/rate (4 pots/rate);
- carrot: 5 plants/pot – 20 plants/rate (4 pots/rate);
- onion: 5 plants/pot – 20 plants/rate (4 pots/rate);
- perennial ryegrass: 5 plants/pot – 20 plants/rate (4 pots/rate);
- oats: 5 plants/pot – 20 plants/rate (4 pots/rate).

A pot was defined as the replicate. Next, the test item was sprayed onto the soil surface with calibrated spraying equipment. The pots were placed on trays. To prevent bias, random assignment of the test and the control pots is recommended. They were rearranged once a week.

Eight rates of the test item were used in the study: 0.2, 0.4, 1.0, 2.6, 6.4, 16.0, 40.0 and 100.0 mL/ha. In case of each species, there was one untreated control group. A separation factor was 2.5.
The volume of distilled water used to prepare the test item at the highest rate corresponded to 300 L/ha.
The test item was sprayed onto the soil using a suitable spraying chamber. Before the test item was applied, the spraying equipment had been calibrated using deionised water in order to select a suitable nozzle providing the most appropriate way of application under the conditions of specified pressure and working quickness.
During the experiment the air temperature and humidity were constantly monitored. The light intensity was measured at the beginning and at the end of the experiment. The CO_2 concentration in the air was also determined. Appropriate soil nutrients were supplemented once a week to maintain good plant vigour (fertilizer: "Florovit" at the concentration of 2.5 mL/L). Top watering was used (30 or 50 mL/pot).
The experiment was finished 14 days after the emergence of 50% of the control seedlings. During the experiment, the plants were observed for emergence (every day and then every 2 – 3 days) and visual phytotoxicity (7 and 14 days after the emergence of 50% of the control seedlings). At the end of the experiment, the plants were counted, cut down, measured, dried to a constant weight at 60°C and weighed.

Test validity criteria:

On the basis of the obtained results, it was stated that the following validity criteria of the study aimed at evaluating the impact of Floras 50 SC on seedling emergence and seedling growth of terrestrial plants were met:
- the seedling emergence in the control (validity criterion: at least 70%) was as follows:
- 100.0% – cabbage
- 100.0% – flax,
- 95.0% – carrot,
- 85.0% – onion,
- 100.0% – perennial ryegrass,
- 90.0% – oats,
- the mean survival of the emerged control seedlings was 100% for each tested plant species (validity criterion: 90%);
- the control seedlings did not exhibit any visible phytotoxic effects;
- environmental conditions for all plants of the same species were identical.

Statistics:

ER10, ER25 and ER50 (number of plants) – Probit analysis using linear max. likelihood regression, Logit analysis using linear max. likelihood regression
ER10, ER25 and ER50 (shoot length) – 4-param. normal CDF, 3-param. normal CDF,
ER10, ER25 and ER50 (plants dry weight) – 4-param. normal CDF, 3-param. normal CDF, Logit analysis using linear max. likelihood regression
ER50 (visual phytotoxicity effects) – probit analysis using linear max. likelihood regression, 3-param. normal CDF
NOER:
- number of plants: Qualitative Trend Analysis by Contrasts (Monotonicity of Rate/Response), Chi2 2x2 Table Test with Bonferroni Correction, Shapiro-Wilk´s Test on Normal Distribution, Levene´s Test on Variance Homogeneity (with Residuals), Trend analysis by Contrasts (Monotonicity of Rate/Response), Williams Multiple Sequential t-test Procedure, Fisher`s Exact Binomial Test with Bonferroni Correction, Non-parametric Trend

analysis by Contrasts (Monotonicity of Rate/Response), Step-down Jonckheere-Terpstra Test Procedure
- shoot length: Shapiro-Wilk´s Test on Normal Distribution, Levene´s Test on Variance Homogeneity (with Residuals), Trend analysis by Contrasts (Monotonicity of Rate/Response), Williams Multiple Sequential t-test Procedure, Non-parametric Trend analysis by Contrasts (Monotonicity of Rate/Response), Step-down Jonckheere-Terpstra Test Procedure
- shoot dry weight: Shapiro-Wilk´s Test on Normal Distribution, Levene´s Test on Variance Homogeneity (with Residuals), Multiple Sequentially-rejective Welsh-t-test After Bonferroni-Holm, Trend analysis by Contrasts (Monotonicity of Rate/Response), Williams Multiple Sequential t-test Procedure, Non-parametric Trend analysis by Contrasts (Monotonicity of Rate/Response), Step-down Jonckheere-Terpstra Test Procedure

Chemical verification of nominal concentration:

The concentration of florasulam in water was determined with a validated analytical methods.
All test suspensions (application rates) as well as the control sample were subjected to chemical analysis in order to confirm the correct preparation of the tested aqueous suspensions.
The validation of analytical method was performed according to SANTE/2020/12830, Rev. 1.

Florasulam method validation.
The following liquid chromatography parameters were used for analysis of florasulam:
- column - Synergi 4μm Fusion-RP 80Å 150x4.6
- mobile phase - acetonitrile and 0.05% solution of orthophosphoric acid in deionized water (60:40, v/v),
- wave length 260 nm,
- flow rate 0.7 mL/min.,
- injected volume 1 μL,
- oven temperature 35°C.

Linearity: Working solutions of florasulam at the concentrations of 0.05, 0.1, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0 and 100.0 μg/mL were injected successively to the chromatographic column and the chromatograms were recorded. The standard curves (peak area versus quantity of the standard) are linear with coefficient (r^2) of 0.9999427 and 0.9999318. The range of linearity of the analytical graphs are from 0.05 mg/L to 5.0 mg/L and from 1.0 to 20.0 mg/L.
Selectivity and specificity: The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experiments conditions. Therefore, the specificity of the method was demonstrated. Furthermore, any interference are directly apparent and would be observed in the chromatograms of the matrix control sample.
Precision: RSD for fortification level = LOQ was 1.9% and for level = 10xLOQ it was 0.5% (dilution method)
RSD for fortification level = LOQ was 4.3% and for level = 10xLOQ it was 0.4% (SPE method)
Accuracy: mean recovery for fortification level = LOQ it was 104.0% and for level = 10xLOQ it was 104.5% (dilution method.
mean recovery for fortification level = LOQ it was 98.6% and for level =

10xLOQ it was 93.9% (SPE method).
Matrix effect: Assessment of matrix effects was performed by comparing the standard preparing in solvent to standard preparing in control matrix at appropriate concentration and the matrix effect was equal to 1.2% (dilution method) and 4.1% (SPE method).
The Limit of Detection (LOD): for dilution method was 0.01 mg/L and for SPE method was 0.005 mg/L.
The Limit of Quantification (LOQ): for dilution method was 0.2 mg/L and for SPE method was 0.001 mg/L.

At exposure initiation, in the fresh test item sample, the concentration of florasulam was in the range of 95.3 – 112.0% of the nominal concentration. The results confirm that the test item concentration was prepared correctly.

Results:

The ER50 and NOER values (mL/ha) determined on the basis of plants number at the end of the experiment, shoot length and shoot dry weight measurements expressed as mL of the test item/ha for all test species are given below.

	Cabbage *Brassica oleracea* var. *capitata*	**Flax** *Linum usitatissimum*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	> 100.0	>100.0	> 100.0	> 100.0	> 100.0	> 100.0
NOER	> 100.0	16.0	≥ 100.0	16.0	≥ 100.0	≥ 100.0
Shoot length (plants without roots)						
ER_{50}	4.6	46.7	49.4	>100.0	> 100.0	> 100.0
NOER	1.0	6.4	6.4	16.0	40.0	40.0
Plant dry weight (plants without roots)						
ER_{50}	5.6	52.9	20.6	>100.0	>100.0	>100.0
NOER	2.6	6.4	6.4	40.0	40.0	6.4
Plant damages at the end of the exposure (phytotoxic effects)						
ER_{50}	12.5	34.3	25.5	>100.0	>100.0	>100.0

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment. During the experiment the phytotoxic symptoms of the test item were noticed in cultivation of all testes plant species. Additionally, the ER_{50} was determined for visual phytotoxicity effects, basis on the results obtained after 21 days of the experiment. The lowest ER_{50} value is 6.4 mL/ha (carrot, plant dry weight)

Reference KCP 10.6.2-2
Report Floras 50 SC. Terrestial Plant Test: Vegetative Vigour Test. Pieczka P., 2022, G-12-22

Guideline(s):	Yes (OECD 227)
Deviations:	Yes Deviations from OECD Guideline No. 227: According to OECD Guideline No. 227 (2006), the light intensity should be 350 ± 50µE/m2/s. However, these values are recommended for tests conducted in greenhouses. The experiment was conducted in a test room, where only artificial lighting was used. The light intensity was between 96.4 – 277.3 µE/m2/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing. The deviation did not affect the results of the experiment. Deviations from the study plan: Volume of suspensions subjected to the chemical analysis was equal to 450 mL each and not 100 mL as it was pointed in the study plan. The change of the volume of aqueous suspensions resulted from the requirements of the analytical method.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	NA

Materials and methods

Materials

Test item:

Name:	Florasulam 50 g/L SC
Batch No.:	RFEAR0501
Production Date:	05.04.20222
Expiry Date:	04.2024
A.i. content:	florasulam 50.3 g/L
Density at 20°C	1.04 g/cm^3

Test system:

Soil:	Sandy loam
Source:	The soil was taken from a place belonging to the Łukasiewicz Research Network - Institute of Industrial Organic Chemistry, Branch Pszczyna (49˚ 59', 780 N; 18˚55', 190 E). The site chosen for soil collection had not been treated with any plant protection products or organic and inorganic fertilisers. The soil was collected from a depth of 20 cm.
Species:	cabbage (*Brassica oleracea* var. *capitata*), flax (*Linum usitatissimum*), carrot (*Daucus carota*), onion (*Allium cepa*), perennial ryegrass (*Lolium perenne*), oats (*Avena sativa*).

Experimental conditions:

Temperature:	18.7 – 24.8°C
Humidity:	51.2 – 87.7%
Light and photoperiod:	16 h light: 8 h dark; light intensity: 96.4 – 277.3 µE/m2 /s
Carbon dioxide concentration:	333 – 353 ppm
Experimental period:	21 days

Test design and treatment:

Six plant species were used. These were cabbage (*Brassica oleracea var. capitata*), flax (*Linum usitatissimum*), carrot *(Daucus carota)*, oats (*Avena sativa*), onion (*Allium cepa*), perennial ryegrass (*Lolium perenne*). Before the experiment started, seeds had been kept under dry conditions, at ambient temperature, and with no access to light. The seed viability had also been examined.

In the experiment the agricultural soil was used. The soil texture showed it was a sandy loam.

Seeds of the test plant species were sown in plastic pots (pot's diameter – 15 cm, pot's surface area – about 177 cm^2) containing the test soil.

There were 10 seeds in each pot for flax, carrot, onion, perennial ryegrass, oats and 6 seeds in each pot for cabbage. The replicate is defined as a pot. The plants were grown to the 2- to 4- true leaf stage. Then, some of them were removed.

As a result, the number of plants per pot as well as the total number of plants per application rate was:

- cabbage: 3 plants/pot – 21 plants/application rate (7 pots/application rate);
- flax: 5 plants/pot – 20 plants/ application rate (4 pots/ application rate);
- carrot: 5 plants/pot – 20 plants/ application rate (4 pots/ application rate);
- onion: 5 plants/pot – 20 plants/ application rate (4 pots/ application rate);
- perennial ryegrass: 5 plants/pot – 20 plants/ application rate (4 pots/ application rate), - oats: 5 plants/pot – 20 plants/ application rate (4 pots/ application rate).

Eight rates of the test item were used in the study: 0.2, 0.4, 1.0, 2.6, 6.4, 16.0, 40.0 and 100.0 mL/ha.

The volume of distilled water used to prepare the test item at the highest rate corresponded to 300 L/ha.

The test item was sprayed onto the soil using a suitable spraying chamber. Before the test item was applied, the spraying equipment had been calibrated using deionised water in order to select a suitable nozzle providing the most appropriate way of application under the conditions of specified pressure and working quickness. The experiment was conducted in a special room where suitable environmental conditions for each species were provided. The air temperature and humidity were constantly measured. The light intensity was controlled at the beginning and at the end of the experiment. The CO2 concentration in the air was also determined. Appropriate soil nutrients were supplemented to maintain good plant vigour (fertilizer: "Florovit" at the concentration of 2.5 mL/L). Top watering was used (30 mL/pot).

The exposure period finished 21 days after the application of the test item. The plants were observed for visual phytotoxicity and mortality 7, 14 and 21 days after the spraying. At the end of the exposure period, the number of plants was determined. Next, they were cut down, measured, dried to a constant weight at 60°C, and weighed.

Test validity criteria:

On the basis of the obtained results, it was stated that the following validity criteria of the study aimed at evaluating the impact of Floras 50 SC on vegetative vigour of terrestrial plants were met:

- the seedling emergence of plants (validity criterion: at least 70%) was as follows:
88.1 – 97.6 – cabbage,
87.5 – 100.0 – flax,
90.0 – 97.5 – carrot,
87.5 – 97.5 – onion,
87.5 – 95.0 – perennial ryegrass,
92.5 – 97.5 – oats,
- the mean plant survival of the control was 100% for all tested species (validity criterion: at least 90%),
- the control plants did not exhibit any visible phytotoxic symptoms,
- environmental conditions for all plants belonging to the same species were identical.

Statistics:

In order to determine ER10, ER25, ER50 the following test were used:
- plant number: Probit analysis using linear max. likelihood regression
- shoot length: Probit analysis using linear max. likelihood regression, 3-param. Normal CDF
- shoot dry weight: Probit analysis using linear max. likelihood regression, 3-param. Normal CDF, 3-param. logistic CDF, Logit analysis using linear max. likelihood regression

ER50 (plant damages) - Probit analysis using linear max. likelihood regression;
In order to determine the NOER values, the following tests were used:
- for the plant number - Qualitative Trend Analysis by Contrasts (Monotonicity of Rate/Response), Tarone´s Test Procedure, Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm, Fisher`s Exact Binomial Test with Bonferroni Correction;
- for the shoot length: Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Multiple Sequentially-rejective Welsh-t-test After Bonferroni-Holm, Trend analysis by Contrasts (Monotonicity of Rate/Response), Williams Multiple Sequential t-test Procedure;
- for the plant shoot dry weight: Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Dunnett`s Multiple t-test Procedure, Trend analysis by Contrasts (Monotonicity of Rate/Response), Williams Multiple Sequential t-test Procedure.
The ER10, ER25, ER50 and NOER were determined using the ToxRat Professional 3.3.0 computer software.

Chemical verification of nominal concentration:

The concentration of florasulam in water was determined with a validated analytical methods.
All test suspensions (application rates) as well as the control sample were subjected to chemical analysis in order to confirm the correct preparation of the tested aqueous suspensions.
The validation of analytical method was performed according to SANTE/2020/12830, Rev. 1.

<u>Florasulam method validation.</u>
The following liquid chromatography parameters were used for analysis of florasulam:
- column - Synergi 4µm Fusion-RP 80Å 150x4.6
- mobile phase - acetonitrile and 0.05% solution of orthophosphoric acid

in deionized water (60:40, v/v),
- wave length 260 nm,
- flow rate 0.7 mL/min.,
- injected volume 1 µL,
- oven temperature 35°C.

Linearity: Working solutions of florasulam at the concentrations of 0.05, 0.1, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0 and 100.0 µg/mL were injected successively to the chromatographic column and the chromatograms were recorded. The standard curves (peak area versus quantity of the standard) are linear with coefficient (r^2) of 0.9999427 and 0.9999318. The range of linearity of the analytical graphs are from 0.05 mg/L to 5.0 mg/L and from 1.0 to 20.0 mg/L.

Selectivity and specificity: The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experiments conditions. Therefore, the specificity of the method was demonstrated. Furthermore, any interference are directly apparent and would be observed in the chromatograms of the matrix control sample.

Precision: RSD for fortification level = LOQ was 1.9% and for level = 10xLOQ it was 0.5% (dilution method)

RSD for fortification level = LOQ was 4.3% and for level = 10xLOQ it was 0.4% (SPE method)

Accuracy: mean recovery for fortification level = LOQ it was 104.0% and for level = 10xLOQ it was 104.5% (dilution method.

mean recovery for fortification level = LOQ it was 98.6% and for level = 10xLOQ it was 93.9% (SPE method).

Matrix effect: Assessment of matrix effects was performed by comparing the standard preparing in solvent to standard preparing in control matrix at appropriate concentration and the matrix effect was equal to 1.2% (dilution method) and 4.1% (SPE method).

The Limit of Detection (LOD): for dilution method was 0.01 mg/L and for SPE method was 0.005 mg/L.

The Limit of Quantification (LOQ): for dilution method was 0.2 mg/L and for SPE method was 0.001 mg/L.

At exposure initiation, in the fresh test item sample, the concentration of florasulam was in the range of 95.3 – 112.0% of the nominal concentration. The results confirm that the test item concentration was prepared correctly.

Results:

The ER_{50} and NOER values (mL/ha) determined on the basis of plants number at the end of the experiment, shoot length, shoot dry weight measurements and plant damage at the end of exposure expressed as mL of the test item/ha for all test species are given below.

	Cabbage *Brassica oleracea* var. *capitata*	**Flax** *Linum usitatissimum*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	>100.0	>100.0	>100.0	>100.0	>100.0	>100.0

NOER	≥100.0	≥100.0	≥100.0	≥100.0	≥100.0	≥100.0
Shoot length (plants without roots)						
ER_{50}	>100.0	9.6	17.3	64.2	>100.0	>100.0
NOER	2.6	2.6	1.0	2.6	16.0	6.4
Plant dry weight (plants without roots)						
ER_{50}	29.5	14.8	6.4	33.4	>100.0	>100.0
NOER	2.6	2.6	0.4	0.4	40.0	≥100.0
Plant damage at the end of exposure						
ER_{50}	22.5	11.7	8.7	>100.0	>100.0	>100.0

A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants

A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

A 2.8 KCP 10.8 Monitoring data