FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: **MEZOFLOR 103 SC**

Product names: **MEZOFLOR 103 SC, FLOCORN 103 SC**

Chemical active substances:

Mesotrione, 100 g/L
Florasulam, 3 g/L

Central

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

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

Submission date: 07/2023

MS Finalisation date: 10/2024, 12/2024, 03/2025

Version history

When	What
07/2023	Initial dRR
12/2023	Additional information in point 9.5.3.2
04/2024	Additional information in points: 9.5.3.2; 9.10.3; A 2.1.2.2; A 2.4.2; A 2.6, and Appendix 1
10/2024	zRMS assessment of dRR
12/2024	The final Registration Report
01/2025	Additional information in points 9.10.4; A 2.4.1 and A 2.4.2 and Appendix 1
03/2025	zRMS assessment of dRR

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9 Ecotoxicology (KCP 10)

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use-No. [e]	**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 [f]	**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 arthropods	Soil organisms	Non-target plants

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use-No. (e)	**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: development stages of the pest or pest group)	**Application**				**Application rate**			**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha (f)	**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 arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	PL	Maize	F	**susceptible weeds:** *Capsella bursa-pastoris* *Galinsoga parviflora* *Thlaspi arense* *Matricaria chamomilla* *Matricaria martima* *Anthemis arvensis* *Viola arvensis* *Centaurea cyanus* *Stellaria media* *Geranium pusillum* *Polygonum convolvulus* *Brassica napus* *Persicaria maculosa"* **moderate susceptible weeds:** *Chenopodium album* *Echinochloa crus-galli* *Galium aparine* *Solanum nigrum* *Capsella bursa-pastoris* *Galinsoga parviflora* *Thlaspi arense*	Foliar spraying	BBCH 12-18	1	N/A	1.0 L/ha	Mesotrione 100 g as/ha Florasulam 3.00 g as/ha	200-300 L/ha	Not relevant		A	C	R	A	A	C	R

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use-No. (e)	**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 (f)	**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 arthropods	Soil organisms	Non-target plants
				Matricaria chamomilla *Matricaria martima* *Anthemis arvensis* *Viola arvensis* *Centaurea cyanus* *Stellaria media* *Geranium pusillum* *Polygonum convolvulus* *Brassica napus* *Persicaria maculosa* *Amaranthus retroflexus* *Anchusa arvensis* **medium resistance:** *Echinochloa crus-galli*																	

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use-No. (e)	**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: development stages of the pest or pest group)	**Application**				**Application rate**			**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha (f)	**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 arthropods	Soil organisms	Non-target plants
				susceptible weeds: *Chenopodium album* *Galium aparine* *Solanum nigrum* *Capsella bursa-pastoris* *Galinsoga parviflora* *Thlaspi arense* *Matricaria chamomilla* *Matricaria martima* *Anthemis arvensis* *Viola arvensis* *Stellaria media* *Geranium pusillum* *Polygonum convolvulus* *Brassica napus* *Persicaria maculosa* *Amaranthus retroflexus* *Anchusa arvensis* **moderate susceptible weeds:** *Echinochloa crus-galli* *Galium aparine* *Solanum nigrum* *Capsella bursa-pastoris*	Foliar spraying	BBCH 12-18	1	-	1.25 L/ha	Mesotrione 125 g as/ha Florasulam 3.75 g as/ha	200-300 L/ha	Not relevant		A	C	R	A	A	C	R

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use-No. (e)	**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 (f)	**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 arthropods	Soil organisms	Non-target plants
				Galinsoga parviflora *Thlaspi arense* *Matricaria chamomilla* *Matricaria martima* *Anthemis arvensis* *Viola arvensis* *Centaurea cyanus* *Stellaria media* *Geranium pusillum* *Polygonum convolvulus* *Brassica napus*															

Remarks columns:

1 Numeration necessary to allow references

2 Use official codes/nomenclatures of EU Member States

3 For crops, the EU and Codex classifications (both) should be used; when relevant, the use situation should be described (e.g. fumigation of a structure)

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

Explanation for column 15 – 21 “Conclusion”

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

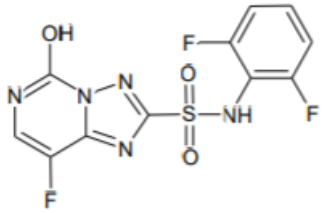
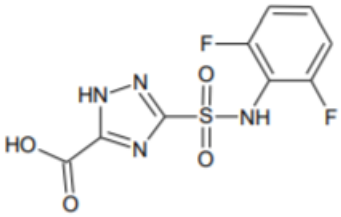
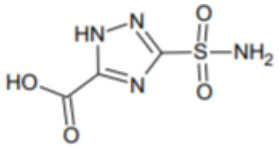
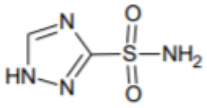
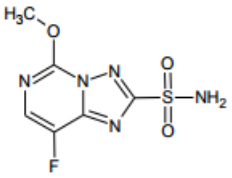
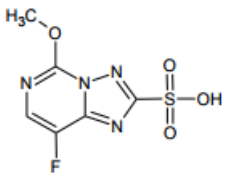
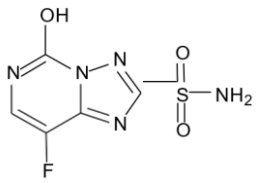
zRMS comment: The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Use is accepted for PL. The refinement risk assessment for mammals should be considered by MSs level. The studies for formulation of **MEZOFLOR 103 SC** for earthworms and *Folsomia candida* and *Hypoaspis aculeifer* with risk assessment was accepted by zRMS only provisionally. The Applicant should complete the calculation the toxicity endpoints based on geometric mean measured concentration with risk assessment. The risk assessment for soil organisms should be considered by MSs level.

9.2 Overall conclusions

9.2.1 zRMS comments

9.2.2 **Information relating to mesotrione metabolites are in line with EU agreed endpoints as reported in EFSA Journal 2016;14(3):4419 and have been considered in the exposure assessment presented in this report.**

Table 9.3-4 Metabolites of florasulam

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
5-OH Florasulam		354.26	Soil: 71.6 % Surface water/sediment: 99 % groundwater: 85%	YES
DFP-ASTCA		304.20	Soil: 17.8 % Surface water/sediment: 8.9 % groundwater :85%	YES
ASTCA		192.13	Soil: 40.0 % Surface water/sediment: 53.8 % groundwater :67%	YES
TSA		248.17	Soil: 15.9 % groundwater :85%	YES
ASTP		247.20	Surface water: 58%	YES
TPSA		248.17	Surface water: 21%	YES
5-OH-ASTP		233.18	Surface water: 29%	YES

zRMS comments:

Information relating to florasulam metabolites are in line with EU agreed endpoints as reported in EFSA Conclusion on the peer review of the pesticide risk assessment of the active substance florasulam EFSA Journal 2015; 13(1):3984 and have been considered in the exposure assessment presented in this report.

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

An estimation of risk indicate low risk for birds and mammals of each range of assessed issues. Calculations conducted due to the influence of MEZOFLOR 103 SC due to the acute and long-term toxicity and reproductive did not indicate any hazardous properties and danger for birds and mammals. There were also no negative effects regarding to drinking water exposure and effect of secondary poisoning. There is no influence to evaluated organism regarding to dangerous to food poisoning.

9.2.4 Effects on aquatic organisms (KCP 10.2)

Taking into consideration risk mitigation calculations for MEZOFLOR 103 SC following risk mitigation measures should be applied:

- **4 m buffer non-spray zone with 4 meter vegetated filter strip.**

Using the above-mentioned precautions, formulation MEZOFLOR 103 SC can be used and will not have a negative impact on aquatic species.

9.2.5 Taking into consideration risk mitigation calculations for MEZOFLOR 103 SC use in maize, following risk mitigation measures should be applied:

- **4 m buffer non-spray zone with 4 meter vegetated filter strip.**

zRMS comment: The evaluation of the risk for aquatic 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" (EFSA Journal 2013;11(7):3290).

Conclusion from the risk assessment based on the active substance mesotrione:
The PECsw/sed calculations for mesotrione have been approved for applications proposed in GAP. PECsw and PECsed calculations were carried out according to the FOCUS recommendations. The Applicant has been used FOCUS models: STEPS1-2 and Step 3. PECsw/sed were also carried out at Step 4 according to FOCUS L&M Guidance. The Applicant used the geometric mean value. In opinion of the zRMS this is acceptable, as being in line with current requirements concerning selection of Kfoc to be used for modelling purposes.

PECsw/sed are acceptable to describe predicted environmental concentrations of mesotrione and its metabolites in surface water and sediment and are appropriate to be used for the subsequent risk assessment for aquatic and sediment organisms.

MS should identify risk reduction measures at the national level.

Taking into consideration risk mitigation calculations for **MEZOFLOR 103 SC** use in maize, following risk mitigation measures should be applied:
- **4 m buffer non-spray zone with 4 meter vegetated filter strip.**

Conclusion from the risk assessment based on the active substance florasulam:

The calculations of PECsw/sed for florasulam and its metabolites submitted by Applicant have been

accept-ed. All input parameters for active substances and its metabolites are in line EFSA conclusion 2015;13(1): 3984. PECsw values were calculated in Step 1 and 2 for active substances and their metabolites for proposed uses in GAP. No further calculation was needed.

The combined risk assessment for mixture **MEZOFLOR 103 SC** was accepted by zRMS.

Conclusion from the risk assessment based on the formulated product:
Taking into consideration risk mitigation calculations for **MEZOFLOR 103 SC** use in maize, following risk mitigation measures should be applied:
- **4 m buffer non-spray zone with 4 meter vegetated filter strip.**

Final risk mitigation measures should be considered at MSs level.

9.2.6 Effects on bees (KCP 10.3.1)

Calculation conducted for MEZOFLOR 103 SC regarding to the oral and contact toxicity also confirm no risk for bees and bumblebees due to the use the formulation: HQ values are lower than the trigger value of 50.
Therefore a low risk to bees and bumblebees is expected from the application of MEZOFLOR 103 SC following application according to the proposed GAP.

9.2.7 Effects on arthropods other than bees (KCP 10.3.2)

A. rhopalosiphi and *T. pyri* are organisms used to designation the initial assessment. $HQ_{in\text{-}field}$ and $HQ_{off\text{-}field}$ values for *A. rhopalosiphi* and *T.pyri* are below the ESCORT 2 trigger of 2. The calculations present an acceptable risk to non-target arthropods, after spray application of MEZOFLOR 103 SC.

9.2.8 Effects on non-target soil meso- and macrofauna (KCP 10.4), zRMS comment:

9.2.9

9.2.10 The toxicity endpoints were based on nominal concentration. At the end on the studies concentration of substances active fell under 80% of nominal. The TWA or geometric mean measured concentration should be calculated over the duration of the test and used if the concentration falls under 80% of nominal. The Applicant should complete the calculation the toxicity endpoints based on geometric mean measured concentration with risk assessment. The new data with toxicity endpoints recalculation and justification that in this case the best solution is to use endpoints based on nominal concentration in risk assessment for soil organisms was provided by Applicant. The recalculation should be treated as additional source information. However, the justification that in this case the best solution is to use endpoints based on nominal concentration in risk assessment for soil organisms was accepted by zRMS. In this case, when the concentration values fall below the limit of quantification of the analytical method, it is not possible to determine reliable toxicity endpoints based on geometrically

measured concentrations. In addition, it can be noted that a decrease in the content of the active substance in the experimental system, caused by the decomposition of this substance (documented low DT_{50}, DT_{90} in soil, confirmed by analytical tests) usually causes a decrease in the toxicity caused by this substance. In the case of unstable substances, precise specification of reliable endpoints based on the measured geometric mean is not possible, especially when the concentration values fall below the limit of quantification of the analytical method. In order to reliably measure the decrease in such rapidly dispersing compounds, more time points of sampling at much shorter intervals (even within the first day) and a much higher number of repetitions may be necessary. Detailed technical guidance on the design of ecotoxicological studies is needed before analytical measurements are routinely included in ecotoxicological studies involving soil invertebrates. Therefore, the revision and validation of the technical guidelines (OECD 222, 232, 226; 2016 a, b, c) is necessary to achieve greater clarity. The study of soil organisms in ecotoxicology differs significantly from, for example, routine aquatic testing procedures, therefore the implementation of such appropriate analysis in the relevant tests cannot be easily adapted or transferred. The risk assessment for soil organisms, in this case, based on toxicity endpoints based on nominal concentration was accepted by zRMS. This should be considered at the level of Member States.

9.2.11 Effects on soil microbial activity (KCP 10.5)

The calculated chronic TER for active substances, their metabolites and formulation are above the trigger value of 5 indicating acceptable chronic risk to earthworms from the proposed uses of MEZOFLOR 103 SC. On the basis of results it was assessed that MEZOFLOR103 SC in considered applications does not pose unacceptable risk to soil microorganisms.

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

Taking into consideration risk mitigation calculations for MEZOFLOR 103 SC – use in maize, following risk mitigation measures should be applied:

- **5 m buffer zone and 75 % drift reduction nozzle,**
- **10 m buffer zone and 50 % drift reduction nozzle,**
- **15 m buffer zone**

Using the above-mentioned precautions, formulation MEZOFLOR 103 SC can be used and will not have a negative impact on non-target terrestrial plants.

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

Not relevant.

9.3 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).

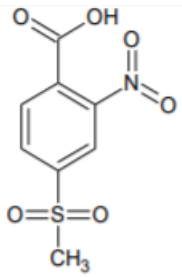
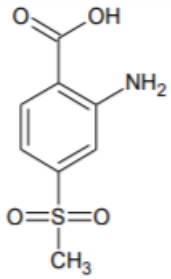
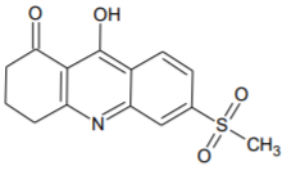
Table 9.3-1: Critical use pattern of MEZOFLOR 103 SC grouped according to dose

Grouping according to dose		
Group	**Intended uses**	**Maximal dose**
1	Maize	1.25 L/ha

9.3.1 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 MEZOFLOR 103 SC is indicated in the table.

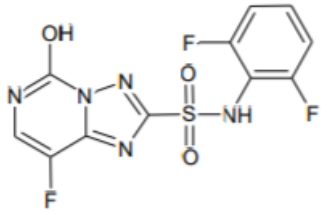
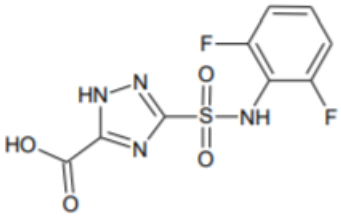
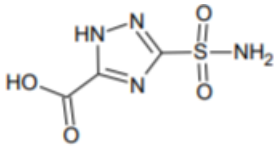
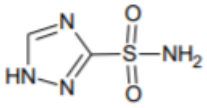
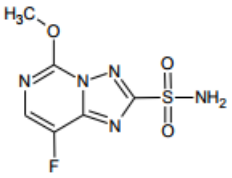
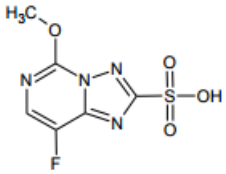
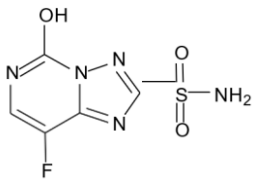
Table 9.3-2 Metabolites of mesotrione

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
MNBA		245	Soil: 57.2 % Surface water/sediment: 7.9 % Groundwater : 100%	YES
AMBA		215	Soil: 9.7 % Surface water/sediment: 24.6 % Groundwater : 25%	YES
SYN 546974		291	Surface water/sediment: 33 %	YES

zRMS comments

Information relating to mesotrione metabolites are in line with EU agreed endpoints as reported in EFSA Journal 2016;14(3):4419 and have been considered in the exposure assessment presented in this report.

Table 9.3-4 Metabolites of florasulam

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
5-OH Florasulam		354.26	Soil: 71.6 % Surface water/sediment: 99 % groundwater: 85%	YES
DFP-ASTCA		304.20	Soil: 17.8 % Surface water/sediment: 8.9 % groundwater :85%	YES
ASTCA		192.13	Soil: 40.0 % Surface water/sediment: 53.8 % groundwater :67%	YES
TSA		248.17	Soil: 15.9 % groundwater :85%	YES
ASTP		247.20	Surface water: 58%	YES
TPSA		248.17	Surface water: 21%	YES
5-OH-ASTP		233.18	Surface water: 29%	YES

zRMS comments:

Information relating to florasulam metabolites are in line with EU agreed endpoints as reported in EFSA Conclusion on the peer review of the pesticide risk assessment of the active substance florasulam EFSA Journal 2015; 13(1):3984 and have been considered in the exposure assessment presented in this report.

9.4 Effects on birds (KCP 10.1.1)

9.4.1 Toxicity data

Avian toxicity studies have been carried out with mesotrione and florasulam and their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents).
However, the provision of further data on the MEZOFLOR 103 SC is not considered essential, because of low risk of mesotrione and florasulam to birds provided in previous studies.

Table 9.4-1: Endpoints and effect values relevant for the risk assessment for birds

Species	Substance	Exposure System	Results	Reference
Bobwhite quail (*Colinus virginianus*)	Mesotrione	Acute	LD50 = 3776 mg/kg bw	EFSA Journal 2016;14(3):4419
Mallard duck (*Anas platyrhynchos*)	Mesotrione	20 weeks (Sub-chronic and reproductive)	NOEL = 20.6 mg/kg bw/d	EFSA Journal 2016;14(3):4419
Japanese quiel	Florasulam	Acute	LD50 = 1046 mg/kg bw	EFSA Journal 2015; 13(1):3984
Mallard duck (*Anas platyrhynchos*)	Florasulam	Long-term	NOEL = 104.6 mg/kg bw/d (10% of LD_{50}) NOEC = 1500 mg/kg feed	EFSA Journal 2015; 13(1):3984

zRMS comments: zRMS confirms that the reported toxicity data in table 9.4-1 are in accordance with the EU agreed endpoints and will be used for risk assessment.

Avian toxicity data for mesotrione in Table 9.4-1 are in line with EU agreed endpoints reported in EFSA Conclusion 2016;14(3):4419.

Avian toxicity data for florasulam in Table 9.4-1 are in line with EU agreed endpoints reported in EFSA Conclusion 2015; 13(1):3984.

9.4.2 Justification for new endpoints

Endpoints for mesotrione presented in EFSA Journal 2016;14(3):4419 and endpoints for florasulam presented in EFSA Journal 2015; 13(1):3984.

9.4.3 Risk assessment for spray applications

The risk assessment for birds was calculated in two ways:

A) according to 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),
B) according to the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2023; 21(2): 7790 (using EFSA birds and mammals calculator),

however, the results obtained in both calculation are similar.

MESOTRIONE

The results of the screening and first-tier risk assessments (acute/reproductive), are summarised in the following tables, for Mesotrione:

A) according to EFSA (EFSA Journal 2009; 7(12): 1438:

Table 9.4-2: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of MEZOFLOR 103 SC in maize

Intended use	**Maize**				
Active substance/product	Mezotroine				
Application rate (g/ha)	1 × 125				
Acute toxicity (mg/kg bw)	3776				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize	Small omnivorous bird	158.8	1	19.85	190
Reprod. toxicity (mg/kg bw/d)	20				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize	Small omnivorous bird	64.8	0.53	4.29	**4.66**

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.

For mesotrione the resulting TER_A values is over the trigger value of 10 for acute toxicity what indicate a low risk for birds. TER_{lt} is lower than 5 for long term toxicity and need further justification in first tier assessment as defined in the EFSA Journal 2009; 7(12):1438.

Table 9.4-3: First tier risk assessment of the long-term/reproductive risk for birds due to the use of MEZOFLOR 103 SC in maize

Intended use	**Maize**				
Active substance/product	Mezotroine				
Application rate (g/ha)	1 × 125				
Reprod. toxicity (mg/kg bw/d)	20				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize BBCH 10-29	Medium granivorous bird “gamebird”	3.0	0.53	0.20	100
Maize BBCH 10-19	Small insectivorous/ worm feeding species “thrush”	5.7	0.53	0.38	53
Maize BBCH 10-29	Small omnivorous bird “lark”	10.9	0.53	0.72	28
Maize BBCH 10-29	medium herbivorous/ granivorous bird “pigeon”	22.7	0.53	1.5	13

Maize BBCH 10-19	Small insectivorous bird "wagtail"	11.3	0.53	0.75	27

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.

For mesotrione the resulting TER_{lt} values is over the trigger value of 5 for long term toxicity what indicate a low risk for birds. No further justification is needed.

zRMS comments: Agreed.

First tier step in the risk assessment

The first step risk assessment for active substance mezotrione performed in maize is agreed by zRMS.

TER_A and TER_{LT} values for the exposure to the active substance when **MEZOFLOR 103 SC** is applied in maize are above the trigger of 10 and 5 for acute and long-term exposure, indicating acceptable risk for birds.

B) According to the EFSA (EFSA Journal 2023; 21(2): 7790 (using EFSA birds and mammals calculator)

Table 9.4-4: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of MEZOFLOR 103 SC in maize

Active substance: MEZOTRIONE Risk: acute							
Crop type	**Acute endpoint (birds) [mg a.s./kg bw]**	**App. rate [kg a.s./ha]**	**TWAf x MAF (moving window)**	**MAF (Acute)**	**DD [mg a.s./kg bw] (acute)**	**TER (acute)**	**Trigger value (acute)**
Crop group 1	3776	0.125	0.53	1	24.3	155.16	10

Active substance: MEZOTRIONE Risk: reproductive						
Crop type	**Reproductive endpoint (birds) [mg a.s./kg bw per day]**	**App.ication rate [kg a.s./ha]**	**TWAf x MAF (moving window)**	**DDD dose [mg a.s./kg bw per day] (reproductive)**	**TER (reproductive)**	**Trigger value (reproductive)**
Crop group 1	20.6	0.125	0.53	3.67	5.62	5

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

For mesotrione the resulting TER_A values is over the trigger value of 10 for acute toxicity what indicate a low risk for birds. TER_{lt} is slightly over than 5 for long term toxicity that's why further justification in first tier assessment was defined.

Table 9.4-5: First tier risk assessment of the long-term/reproductive risk for birds due to the use of MEZOFLOR 103 SC in maize

Active substance: MEZOTRIONE							
Crop: Maize and millet crop							
BBCH: 10-19							
Application rate [kg a.s./ha]: 0.125							
Feeding guild	**Acute endpoint (birds) [mg a.s./kg bw]**	**Reproductive endpoint (birds) [mg a.s./kg bw per day]**	**Daily energy expenditure [kJ/day]**	**Food intake rate [g fresh weight/day]**	**Daily dietary dose [mg a.s./kg bw per day] (reproductive)**	**TER (reproductive)**	**Trigger value (reproductive)**
Medium omnivorous	3776	20.6	374	214	1.08	19.1	5
Small insectivorous	3776	20.6	67.1	12.5	0.289	71.2	5
Small omnivorous	3776	20.6	99.9	13.5	0.702	29.3	5
Large herbivorous	3776	20.6	701	412	0.81	25.4	5
Granivorous	3776	20.6	54.4	3.48	0.632	32.6	5

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

For mesotrione the resulting TER_{lt} values is over the trigger value of 5 for long term toxicity what indicate a low risk for birds. No further justification is needed.

zRMS comments: Recently EFSA issued a new guidance document for birds and mammals: Birds & mammals: Risk assessment for Birds and Mammals approved: 15 December 2022 doi: 10.2903/j.efsa.2023.7790. It was discussed at the last meeting of the central zone in the field of ecotoxicology (Warsaw, 12.2023), where it was agreed that:

- During the transitional period (new GD noted but not yet into force) the Applicants will have to make decision which GD to follow (2009 or 2023), but without mixing the both documents (at least until the official implementation schedule is available).;
- The exposure may be refined using EFSA (2023) but only with regard to parameters indicated in EFSA (2009) as provisional or based on the limited data;
- In case some parameters are taken from EFSA (2023) to refine the exposure, this has to be done for both, birds and mammals (even if refinement was necessary for only one group) and for all crops listed in the GAP. This requirement may be waived when it is demonstrated that worst case situation was covered in the risk assessment based entirely on EFSA (2009) indications.

In our opinion due to new GD noted but not yet into force the risk assessment for birds based on EFSA 2013 should be treated as additional source of information. It should be considered by MSs level.

FLORASULAM

For screening step for florasulam the resulting TER_A values is over the trigger value of 10 for acute toxicity and TER_{lt} is over than 5 for long term toxicity, what indicate a low risk for birds. No further justification is needed as defined in the EFSA Journal 2009; 7(12):1438 and EFSA (EFSA Journal 2023; 21(2):

7790. The results are summarized in tables below:

A) According to the EFSA (EFSA Journal 2009; 7(12): 1438:

Table 9.4-6: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of MEZOFLOR 103 SC in maize

Intended use	**Maize**				
Active substance/product	Florasulam				
Application rate (g/ha)	1 × 3.75				
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**
Maize	Small omnivorous bird	158.8	1	0.6	1743
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}**
Maize	Small omnivorous bird	64.8	0.53	0.13	804

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

zRMS comments: Agreed.

First tier step in the risk assessment

The first step risk assessment for active substance florasulam performed in maize is agreed by zRMS.

TER_A and TER_{LT} values for the exposure to the floramsulam when **MEZOFLOR 103 SC** is applied in maize are above the trigger of 10 and 5 for acute and long-term exposure, indicating acceptable risk for birds.

B) According to the EFSA (EFSA Journal 2023; 21(2): 7790 (using EFSA birds and mammals calculator)

Table 9.4-7: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of MEZOFLOR 103 SC in maize

FLORASULAME Risk: acute							
Crop type	**Acute endpoint (birds) [mg a.s./kg bw]**	**App. rate [kg a.s./ha]**	**TWAf x MAF (moving window)**	**MAF (Acute)**	**DD [mg a.s./kg bw] (acute)**	**TER (acute)**	**Trigger value (acute)**
Crop group 1	1046	0.00375	0.53	1	0.73	1432.75	10

FLORASULAME Risk: reproductive						
Crop type	**Reproductive endpoint (birds) [mg a.s./kg bw per day]**	**App. rate [kg a.s./ha]**	**TWAf x MAF (moving window)**	**DDD[mg a.s./kg bw per day] (reproductive)**	**TER (reproductive)**	**Trigger value (reproductive)**
Crop group 1	104.6	0.00375	0.53	0.11	951	5

zRMS comments: Recently EFSA issued a new guidance document for birds and mammals: Birds & mammals: Risk assessment for Birds and Mammals approved: 15 December 2022 doi: 10.2903/j.efsa.2023.7790. Due to new GD noted but not yet into force the risk assessment for birds based on EFSA 2013 should be treated as additional source of information. It should be considered by MSs level.

Risk Assessment for combined exposure for birds:

A) According to the EFSA (EFSA Journal 2009; 7(12): 1438:

According to the EFSA Journal (2009)[1], the simultaneous exposure of animals to residues of two or more potential toxic substances should be considered in the risk assessment. Therefore, for the assessment of acute effects, a surrogate LD_{50} for the mixture of active substances with known toxicity was derived assuming dose additivity of toxicity. For the calculation, the following equation was used:

$$LD_{50}\,(\text{mix}) = \left(\sum_{i} \frac{X\,(a.s_i)}{LD_{50}\,(a.s_i)} \right)^{-1}$$

With:

$X\,(a.s._i)$ = fraction of each a.s. in the mixture

$LD_{50}\,(a.s._i)$ = acute toxicity value for each a.s.

Acute risk from combined exposure

The active substance content of the formulation MEZOFLOR 103 SC addressed in this dossier is 10% (w/v) mesotrione and 0.3% (w/v) florasulam, making up a total of 103 g a.s./L product.

According to GAP, the maximum application rate is 1.25 L/ha, therefore, an application rate of g 128.75 g a.s./ha was considered in the assessment.

Acute risk assessment:

Acute LD_{50} for the mixture of active substances for birds.

Test substance	**Concentration of active substance in formulation**	**Fraction of active substance in the formulation mixture[a]**	**Acute toxicity endpoint (mg as/kg bw)**	**Fraction of active substance/LD_{50} for the active sub-**	**LD_{50} mix (mg/kg bw)**

[1] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. [139 pp.].

	(g/L)			stance	
Florasulam	3	0.029	1046	0.0000277	3514
Mesotrione	100	0.97	3776	0.0002569	
Total	103			0.0002846	

[a] Concentration of an active substance in the formulation, divided by, the total concentration of all active substances in the formulation.

Comparison of the measured and predicted endpoints using the acute toxicity data for birds.

Test substance	Concentration of active substance in formulation (g/L)	Fraction of active substance in the formulation mixture	Acute toxicity endpoint (mg as/kg bw)	Tox per fraction a.s.	Tox per fraction mix	Deviation (%)
Florasulam	3	0.029	1046	36 069	3514	90.3
Mesotrione	100	0.97	3776	3 893		9.8
Total	103					

[a] Concentration of an active substance in the formulation, divided by, the total concentration of all active substances in the formulation.

According to EFSA Journal 2009; 7(12):1438, if deviation between tox per fraction a.s. and tox per fraction mix is less or equal to 10, then this active substance contributes to over 90% of mixture toxicity. Therefore there is no need to calculate mix tox and risk assessment for single active substance should be performed. Low risk for both active substances was shown according to calculations in Tables 9.4-2, 9.4-3, 9.4-6.

zRMS comment: Agreed. No additional actions are required.

Long-term risk assessment for mixture toxicity

Long-term NOEL for the mixture of active substances for birds.

Test substance	Concentration of active substance in formulation (g/L)	Concentration of active substance in formulation (g/L)	Long term toxicity endpoint (mg as/kg bw)	Fraction of active substance/NOEL for the active substance	$NOEL_{mix}$ (mg/kg bw)
Florasulam	3	0.029	104.6	0.000277	20.5
Mesotrione	100	0.97	20	0.0485	
Total	103			0.048777	

[a] Concentration of an active substance in the formulation, divided by, the total concentration of all active substances in the formulation.

Comparison of the measured and predicted endpoints using the long-term toxicity data for birds.

Test substance	Concentration of active substance in formulation (g/L)	Fraction of active substance in the formulation mixture[a]	Long term toxicity endpoint (mg as/kg bw)	Tox per fraction a.s.	Tox per fraction mix	Deviation (%)
Florasulam	3	0.029	104.6	3 607	20.5	99.4
Mesotrione	100	0.97	20	20.6		0.5
Total	725					

[a] Concentration of an active substance in the formulation, divided by, the total concentration of all active substances in the formulation.

According to EFSA Journal 2009; 7(12):1438, if deviation between tox per fraction a.s. and tox per fraction mix is less or equal to 10, then this active substance contributes to over 90% of mixture toxicity. Therefore there is no need to calculate mix tox and risk assessment for single active substance should be performed. Low risk for both active substances was shown according to calculations in Tables 9.4-2 – 9.4.7.

zRMS comment: Agreed. No additional actions are required.

B) According to the EFSA (EFSA Journal 2023; 21(2): 7790 (using EFSA birds and mammals calculator),

the combined risk for a mixture of 2 components: Mezotrione and Florasulame, was calculated according to the formula:

$$TER_{combi} = \left(\sum_{i=1}^{n} \frac{1}{TER_i}\right)^{-1}$$

This approach should be followed as a default whenever experimental studies with the formulated mixture are not available for either birds or mammals. Trigger values are the same used in the single substance assessment (10 for acute, 5 for reproductive). Calculated $TER_{combi/acute}$ and $TER_{combi/reproductive}$ value for generic species of birds for MEZOFLOR 103 SC are presented in table below:

Table 9.4-8: TER combi acute and TER combi reproductive for generic species of birds

Feeding guild	Application rate [kg a.s./ha]	Number of applications	Model deviation ratio (MDR, acute)	TER (combi, acute)	Model deviation ratio (MDR, reproductive)	TER (combi, reproductive)
Medium omnivorous	0.12875	1	1	770	1	19
Small insectivorous	0.12875	1	1	1800	1	71
Small omnivorous	0.12875	1	1	1100	1	29
Large herbivorous	0.12875	1	1	1000	1	25
Granivorous	0.12875	1	1	1500	1	33

All calculated value ($TER_{combi/acute}$ and $TER_{combi/reproductive}$) for birds, are above the trigger values (10 and 5). Low risk for both active substances was shown according to calculations in Tables 9.4-4, 9.4.5 and 9.4.7.

zRMS comments: Recently EFSA issued a new guidance document for birds and mammals: Birds & mammals: Risk assessment for Birds and Mammals approved: 15 December 2022 doi: 10.2903/j.efsa.2023.7790. Due to new GD noted but not yet into force the risk assessment for birds based on EFSA 2013 should be treated as additional source of information. It should be considered by MSs level.

9.4.3.1 Higher-tier risk assessment

Not relevant.

9.4.3.2 Drinking water exposure

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

Leaf scenario

Since MEZOFLOR 103 SC is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not have to be considered.

Puddle scenario

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

With a K(f)oc of up to 156.7, mesotrione belongs to the group of less sorptive substances.

Effective application rate (g/ha)	=	125		
Acute toxicity (mg/kg bw)	=	3776	quotient	= 0.03
Reprod. toxicity (mg/kg bw/d)	=	20	quotient	= 6.25

With a K(f)oc of 10.53, florasulam belongs to the group of less sorptive substances.

Effective application rate (g/ha)	=	3.75		
Acute toxicity (mg/kg bw)	=	1046	quotient	= 0.004
Reprod. toxicity (mg/kg bw/d)	=	104.6	quotient	= 0.04

zRMS comment: .

No specific calculations of exposure and TER are necessary for risk to birds through drinking water (leaf scenario) since **MEZOFLOR 103 SC** is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later.

No specific calculations of exposure and TER are necessary for risk to birds through drinking water (puddle scenario) since the calculated ratios of effective application rate to acute and chronic effect endpoints are below the trigger value of 50. No additional actions are required.

9.4.3.3 Effects of secondary poisoning

The log P_{ow} of mesotrione amounts to 0.11 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

The log P_{ow} of florasulam amounts to up to 1.0 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

zRMS comment:
Agreed. The Applicants' approach in evaluation of the risk of secondary poisoning is in line with EFSA (2009). Compounds selected for this assessment are agreed by the zRMS. Evaluation was not triggered for mesotrione and florasulam due to their log Pow <3.
No additional actions are required.

Risk assessment for earthworm-eating birds via secondary poisoning

Not required.

Risk assessment for fish-eating birds via secondary poisoning

Not required.

9.4.3.4 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.4.5 Overall conclusions

An estimation of risk indicate low risk for birds. Calculations conducted due to the influence formulation MEZOFLOR 103 SC due to the acute and long-term toxicity and reproductive did not indicate any hazardous properties and danger for birds. There was also no negative effects regarding to drinking water exposure and effect of secondary poisoning. There is no influence to evaluated organism regarding to dangerous to food poisoning.

zRMS comment: In the first step the TER_A and TER_{LT} values for mesotrione and florasulam exceeds the trigger value set by Commission regulation (EU) 546/2011 for acceptability of effects.

Based on the Tier 1 risk assessment for birds all the TER_A and TER_{LT} values for mesotrione and florasulam are greater than the Annex VI trigger of 10 and 5, respectively, indicating that **MEZOFLOR 103 SC** presents no unacceptable acute and long-term risk to birds according to the intended uses.

Further refinement for acute and long-term risk assessment is not required. Acute and long-term risk assessment was accepted by zRMS.

9.5 Effects on terrestrial vertebrates other than birds (KCP 10.1.2)

9.5.1 Toxicity data

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

However, the provision of further data on the formulation MEZOFLOR 103 SC is not considered essential, because risk assessment can be conducted based on data for active substances.

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

Table 9.5-1: Endpoints and effect values relevant for the risk assessment for mammals

Species	Substance	Exposure System	Results	Reference
Rat	Mesotrione	Acute	LD50 > 5000 mg/kg bw	EFSA Journal 2016;14(3):4419
Rat	Mesotrione	Long-term	NOEL = 1.2 mg/kg bw/d* NOEL = 0.3 mg/kg bw/d*	Renewal Assessment Report for mesotrione
Rat	Florasulam	Acute	LD50 > 5000 mg/kg bw	EFSA Journal 2015; 13(1):3984
Rat	Florasulam	Long-term	NOEL = 100 mg/kg bw/d	EFSA Journal 2015; 13(1):3984

~~* Using of this endpoint is justified in the point below.~~

*For details, please see the consideration below.

zRMS comments:

Mammalian toxicity data for mesotrione in table 9.5-1 are in line with EU agreed endpoints reported in EFSA Journal 2016;14(3):4419.

Mammalian toxicity data for florasulam in table 9.5-1 are in line with EU agreed endpoints reported in EFSA Journal 2015; 13(1):3984.

9.5.2 Justification for new endpoints

Endpoints for mesotrione presented in EFSA Journal 2016;14(3):4419 and endpoints for florasulam presented in EFSA Journal 2015; 13(1):3984.

~~For mesotrione a value of 1.2 mg/kg bw/d was used for calculations as a more reliable endpoint. According to residue studies for mesotrione, this active substance degrades to fast to give mammals a chance for long exposure. Therefore the strictest value of 0.3 mg/kg bw/d seems not reliable for ecotoxicological risk assessment~~. For mesotrione a value of 0.3 mg/kg bw/d was used for the long term risk assessment calculations as a recommended reliable endpoint.

zRMS comments: Agreed.

9.5.3 Risk assessment for spray applications

The risk assessment is calculated in two ways:

A) according to 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),

B) according to the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2023; 21(2): 7790,
however, the results obtained in both calculation are similar.

9.5.3.1 Screening and first-tier assessment (screening/generic focal species)

MESOTRIONE

The results of the screening and first-tier risk assessments (acute/reproductive), are summarised in the following tables, for Mesotrione:

A) according to EFSA (EFSA Journal 2009; 7(12): 1438:

Table 9.5-2: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of MEZOFLOR 103 SC in maize

Intended use	Maize				
Active substance/product	mesotrione				
Application rate (g/ha)	1 × 125				
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**
BBCH 12-18	Small herbivorous mammal	136.4	1	17.05	293
Reprod. toxicity (mg/kg bw/d)	~~1.2~~ 0.3				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
BBCH 12-18	Small herbivorous mammal	72.3	0.53	4.79	~~**0.25**~~ 0.06

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.

For mesotrione the resulting TER_A values is over the trigger value of 10 for acute toxicity what indicate a low risk for mammals. TER_{lt} is lower than 5 for long term toxicity and need further justification in first tier assessment as defined in the EFSA Journal 2009; 7(12):1438.

zRMS comments: In the screening step the TER_A values for mesotrione exceeds the trigger value set by Commission regulation (EU) 546/2011 for acceptability of effects. Further refinement for acute risk

assessment is not required. Acute risk assessment was accepted by zRMS. For mesotrione the TER_{LT} values from the tier 1 reproductive risk assessment are below the trigger for all scenarios. The refinement for long-term risk assessment is required. The long-term toxicity endpoints = 0.3 mg/kg bw/d should be used in risk assessment for mammals.

Table 9.5-3: First-tier assessment of the long-term/reproductive risk for mammals due to the use of MEZOFLOR 103 SC in maize

Intended use	Maize				
Active substance/product	mesotrione				
Application rate (g/ha)	1 × 125				
Reprod. toxicity (mg/kg bw/d)	~~1.2~~ 0.3				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
BBCH 10-19	Small insectivorous mammal "shrew"	4.2	0.53	0.28	~~**4.28**~~ **0.07**
BBCH 10-29	Small herbivorous mammal "vole"	72.3	0.53	4.79	~~**0.25**~~ **0.06**
BBCH 10-29	Small omnivorous mammal "mouse"	7.8	0.53	0.52	~~**2.3**~~ **0.58**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily
Since for each focal species TER_{lt} is below 5, higher-tier risk assessment is necessary.

zRMS comments: In the first step the TER_{LT} values from reproductive risk assessment for mesotrione are below the trigger for all scenarios. The refinement for long-term risk assessment is required. The long-term toxicity endpoints = 0.3 mg/kg bw/d should be used in risk assessment for mammals.

B) According to the EFSA (EFSA Journal 2023; 21(2): 7790 (using EFSA birds and mammals calculator)

Table 9.5-4: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of MEZOFLOR 103 SC in maize

Active substance: MEZOTRIONE Risk: acute									
Crop type	**Acute endpoint (mammals) [mg a.s./kg bw]**	**App. rate [kg a.s./ha]**	**Food intake rate [g fresh weight/day]**	**Residue per unit dose [mg a.s./kg]**	**TWAf x MAF (moving window)**	**MAF (Acute) (Acute)**	**DD [mg a.s./kg bw] (acute)**	**TER (acute)**	**Trigger value (acute)**
Crop group 1	5000	0.125	31.4	118	0.53	1	20.1	> 248.64	10

Active substance: MEZOTRIONE Risk: reproductive								
Crop type	**Reproductive endpoint (mammals) [mg a.s./kg bw per day]**	**App. rate [kg a.s./ha]**	**Food intake rate [g fresh weight/day]**	**Residue per unit dose [mg a.s./kg]**	**TWAf x MAF(moving window)**	**DDD [mg a.s./kg bw per day] (reproductive)**	**TER (reproductive)**	**Trigger value (reproductive)**

Crop group 1	1.2	0.125	31.4	47.2	0.53	4.27	**0.28**	**5**

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

For mesotrione the resulting TER_A values is over the trigger value of 10 for acute toxicity what indicate a low risk for mammals. TER_{lt} is lower than 5 for long term toxicity and need further justification in first tier assessment as defined in the EFSA Journal 2023; 21(2): 7790.

Table 9.5-5: First-tier assessment of the long-term/reproductive risk for mammals due to the use of MEZOFLOR 103 SC in maize

Active substance: MEZOTRIONE						
Crop: Maize and millet crop						
BBCH: 10-19						
Application rate [kg a.s./ha]: 125						
Feeding guild	Reproductive endpoint (mammals) [mg a.s./kg bw per day]	Daily energy expenditure [kJ/day]	Food intake rate [g fresh weight/day]	DDD [mg a.s./kg bw per day] (reproductive)	TER (reproductive)	Trigger value (reproductive)
Medium herbivorous	1.2	1220	645	0.802	**1.5**	**5**
Small insectivorous	1.2	17.6	2.85	0.0991	12.1	5
Small omnivorous	1.2	61.3	5.99	0.473	**2.54**	**5**
Granivorous	1.2	61.3	3.73	0.324	**3.7**	**5**

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.

Since for 3 out of 4 species TER_{lt} is below 5, higher-tier risk assessment is necessary.

According to EFSA Journal 2023; 21(2): 7790, the exposure to small mammals in the TAI ('Terrestrial Area of Interest') for the 1 tier was calculated. To calculate the exposure to small mammals in the TAI, the application rate (kg a.s./ha) is adjusted by the spray drift % at a minimum distance from the spray equipment for standard horizontal sprayers (default distance at Tier 1 is 1 m). Results are presented in table below.

Table 9.5-6: First-tier exposure to small mammals in the TAI ('Terrestrial Area of Interest')

Crop: Maize and millet crop								
Fielding guild	**Name of the active substance**	**Spray drift [%]**	**Dietary dose [mg a.s./kg bw] (acute)**	**TER (acute)**	**Trigger value (acute)**	**Daily dietary dose [mg a.s./kg bw per day] (reproductive)**	**TER (reproductive)**	**Trigger value (reproductive)**
Small insectivorous	Mezotrione	2.77	0.027	> 182328.54	10	0.002	600	5

Small herbivorous	Mezotrione	2.77	0.51	> 9744.34	10	0.093	13	5
Granivorous	Mezotrione	2.77	0.039	> 126659.24	10	0.0097	120	5
Small insectivorous	Florasulame	2.77	0.00081	> 6140564.72	10	6e-05	1700000	5
Small herbivorous	Florasulame	2.77	0.015	> 328175.29	10	0.0028	36000	5
Granivorous	Florasulame	2.77	0.0012	> 4265702.33	10	0.00029	350000	5

zRMS comments: Recently EFSA issued a new guidance document for birds and mammals: Birds & mammals: Risk assessment for Birds and Mammals approved: 15 December 2022 doi: 10.2903/j.efsa.2023.7790. Due to new GD noted but not yet into force the risk assessment for mammals based on EFSA 2013 should be treated as additional source of information. It should be considered by MSs level.

FLORASULAM

For screening step for florasulam the resulting TER_A values is over the trigger value of 10 for acute toxicity and TER_{lt} is over than 5 for long term toxicity, what indicate a low risk for birds. No further justification is needed as defined in the EFSA Journal 2009; 7(12):1438 and EFSA (EFSA Journal 2023; 21(2): 7790. The results are summarized in tables below:

~~**A) According to the EFSA (EFSA Journal 2023; 21(2): 7790 (using EFSA birds and mammals calculator)**~~

A) according to EFSA (EFSA Journal 2009; 7(12): 1438:

Table 9.5-7: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of MEZOFLOR 103 SC in maize

Active substance: FLORASULAME **Risk: acute**									
Crop type	Acute endpoint (mammals) [mg a.s./kg bw]	App. rate [kg a.s./ha]	Food intake rate [g fresh weight/day]	Residue per unit dose [mg a.s./kg]	TWAf x MAF(moving window)	Multiple application factor (Acute)	Dietary dose [mg a.s./kg bw] (acute)	Toxicity exposure ratio (acute)	Trigger value (acute)
Crop group 1	5000	0.00375	31.4	118	0.53	1	0.603	> 8288.07	10
Active substance: FLORASULAME **Risk: reproductive**									

Crop type	Reproductive end-point (mammals) [mg a.s./kg bw per day]	Application rate [kg a.s./ha]	Number of applications	Food intake rate [g fresh weight/day]	Residue per unit dose [mg a.s./kg]	TWAf x MAF(moving window)	Daily dietary dose [mg a.s./kg bw per day] (reproductive)	Toxicity exposure ratio (reproductive)	Trigger value (reproductive)
Crop group 1	100	0.00375	1	31.4	47.2	0.53	0.128	781	5

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

zRMS comments: Agreed.

9.5.3.2 Higher-tier risk assessment

~~According to available data, presented in Additional Report to the DAR for Terbuthylazine (2010, United Kingdom), an appropriate focal species for risk assessment calculations for products to be used in maize, especially at the early stage of development is wood mouse (small omnivorous). Therefore it is used as a focal species for third tier risk assessment calculations.~~

Diet composition and **the body wight** of the focal species- wood mouse is based on information included in annex b of **EFSA Guidance Document on Risk Assessment for Birds and Mammals (2023)**, for the small omnivorous mammals in maize and millet crop, post emergence. **This data align with requirement included in EFSA Journal 2009; 7(12): 1438.**

~~The PT value is based on GLP field study (Grimm T., 2013). The study involved live trapping – radio tracking of wood mouse at post-emergence. The results of the radiotracking for the individuals having used maize fields as foraging habitat, indicated a **90th percentile PT of 0.08** for wood mouse (average of all sessions). This value was used in calculation for wood mouse in the post-emergence use in maize. The RUD and PD values were used in calculation based on Annex E, and B respectively, whereas DV according to Annex D of **EFSA Guidance Document on Risk Assessment for Birds and Mammals (2023). This data align with requirement included in EFSA Journal 2009; 7(12): 1438**~~

~~According to the EFSA (EFSA Journal 2009; 7(12): 1438 and EFSA (EFSA Journal 2023; 21(2): 7790, calculation for third tier for focal species- wood mouse, are presented in table below~~

~~**Table 9.5-4: Higher-tier risk assessment of long-term/reproductive risk for mammals due to the use of MEZOFLOR 103 SC in maize**~~

~~Active substance: MEZOTRIONE~~										
~~Reprod. toxicity (mg/kg bw/d): 1.2~~										
~~Application rate (kg/ha): 1 x 0.125~~										
~~**Focal species**~~	~~**Food category, % in diet**~~	~~**FIR/bw**~~	~~**RUDm (geo mean) (mg/kg food)**~~	~~**PD**~~	~~**PT**~~	~~**Deposition value (DV)**~~	~~**MAF**~~	~~**fTWA**~~	~~**DDDm (mg/kg bw/d)**~~	~~**TERlt**~~
~~Wood mouse~~	~~Ground-dwelling arthropods~~	~~0.34~~	~~2.8~~	~~0.25~~	~~0.08~~	~~0.75~~	~~1~~	~~0.53~~	~~0.00095~~	
	~~Ground seeds~~	~~0.34~~	~~40.2~~	~~0.5~~	~~0.08~~	~~0.75~~	~~1~~	~~0.53~~	~~0.0272~~	

	~~Monocotyledon foliage~~	~~0.34~~	~~47.2~~	~~0.25~~	~~0.08~~	~~1~~	~~1~~	~~0.53~~	~~0.0213~~	
								~~whole diet~~	~~0.049~~	~~24.5~~

~~FIR/bw: Food intake rate per body weight; RUD: residue unit dose; DDD: daily dietary dose; PD:proportion in diet ; DV: deposition value; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.~~

~~TER value for a wood mouse is over the trigger value of 5 for reproductive toxicity what indicate an acceptable risk to mammals.~~

The results from field studies regarding of the use of maize crops by small mammals, describe by Wolf, 2005, and Grimm et al., 2013, indicate that the omnivorous wood mouse can be an appropriate focal species for early and freshly emerged maize. In study presented by Grimm et al., 2013, there were a total of 1560 trapnights between BBCH 00-09 and 720 trapnights between BBCH 10-12 in maize fields. The only in-field trapped wood mouse individual gives a 0.14 capture/100 trapnights trapping efficiency for the post-emergence period until BBCH 12.

On the basis of results of the study by Grimm et al. (2013) and studies available in the course of the EU renewal of mesotrione, wood mouse is considered as relevant focal species with agreed **PT value of 0.139** for early stages of maize. Although acceptable risk for the long-term assessment for wood mouse, could be concluded with consideration of the: refined PT and, the residue decline in maize plants.

Refinement of PT value and focal species

zRMS comment: The PT value of 0.139 for wood mouse was accepted at the EU level during mesotrione evaluation. The focal species for maize at early BBCH growth stages such as wood mouse and brown hare were accepted by zRMS.

Normally a DT_{50} of 10 days is assumed in the birds and mammals risk assessment as a default value. By using data from measured residues on plant material, more realistic exposure estimations can be derived for the ecotoxicological risk assessment for herbivorous and omnivorous mammals.

Short-term residue decline studies were performed in maize plants by Schneider, (2016, Report No. R5116). The results confirm the very fast dissipation of residues of mesotrione and its major metabolite MNBA in maize, leading to DT_{50} values of 14 hours (Hazlerigg & Garrat, 2016, Report No. E2016-13). Taking into account the measured DT50, long-term time weighted average value (f_{TWA}) is recalculated to be 0.04 .These values are used in the refined risk assessment of the small omnivorous mammal ‘mouse’ (for part of diet based on monocots) and herbivorous species ‘brown hare’. EU agreed NOAEL of 0.3 mg a.s./kg bw/d was used. Respective calculations for wood mouse are presented below.

Table 9.5-5: Higher-tier risk assessment of long-term/reproductive risk for mammals due to the use of MEZOFLOR 103 SC in maize, for wood mouse

Active substance: MEZOTRIONE
Reprod. toxicity (mg/kg bw/d): 0.3
Application rate (kg/ha): 1 x 0.125

Focal species	Food category, % in diet	SVm	PD	PT	fTWAx MAF	DDDm (mg/kg bw/d)	TERlt
Wood mouse	Ground-dwelling arthropods	7.8	0.25	0.139	0.53	0.018	
	Ground seeds		0.5	0.139	0.53	0.036	
	Monocotyledon foliage		0.25	0.139	0.04	0.0013	
	whole diet					0.055	5.45

In addition to wood mouse, European brown hare (*Lepus europaeus*) was detected within the germinated maize fields (BBCH 10-14) in Austria with abundance of 0.12 individuals/hectare (5 transect tracks comprising 65.4 ha, 10 transect counts during the whole study; Wolf, 2005).

On the basis of results of the studies available in the course of the EU renewal of mesotrione, brown hare is considered as relevant focal species for early stages of maize. The risk assessment for this species exposed to mesotrione was performed with consideration of the refined FIR/bw and refined fTWA values (0.04). As a worst case it was assumed that the diet consists of 100% maize shoots. A generic field study monitoring the use of maize crops by the brown hare was performed by Grimm and Katzschner, 2019. The **PT** value, used for the risk assessment was **0.62.**

zRMS comment: zRMS agrees with the refined PT value (PT = 0.139) for wood mouse and PT value (PT = 0.62) for brown hare.

The refinement PT value for wood mouse and brown hare should be considered by MSs level.

Table 9.5-5: Higher-tier risk assessment of long-term/reproductive risk for mammals due to the use of MEZOFLOR 103 SC in maize, for brown hare

Active substance: MEZOTRIONE

Reprod. toxicity (mg/kg bw/d): 0.3

Application rate (kg/ha): 1 x 0.125

Focal species	Food category, % in diet	FIR/ bw	RUDm (geo mean) (mg/kg food)	PD	PT	Deposition value (DV)	MAF	fTWA	DDDm (mg/kg bw/d)	TERlt
Brown hare	100% maize shoots	0.334	54.2	1	0.62	1	1	0.04	0.0561	
	whole diet								0.0561	5.34

zRMS comment: A higher tier risk assessment based on the refinement parameters such as foliage residue dissipation (DT_{50}) was corrected by RMS.

zRMS agrees with the refined PT value (PT = 0.139) for wood mouse and PT value (PT = 0.62) for brown hare.

The refinement PT value for wood mouse and brown hare should be considered by MSs level.

Refinement of DT_{50}

The f_{TWA} value used to refine the risk to mammals was calculated based on the residue level decrease

study for the active substance mesotrione in corn (DT_{50}= 14h), authored by Schneider E. from 2016: "Determination of mesotrione and its metabolite (MNBA) residue In maize following treatment with Mesotrione 100 SC under field conditions in northern and southern France in 2015, together with the report by Hazlerigg C.: "A kinetic analysis of the dissipation of mesotrione in maize". The Applicant does not have the full report: "A kinetic analysis of the dissipation of mesotrione in maize". The risk calculation used unprotected data contained in the TEMSA SC product documentation (mesotrione, 100g/L), in which the report was described in detail. Authorization of the TEMSA SC product was renewed in Poland in accordance with the decision (MRiRW Permit No. R-190/2015 of 29.10.2015 renewed by MRiRW Decision No. R – 25 /2021o of 23.06.2021) of 23.06.2021. These studies were presented in connection with the need to refine the risk assessment for two mammalian species: *Apodemus sylvaticus* and *Lepus europaeus*, the data were necessary to obtain an acceptable risk assessment for the product TEMSA SC (mesotrione, 75-125 g/ha), in the process of renewal of the authorization in Poland.

The possibility of using these residue studies to refine risk assessments should be considered at Member State level.

Normally a DT_{50} of 10 days is assumed in the birds and mammals risk assessment as a default value. For this product **MEZOFLOR 103 SC** however, a lower DT_{50} could be expected based on five plant residue trials that were conducted in Europe (see dR Part B7 for a description of these studies). A kinetic analysis of the dissipation of mesotrione in maize was conducted by enviresearch and a report of this study was submitted to support this refinement of the DT_{50} of mesotrione on plants (Hazlerigg & Garratt, 2016).

FOCUS (2006, 2014) degradation kinetics guidance was applied to calculate DT_{50} endpoints for mesotrione modelling from residues measured in five plant residue trials in Europe. The data were described reasonably well by either SFO kinetics or bi-phasic FOMC kinetics and acceptable endpoints were derived for all five studies.

The calculated DT_{50} values and statistics for the decline of mesotrione in maize are shown in the table below. The DT_{50} values ranged from 10.1 to 21.9 hours. The final DT_{50} recommended for modelling is the geomean of 14 hours. The DT_{50} = 14 hours as geometric mean was also proposed by Applicant. However, according to the harmonization arrangements for Poland, when the tests include 4 - 9 locations - maximum values can be used DT_{50}. The worst case is DT_{50} = 21.9 hours and this value should be used in risk assessment. Estimated new f_{TWA} = 0.063 based on residue decline study will be used as a risk refinement for reproductive risk to mammals in post-emergence use. MAFm * TWA (refined DT_{50}) = 0.063 should be used in risk assessment. The registration dossier included 5 studies on the dynamics of residue depletion in maize (4 in the N-EU zone and 1 in the S-EU zone), on the basis of which the DT_{50} value was determined. In the opinion of RMS, one study of the southern zone residues (B5116 EF1) undermines the validity of using the geometric mean and should be excluded from the analysis. It is most reasonable to use the maximum value can be used DT_{50}.

Trial	Site	European area	Crop, variety	Trial type
B5116 AN1	Seebach, Alsace, France	North	Maize, Karedas	Decline curve
B5116 MA1	Donnelay, Lorraine, France	North	Maize, P3184	Decline curve
B5116 BM1	Thorée les Pins, Pays de la Loire, France	North	Maize, P9074	Decline curve
B5116 ND1	Hérin, Nord Pas de Calais, France	North	Maize, Ramses	Decline curve
B5116 EF1	Saint-Livrade, Aquitaine, France	South	Maize, Roberi	Decline curve

Table: Summary of fitted parameters for the decline of mesotrione.

Study	Kinetic model	t-test	χ2-error	Visual fit	DT50 (hours)
B5116 AN1	FOMC	n/a	7.93	Good	13.3 *
B5116 MA1	SFO	Pass	6.92	Good	21.9
B5116 BM1	SFO	Pass	14.3	Medium	10.1
B5116 ND1	FOMC	n/a	6.74	Good	15.2 *
B5116 EF1	FOMC	n/a	10.9	Good	12.1 *
Geomean of all trials					**14.0**

* Pseudo first-order DT_{50} calculated as FOMC DT_{90}/3.32 (FOCUS 2006, 2014)

Estimated new f_{TWA} = 0.063 based on residue decline study will be used as a risk refinement for reproductive risk to mammals in post-emergence use. The worst case is DT_{50} = 21.9 hours. Refinement of DT_{50} should be considered at MSs level.

Based on EFSA Conclusion 2016 voles are not representative species in maize. The focal species for maize at early BBCH growth stages such as wood mouse and brown hare were accepted by zRMS.

The focal species in maize should be considered by MSs level.

<u>Maize 1 x 125 g a.s./ha</u>

The refinement risk assessment for mammals was corrected by zRMS.

Intended use	Maize
Active substance/product	Mesotrione
Application rate (g a.s./ha)	1 × 125
Reprod. toxicity (mg/kg bw/d)	0.3
TER criterion	5

Crop scenario Growth stage	Indicator/generic focal species	SV_m	PT	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Maize BBCH 10-29	Brown hare *Lepus europaeus* (100% grass)	17.3[1)]	0.62	1 x 0.063	0.084	**3.57**

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.

1) SVm from EFSA B&M guidance (2009) for Brown hare (grassland scenario)

Intended use	Maize
Active substance/product	Mesotrione
Application rate (g a.s./ha)	1 × 125
Reprod. toxicity (mg/kg bw/d)	0.3
TER criterion	5

Crop scenario Growth stage	Indicator/generic focal species	SV_m	PT	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Maize BBCH 10-29	*Apodemus sylvaticus*	7.8[1)]	0.139	1 x 0.063	0.0085	35.29

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.

1) SVm from EFSA B&M guidance (2009) for Brown hare (grassland scenario)

Based on the new f_{TWA} and refinement PT value application for brown hare is still unacceptable, therefore refinement of reproductive risk assessment for the herbivorous brown hare (*Lepus europaeus*) and the small omnivorous for Rabitt exposed to mesotrione is required as TER_{LT} is below the trigger of 5 (TER_{lt} = 3.57).

Maize 1 x 100 g a.s./ha

The refinement risk assessment for mammals was corrected by zRMS.

Intended use	Maize
Active substance/product	Mesotrione
Application rate (g a.s./ha)	1 × 100
Reprod. toxicity (mg/kg bw/d)	0.3
TER criterion	5

Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**PT**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize BBCH 10-29	Brown hare *Lepus europaeus* (100% grass)	17.3[1)]	0.62	1 x 0.063	0.068	**4.41**

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.

[2)] SVm from EFSA B&M guidance (2009) for Brown hare (grassland scenario)

Intended use	Maize
Active substance/product	Mesotrione
Application rate (g a.s./ha)	1 × 100
Reprod. toxicity (mg/kg bw/d)	0.3
TER criterion	5

Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**PT**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize BBCH 10-29	*Apodemus sylvaticus*	7.8[1)]	0.139	1 x 0.063	0.0068	44.12

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.

[2)] SVm from EFSA B&M guidance (2009) for Brown hare (grassland scenario)

Based on the new f_{TWA} and refinement PT value application for brown hare is still unacceptable, therefore refinement of reproductive risk assessment for the herbivorous brown hare (*Lepus europaeus*) and the small omnivorous for Rabitt exposed to mesotrione is required as TER_{LT} is below the trigger of 5 (TER_{lt} = 4.41).

The refinement risk assessment for mammals should be considered by MSs level.

With regard to the long-term endpoint to be used in the risk assessment, the following text is included in the RAR (2015) : *„There is no new data presented regarding the reproductive/developmental toxicity to mammals. However, the relevant reproductive endpoint for the ecotoxicology risk assessment has been considered and is dis-cussed further under section B.6 (Toxicology and metabolism). The NOAEL for reproductive perfor-mance is 1.2 mg/kg bw/d. It is concluded that the NOAEL of 1.2 mg/kg bw/d specific to F0 and F1 is ap-propriate for the reproductive risk assessment. Given the proposed GAP and persistence of mesotrione, effects observed in the F2 generation are considered less relevant. In this NOAEL value both of the effects on litter size and plasma tyrosine level have been considered, along with the uncertainties of lacking data on female's tyrosine level. It should be noted that this value is accompanied by a 6.8 % reduction in rat litter size but that this reduction in litter size is not statistically significant"*

Mesotrione is a herbicide applied to maize mostly once per season at BBCH 12-18, therefore the second generation of animals (F2) is unlikely to be exposed in the wild and the effects seen in this generation are not appropriate for ecotoxicological risk assessment. The litter size effects on which the EFSA NOAEL is based correspond to more than 20 weeks of exposure to the test substance. Rats are repeatedly exposed to the active substance over several weeks from F0 to mature F1 animals. According to OECD 416 "Two-Generation Reproduction Toxicity Study", the test substance should be administered via the diet or drinking water, preferably 7 days per week, and dosing should be continued for at least 10 weeks

prior to the mating period and also during the 2-week mating period. In addition, the short-term residue degradation studies conducted on maize plants show that the worst case DT50 is approximately 21 hours.

In conclusion, the total exposure in the multi-generation laboratory experiment is therefore much higher than the possible real exposure in a treated field, as mesotrione should only be applied once per season, and therefore developmental data from the F1 generation are considered more appropriate for the calculation of the long-term mammalian endpoint for the environmental risk assessment.

It is proposed here to use the NOAEL 1.2 mg/kg bw/d from the F1 generation data, which is more relevant for the ecotoxicological risk assessment due to the use pattern of mesotrione.

The refinement risk assessment for Brown hare *Lepus europaeus*, using NOEL= 1.2 mg/kg bw/d from the F1 generation data.

zRMS comment: Based on the new f_{TWA} and refinement PT value - application for brown hare is still unacceptable, therefore refinement of reproductive risk assessment for the herbivorous brown hare (*Lepus europaeus*) and the small omnivorous for Rabitt exposed to mesotrione based on refined NOEAL of 1.2 mg a.s./kg bw.

The information available in the mesotrione RAR of 2015 (Vol. 3CA, B.6) indicates that in fact, slightly reduced pup survival at 10 ppm (1.2 mg a.s./kg bw/d) was incidental and not treatment related, as at the next higher dose (100 ppm) the pup survival was at the level comparable with control values. For this reason it seems that for purposes of the ecological risk assessment NOAEL of 1.2 mg a.s./kg bw/d could be considered relevant and was actually proposed by the RMS (UK).

Mesotrione effects on F1 generation from the multigeneration study in rats (RAR (2015))

Parameter	**Generation**	**Dose Level (ppm)**				
		0	**2.5**	**10**	**100**	**2500**
Gestation length (d)	F1	22.3	22.3	22.4	22.8**	22.9**
Litter size (no. pups)	F1	11.7	12.4	10.9	10.3	9.2**
Litter weight (g) Day 0	F1	70.4	72.2	65.9	63.4	57.1**
Pup survival (%)	F1	92.4	89.9	85.2**	89.7	77.6**

* significantly different to control ($p<0.05$), ** ($p<0.01$)

Conclusion: F1 results indicate a clear effect at dose levels of 2500 ppm, with all results being significantly different to the control. A reduction in litter size by 6.8% is seen in animals treated at 10 ppm and by 11.1% for those treated at 100 ppm when compared to the control group. Litter weight is similarly reduced at these doses, but this effect is a consequence of the reduced litter size. A significant reduction in pup survival is seen at 10 ppm but this is not dose-related and is therefore not considered to be of toxicological concern. Based on F1 developmental data, a NOAEL of 10 ppm (1.2 mg/kg bw/d) is therefore proposed. It is here proposed to use the NOAEL of 10 ppm (corresponding to 1.2 mg/kg bw/d) from the F1 generation data, being in more relevant in the ecotoxicology risk assessment because of the use pattern of mesotrione (it is applied once per season, thus results from the single generation are more appropriate than results from a second generation after more than 20 weeks of exposure) and results from different generations indicate that mesotrione effects were not the result of exposure during a critical developmental phase. On the other hand this issue was discussed at Pesticides Peer Review experts Meeting 136 in December 2015, where it was decided that the observed effects (e.g., litter size and pup survival) on the F2 generation should not disregard.

Higher-tier assessment of the long term risk for mammals due to the mesotrione use of MEZOFLOR 103 SC in maize

\Intended use		maize
Active substance/product		mesotrione

Application rate (g/ha)		1 × 125						
Reprod. toxicity (mg/kg bw/d)		1.2						
TER criterion		5						
Crop scenario Growth stage	**Indicator/generic focal species**	**Fir/bw**	**RUD**	**SV_m**	**MAF_m × TWA***	**PT**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize	Rabbit (100 % plant material)	0.334	54.2	-	0.063	0.62	0.088	13.63

* **The worst case is DT_{50} = 21.9 hours**

Higher-tier assessment of the long term risk for mammals due to the mesotrione use of MEZOFLOR 103 SC in maize

\Intended use		maize						
Active substance/product		mesotrione						
Application rate (g/ha)		1 × 100						
Reprod. toxicity (mg/kg bw/d)		1.2						
TER criterion		5						
Crop scenario Growth stage	**Indicator/generic focal species**	**Fir/bw**	**RUD**	**SV_m**	**MAF_m × TWA***	**PT**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize	Rabbit (100 % plant material)	0.334	54.2	-	0.063	0.62	0.07	17

* **The worst case is DT_{50} = 21.9 hours**

The trigger value for rabbit and *Apodemus sylvaticus* are above the trigger of 5.
Therefore, further refinement is not required for this species as the TER_{LT} is above the trigger of 5 indicating acceptable risk to mammals.

Based on the risk refinement based on refinement toxicity endpoints, F_{twa} value and PT value it can be concluded that application of **MEZOFLOR 103 SC** according to the label will not pose no unacceptable reproductive risk to mammals.

The refinement risk assessment for mammals should be considered by MSs level.

Risk Assessment for combined exposure for mammals

According to the EFSA Journal (2009)[2], the simultaneous exposure of animals to residues of two or more potential toxic substances should be considered in the risk assessment. Therefore, for the assessment of acute effects, a surrogate LD_{50} for the mixture of active substances with known toxicity was derived assuming dose additivity of toxicity. For the calculation, the following equation was used:

[2] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. [139 pp.].

$$LD_{50}\,(\text{mix}) = \left(\sum_{i} \frac{X\,(a.s_{\cdot i})}{LD_{50}\,(a.s_{\cdot i})} \right)^{-1}$$

With:

$X\,(a.s._i)$ = fraction of each a.s. in the mixture

$LD_{50}\,(a.s._i)$ = acute toxicity value for each a.s.

Acute risk from combined exposure for mammals

The active substance content of the formulation MEZOFLOR 103 SC addressed in this dossier is 10% (w/v) mesotrione and 3% (w/v) florasulam, making up a total of 103 g a.s./kg product.
According to GAP, the maximum application rate is 1.25 L/ha, therefore, an application rate of 128.75 g a.s./ha was considered in the assessment.

Acute LD_{50} for the mixture of active substances.

Test substance	Concentration of active substance in formulation (g/L)	Fraction of active substance in the formulation mixture[a]	Acute toxicity endpoint (mg as/kg bw)	Fraction of active substance/LD_{50} for the active substance	LD_{50} mix (mg/kg bw)
Florasulam	3	0.029	5000	0.0000058	5000
Mesotrione	100	0.97	5000	0.0001942	
Total	103			0.0002	

[a] Concentration of an active substance in the formulation, divided by, the total concentration of all active substances in the formulation.

Comparison of the measured and predicted endpoints using the acute toxicity data for mammals.

Test substance	Concentration of active substance in formulation (g/L)	Fraction of active substance in the formulation mixture	Acute toxicity endpoint (mg as/kg bw)	Tox per fraction a.s.	Tox per fraction mix	Deviation (%)
Florasulam	3	0.029	5000	172 413	5000	97.1
Mesotrione	100	0.97	5000	5 154		3.0
Total	103					

[a] Concentration of an active substance in the formulation, divided by, the total concentration of all active substances in the formulation.

According to EFSA Journal 2009; 7(12):1438, if deviation between tox per fraction a.s. and tox per fraction mix is less or equal to 10, then this active substance contributes to over 90% of mixture toxicity. Therefore there is no need to calculate mix tox and risk assessment for single active substance should be performed. Low risk for both active substances was shown according to calculations in Tables 9.5-2 – 9.5-6.

Long-term risk assessment for mixture toxicity for mammals

Long-term NOEL for the mixture of active substances.

Test substance	Concentration of active substance in formulation (g/L)	Concentration of active substance in formulation mixture[a] (g/L)	Long term toxicity endpoint (mg as/kg bw)	Fraction of active substance/NOEL for the active substance	$NOEL_{mix}$ (mg/kg bw)
Florasulam	3	0.029	100	0.00029	~~1.24~~ 0.30956
Mesotrione	100	0.97	~~1.2~~ 0.3	~~0.8083~~ 3.23	

Total	103			~~0.8086~~ 3.2303	

[a] Concentration of an active substance in the formulation, divided by, the total concentration of all active substances in the formulation.

Comparison of the measured and predicted endpoints using the long-term toxicity data for mammals.

Test substance	Concentration of active substance in formulation (g/L)	Fraction of active substance in the formulation mixture[a]	Long term toxicity endpoint (mg as/kg bw)	Tox per fraction a.s.	Tox per fraction mix	Deviation (%)
Florasulam	3	0.029	100	~~345~~ 3450	~~1.24~~ 0.30956	~~99.6~~ 99.9
Mesotrione	100	0.97	~~1.2~~ 0.3	~~1.237~~ 0.3092		~~0.25~~ 0.1
Total	103					

[a] Concentration of an active substance in the formulation, divided by, the total concentration of all active substances in the formulation.

According to EFSA Journal 2009; 7(12):1438, if deviation between tox per fraction a.s. and tox per fraction mix is less or equal to 10, then this active substance contributes to over 90% of mixture toxicity. Therefore there is no need to calculate mix tox and risk assessment for single active substance should be performed. ~~Low risk for both active substances was shown according to calculations in Tables 9.5-2 – 9.5-4.~~

Based on the new f_{TWA} and refinement PT value application for brown hare is still unacceptable, therefore refinement of reproductive risk assessment for the herbivorous brown hare (*Lepus europaeus*) and the small omnivorous for Rabitt exposed to mesotrione is required as TER_{LT} is below the trigger of 5 (TER_{lt} = 4.41).

The refinement risk assessment for mammals should be considered by MSs level.

According to EFSA Journal 2023; 21(2): 7790, the combined risk for a mixture of 2 components: Mezotrione and Florasulame, was calculated according to the formula:

$$TER_{combi} = \left(\sum_{i=1}^{n} \frac{1}{TER_i}\right)^{-1}$$

This approach should be followed as a default whenever experimental studies with the formulated mixture are not available for either birds or mammals. Trigger values are the same used in the single substance assessment (10 for acute, 5 for reproductive). Calculated $TER_{combi/acute}$ and $TER_{combi/reproductive}$ value for generic species of birds for MEZOFLOR 103 SC are presented in table below:

	$TER_{A/lt}$ mesotrione	**$TER_{A/lt}$ florasulame**	TER (combi, acute)	TER (reproductive)
Screening step	>248.64	8288.07	770	-
Higher tier/focal species	47.6	165837.4	-	50

zRMS comments: Recently EFSA issued a new guidance document for birds and mammals: Birds & mammals: Risk assessment for Birds and Mammals approved: 15 December 2022 doi: 10.2903/j.efsa.2023.7790. Due to new GD noted but not yet into force the risk assessment for mammals based on EFSA 2013 should be treated as additional source of information. It should be considered by

MSs level.

9.5.3.3 Drinking water exposure

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

Puddle scenario

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

With a K(f)oc of up to 156.7, mesotrione belongs to the group of less sorptive substances.

Effective application rate (g/ha)	=	125		
Acute toxicity (mg/kg bw)	=	5000	quotient =	0.025
Reprod. toxicity (mg/kg bw/d)	=	1.2	quotient =	**104**

With a K(f)oc of 10.53, florasulam belongs to the group of less sorptive substances.

Effective application rate (g/ha)	=	3.75		
Acute toxicity (mg/kg bw)	=	5000	quotient =	0.00075
Reprod. toxicity (mg/kg bw/d)	=	100	quotient =	0.0375

Since, the result for reproductive toxicity for mesotrione is over 50, further justification is necessary.

zRMS comment: Agreed.

A) According to EFSA Journal 2009; 7(12):1438:

Table 9.5-9: Assessment of the risk for mammals due to exposure to mesotrione via contaminated drinking water in puddles

Intended use	Maize				
Active substance	mesotrione				
Application rate (g/ha)	1 × 125				
Reprod. toxicity (mg/kg bw/d)	~~1.2~~ 0.3				
TER criterion	5				
Soil-relevant applic. rate (g/ha)	**Koc (L/kg)**	**PEC_{puddle} (mg/L)**	**DW uptake (L/kg bw/d)**	**Daily dose (mg/kg bw/d)**	**TER_{lt}**
93.75*	14	0.229	0.24	0.055	~~21.8~~ 5.45

PEC_{puddle}: concentration in puddles; DW: drinking water; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.
* 125 mg/ha including interception factor of 25%

zRMS comment: Agreed.

B) According to EFSA Journal 2023; 21(2): 7790 (using EFSA birds and mammals calculator).

PEC_{puddle} was calculated according to the formula

$$PEC_{puddle} = \frac{AReff/10}{1000\ x\ (0.02 + K_{oc}\ x\ 0.015)}$$

Table 9.5-10: Assessment of the risk for mammals due to exposure to mesotrione via contaminated drinking water in puddles

Active substance: MEZOTRIONE					
Crop: Maize and millet crop					
Application rate [g a.s./ha]: 125					
Koc (L/kg):14					
Reproductive endpoint [mg a.s./kg bw per day]	refined PEC	Screening - low risk?	Exposure	TER (reproductive)	Trigger value (reproductive)
1.2	0.307	No	0.07368	16	5

TER_{lt} values is over the trigger value of 5 for reproductive toxicity what indicate a low risk for mammals from consumption of contaminated water in puddles No further justification is needed.

zRMS comments: Recently EFSA issued a new guidance document for birds and mammals: Birds & mammals: Risk assessment for Birds and Mammals approved: 15 December 2022 doi: 10.2903/j.efsa.2023.7790. Due to new GD noted but not yet into force the risk assessment for mammals based on EFSA 2013 should be treated as additional source of information. It should be considered by MSs level.

9.5.3.4 Effects of secondary poisoning

The log P_{ow} of mesotrione amounts to 0.11 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

The log P_{ow} of florasulam amounts to up to 1.0 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

zRMS comment: Agreed.

Risk assessment for earthworm-eating mammals via secondary poisoning

Not required.

Risk assessment for fish-eating mammals via secondary poisoning

Not required.

9.5.3.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.5.5 Overall conclusions

An estimation of risk indicate low risk for mammals. Calculations conducted due to the influence formulation MEZOFLOR 103 SC due to the acute and long-term toxicity and reproductive did not indicate any hazardous properties and danger for mammals. There was also no negative effects regarding to drinking water exposure and effect of secondary poisoning. There is no influence to evaluated organism regarding to dangerous to food poisoning.

zRMS comment: In the screening step the TER_A values for mesotrione and florasulam exceeds the trigger value (10), indicating that **MEZOFLOR 103 SC** presents an acceptable acute risk to mammals.

The TER_{LT} values from the tier 1 reproductive risk assessment for florasulam are above the trigger of 5 for the use on maize, indicating that **florasulam** presents an acceptable long-term risk to mammals.

The TER_{LT} values from the tier 1 reproductive risk assessment for mesotrione are below the trigger of 5 for the use on maize, indicating that **mesotrione** presents an unacceptable long-term risk to mammals.

A higher tier long-term risk assessment based on the following refinement parameters: foliage residue dissipation (DT_{50}) and ecological data on PT values as well as ecological toxicity endpoints for mammals should be considered by MSs level.

Refinement long-term risk assessment should be considered at MS level.

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

Not relevant

9.7 Effects on aquatic organisms (KCP 10.2)

9.7.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with mesotrione, florasulam and their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related doc-

uments.

Effects on aquatic organisms of MEZOFLOR 103 SC were not evaluated as part of the EU assessment of mesotrione and florasulam. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Table 9.7-1: Endpoints and effect values relevant for the risk assessment for aquatic organisms – mesotrione, florasulam and their relevant metabolites

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss, Lepomis macrochirus	Florasulam	96 h, s	LC_{50} > 100 mg a.s./L $_{mm}$	EFSA Journal 2015; 13(1):3984
Pimephales promelas	Florasulam	33 d (ELS), f	NOEC = 2.9 mg a.s./L $_{mm}$	EFSA Journal 2015; 13(1):3984
Daphnia magna	Florasulam	48 h, s	EC_{50} > 292 mg a.s./L $_{m}$	EFSA Journal 2015; 13(1):3984
Palaemonetes pugio	Florasulam	48 h, s	EC_{50} > 120 mg a.s./L $_{m}$	EFSA Journal 2015; 13(1):3984
Daphnia magna	Florasulam	21 d, ss	NOEC = 23.4 mg a.s./L $_{nom}$	EFSA Journal 2015; 13(1):3984
Chironomus riparius	Florasulam	28 d, ss	NOEC = 10.0 mg a.s./L $_{nom}$	EFSA Journal 2015; 13(1):3984
Pseudokirchneriella subcapitata	Florasulam	72 h, s	E_rC_{50} = 0.00894 mg a.s./L $_{mm}$	EFSA Journal 2015; 13(1):3984
Lemna gibba	Florasulam	14 d, ss	E_rC_{50} = 0.00118 mg a.s./L $_{im}$	EFSA Journal 2015; 13(1):3984
Oncorhynchus mykiss	5-OH-florasulam	96 h, s	LC_{50} > 91 mg a.s./L $_{nom}$	EFSA Journal 2015; 13(1):3984
Daphnia magna	5-OH-florasulam	48 h, s	EC_{50} > 96.7 mg a.s./L $_{mm}$	EFSA Journal 2015; 13(1):3984
~~*Pseudokirchneriella subcapitata*~~ *Raphidocelis subcapitata*	5-OH-florasulam	72 h, s	E_rC_{50} = 21.57 mg a.s./L $_{mm}$	EFSA Journal 2015; 13(1):3984
Lemna gibba	5-OH-florasulam	7 d, ss	E_rC_{50} = 0.0378 mg a.s./L $_{mm}$	EFSA Journal 2015; 13(1):3984
Daphnia magna	DFP-ASTCA	48 h, s	EC_{50} > 0.030 mg a.s./L $_{mm}$	EFSA Journal 2015; 13(1):3984
~~*Pseudokirchneriella subcapitata*~~ *Raphidocelis subcapitata*	DFP-ASTCA	72 h, s	E_rC_{50} = 96 mg a.s./L $_{nom}$	EFSA Journal 2015; 13(1):3984
Lemna gibba	DFP-ASTCA	7 d, ss	E_rC_{50} > 100 mg a.s./L $_{nom}$	EFSA Journal 2015; 13(1):3984
Daphnia magna	ASTCA	48 h, s	EC_{50} > 0.030 mg a.s./L $_{nom}$	EFSA Journal 2015; 13(1):3984
~~*Pseudokirchneriella subcapitata*~~ *Raphidocelis*	ASTCA	72 h, s	E_rC_{50} > 9.2 mg a.s./L $_{mm}$	EFSA Journal 2015; 13(1):3984

Species	Substance	Exposure System	Results	Reference
subcapitata				
Lemna gibba	ASTCA	14 d, ss	E_rC_{50} > 10.2 mg a.s./L $_{nom}$	EFSA Journal 2015; 13(1):3984
Daphnia magna	TSA	48 h, s	EC_{50} > 0.030 mg a.s./L $_{mm}$	EFSA Journal 2015; 13(1):3984
~~*Pseudokirchneriella subcapitata*~~ *Raphidocelis subcapitata*	TSA	72 h, s	E_rC_{50} > 94 mg a.s./L $_{mm}$	EFSA Journal 2015; 13(1):3984
Lemna gibba	TSA	7 d, ss	E_rC_{50} > 100 mg a.s./L $_{nom}$	EFSA Journal 2015; 13(1):3984
~~*Pseudokirchneriella subcapitata*~~ *Raphidocelis subcapitata*	TPSA	72 h, s	E_rC_{50} > 100 mg a.s./L $_{nom}$	EFSA Journal 2015; 13(1):3984
Lemna gibba	TPSA	7 d, ss	E_rC_{50} > 100 mg a.s./L $_{nom}$	EFSA Journal 2015; 13(1):3984
~~*Pseudokirchneriella subcapitata*~~ *Raphidocelis subcapitata*	5-OH-ASTP	72 h, s	E_rC_{50} > 100 mg a.s./L $_{nom}$	EFSA Journal 2015; 13(1):3984
Lemna gibba	5-OH-ASTP	7 d, ss	E_rC_{50} > 100 mg a.s./L $_{nom}$	EFSA Journal 2015; 13(1):3984
~~*Pseudokirchneriella subcapitata*~~ *Raphidocelis subcapitata*	ASTP	72 h, s	E_rC_{50} > 100 mg a.s./L $_{nom}$	EFSA Journal 2015; 13(1):3984
Lemna gibba	ASTP	7 d, ss	E_yC_{50} = 88 mg a.s./L $_{mm}$	EFSA Journal 2015; 13(1):3984
Oncorhynchus mykiss	Mesotrione	96-h, s	LC_{50} > 120 mg a.s./L $_{nom}$	EFSA Journal 2016;14(3):4419
Pimephales promelas	Mesotrione	36 d, f	NOEC – 12.5 mg a.s./L $_{nom}$	EFSA Journal 2016;14(3):4419
Daphnia magna	Mesotrione	48 h, s	EC_{50} > 622 mg a.s./L $_{nom}$	EFSA Journal 2016;14(3):4419
Daphnia magna	Mesotrione	21 d, ss	NOEC = 180 mg a.s./L $_{nom}$	EFSA Journal 2016;14(3):4419
~~*Pseudokirchneriella subcapitata*~~ *Raphidocelis subcapitata*	Mesotrione	120 h, s	E_rC_{50} = 13 mg a.s./L $_{nom}$	EFSA Journal 2016;14(3):4419
Lemna gibba	Mesotrione	14 d, ss	E_bC_{50} (for frond no.)= 0.022 mg a.s./L (nom) E_bC_{50} (for dry weight)= 0.0077 mg a.s./L (nom)	EFSA Journal 2016;14(3):4419
Oncorhynchus mykiss	MNBA	96-h, s	LC_{50} > 120 mg a.s./L $_{nom}$	EFSA Journal 2016;14(3):4419

Species	Substance	Exposure System	Results	Reference
Daphnia magna	MNBA	48 h, s	EC_{50} = 130 mg a.s./L $_{nom}$	EFSA Journal 2016;14(3):4419
~~*Pseudokirchneriella subcapitata*~~ *Raphidocelis subcapitata*	MNBA	72 h, s	E_rC_{50} = 42 mg a.s./L $_{nom}$	EFSA Journal 2016;14(3):4419
Lemna gibba	MNBA	7 d, ss	E_rC_{50} > 97 mg a.s./L $_{mm}$	EFSA Journal 2016;14(3):4419
Oncorhynchus mykiss	AMBA	96-h, s	LC_{50} = 150 mg a.s./L $_{nom}$	EFSA Journal 2016;14(3):4419
Daphnia magna	AMBA	48 h, s	EC_{50} = 160 mg a.s./L $_{nom}$	EFSA Journal 2016;14(3):4419
~~*Pseudokirchneriella subcapitata*~~ *Raphidocelis subcapitata*	AMBA	72 h, s	E_rC_{50} = 7.7 mg a.s./L $_{nom}$	EFSA Journal 2016;14(3):4419
Lemna gibba	AMBA	7 d, ss	E_rC_{50} > 90 mg a.s./L $_{mm}$	EFSA Journal 2016;14(3):4419
Lemna gibba	SYN546974	7 d, ss	E_rC_{50} (for both)>95 mg a.s./L mm E_rC_{50} (for fronf no.) =93 mg a.s./L mm	EFSA Journal 2016;14(3):4419

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

Table 9.7-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – MEZOFLOR 103 SC

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	MEZOFLOR 103 SC	96 h, s	LC_{50} > 100 mg/L $_{nom}$	[redacted]
Daphnia magna	MEZOFLOR 103 SC	48 h, s	EC_{50} > 100 mg/L $_{nom}$	Czarnecka M., 30.07.2021/ W-56-20
~~*Pseudokirchneriella subcapitata*~~ *Raphidocelis subcapitata*	MEZOFLOR 103 SC	72 h, s	E_rC_{50} = 45.89 mg/L $_{nom}$	Nierzędska E., 26.07.2021/ W-57-20
Navicula pelliculaos	MEZOFLOR 103 SC	72 h, s	E_rC_{50} > 100 mg/L $_{nom}$	Nierzędska E., 26.07.2021/ W-58-20
Lemna gibba	MEZOFLOR 103 SC	7 d, ss	E_rC_{50} = 2.5 mg/L $_{nom}$	Czarnecka M., 16.09.2021/ W-59-20

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

9.7.2 Justification for new endpoints

According to COMMISSION REGULATION (EU) No 284/2013 of 1 March 2013, for formulated prod-

ucts, at least studies for fish, invertebrates and algae must be performed. In case of herbicides additionally test for macrophytes has to be done.

9.7.3 Risk assessment

The evaluation of the risk for aquatic and sediment-dwelling organisms was performed in accordance with the recommendations of the "Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters in the context of Regulation (EC) No 1107/2009", as provided by the Commission Services (SANTE-2015-00080, 15 January 2015).
The relevant global maximum FOCUS Step 1, 2 and 3,4 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the table below.
Since the product MEZOFLOR 103 SC shows a lot lower toxicity than technical active substances mesotrione and florasulam, the risk assessment is performed with appropriate endpoint obtained in studies with those active substances.

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

Table 9.7-3: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mesotrione for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of MEZOFLOR 103 SC in maize (soil pH <7)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Macrophytes**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba.***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	E_rC_{50}
(µg/L)		120 000	12 500	622 000	180 000	13 000	7.7
AF		100	10	100	10	10	10
RAC (µg/L)		1 200	1 250	6 220	18 000	1 300	0.77
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1							
	35.62	0.030	0.028	0.006	0.002	0.027	**46.260**
Step 2							
N-Europe	5.29	0.004	0.004	0.001	0.000	0.004	**6.870**
S-Europe	9.97	0.008	0.008	0.002	0.001	0.008	**12.948**
Step 3							
D3/ditch	0.6559	0.001	0.001	< 0.001	< 0.001	0.001	0.852
D4/pond	0.07121	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.092
D4/stream	0.5649	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.734
D5/pond	0.04820	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.063
D5/stream	0.5976	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.776
D6/ditch	0.6553	0.001	0.001	< 0.001	< 0.001	0.001	0.851
R1/pond	0.09593	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.125
R1/stream	2.014	0.002	0.002	< 0.001	< 0.001	0.002	**2.616**

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Macrophytes
R2/stream	1.564	0.001	0.001	< 0.001	< 0.001	0.001	**2.031**
R3/stream	4.181	0.003	0.003	0.001	< 0.001	0.003	**5.430**
R4/stream	4.523	0.004	0.004	0.001	< 0.001	0.003	**5.874**

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 9.7-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mesotrione for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of MEZOFLOR 103 SC in maize (soil pH >7)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Macrophytes**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba.***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	E_rC_{50}
(µg/L)		120 000	12 500	622 000	180 000	13 000	7.7
AF		100	10	100	10	10	10
RAC (µg/L)		1 200	1 250	6 220	18 000	1 300	0.77
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1							
	41.87	0.035	0.033	0.007	0.002	0.032	**54.377**
Step 2							
N-Europe	4.34	0.004	0.003	0.001	< 0.001	0.003	**5.636**
S-Europe	7.99	0.007	0.006	0.001	< 0.001	0.006	**10.377**
Step 3							
D3/ditch	0.6560	0.001	0.001	< 0.001	< 0.001	0.001	0.852
D4/pond	0.02653	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.034
D4/stream	0.5618	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.730
D5/pond	0.02649	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.034

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Macrophytes
D5/stream	0.5862	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.761
D6/ditch	0.6552	0.001	0.001	< 0.001	< 0.001	0.001	0.851
R1/pond	0.02868	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.037
R1/stream	1.324	0.001	0.001	< 0.001	< 0.001	0.001	**1.719**
R2/stream	3.193	0.003	0.003	0.001	< 0.001	0.002	**4.147**
R3/stream	3.975	0.003	0.003	0.001	< 0.001	0.003	**5.162**
R4/stream	4.32	0.004	0.003	0.001	< 0.001	0.003	**5.610**

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

For the intended use in maize, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for *Lemna gibba* as characterised by EC_{50} of 7.7 µg/L in connection with an assessment factor of 10) in four FOCUS Step 3 scenarios. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies.

FOCUS Step 4

Table 9.7-5: Global maximum PEC_{sw} values for mesotrione (pH < 7), following single application of MEZOFLOR 103 SC to maize according to the central EU zone GAP according to surface water Step 4

RAC = 0.77 µg/L	Scenario	STEP 4 mesotrion	
Nozzle reduction	Vegetative strip (m)	1	3
	No spray buffer (m)	1	3
None	R1 Stream	0.72	0.3012
None	R2 Stream	0.9441	0.3997
None	R3 Stream	**1.211**	0.4195
None	R4 Stream	**1.923**	0.6654

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

Table 9.7-6: Global maximum PEC_{sw} values for mesotrione (pH > 7), following single application of MEZOFLOR 103 SC to maize according to the central EU zone GAP according to surface water Step 4

RAC = 0.77 µg/L	Scenario	STEP 4 mesotrion	
Nozzle reduction	Vegetative strip (m)	3	4
	No spray buffer (m)	3	4
None	R1 Stream	0.2932	0.2287
None	R2 Stream	0.3998	0.3118
None	R3 Stream	0.4195	0.3273
None	R4 Stream	**0.924**	0.2324

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

Table 9.7-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for MNBA for each organism group based on FOCUS Steps 1 calculations for the use of MEZOFLOR 103 SC in maize

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Macrophytes**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba.***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}	E_rC_{50}
(µg/L)		120 000	130 000	42 000	97 000
AF		100	100	10	10
RAC (µg/L)		1 200	1 300	4 200	9 700
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**				
Step 1					
	19.59	0.016	0.015	0.005	0.002

Table 9.7-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AMBA (pH <7 and pH >7) for each organism group based on FOCUS Steps 1 calculations for the use of MEZOFLOR 103 SC in maize

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Macrophytes**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba.***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}	E_rC_{50}
(µg/L)		150 000	160 000	7 700	90 000
AF		100	100	10	10
RAC (µg/L)		1 500	1 600	770	9 000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**				
Step 1					
pH < 7	8.12	0.005	0.005	0.011	< 0.001
pH > 7	8.99	0.006	0.006	0.012	< 0.001

Table 9.7-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for SYN546974 for each organism group based on FOCUS Steps 1 calculations for the use of MEZOFLOR 103 SC in maize

Group		**Macrophytes**
Test species		***Lemna gibba.***
Endpoint		E_rC_{50}
(µg/L)		95 000
AF		10
RAC (µg/L)		9 500
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**	
Step 1		
	0.65	> 0.001

Table 9.7-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for florasulam for each organism group based on FOCUS Steps 1, 2 calculations for the use of MEZOFLOR 103 SC in maize

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**		**Algae**	**Macrophytes**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Palaemonetes pugio***	***Daphnia magna***	***Chironomus riparius***	***Pseudokirchn. subcapitata***	***Lemna gibba.***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	NOEC	E_rC_{50}	E_rC_{50}
(µg/L)		100 000	2 900	120 000	23 400	10 000	8.94	1.18
AF		100	10	100	10	10	10	10
RAC (µg/L)		1 000	290	1 200	2 340	1 000	0.894	0.118
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**							
Step 1								
	1.27	0.001	0.004	0.001	0.001	0.001	**1.421**	**10.763**
Step 2								
N-Europe	0.06	0.000	0.000	0.000	0.000	0.000	0.067	0.508
S-Europe	0.09	0.000	0.000	0.000	0.000	0.000	0.101	0.763

Table 9.7-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for 5-OH florasulam for each organism group based on FOCUS Steps 1 calculations for the use of MEZOFLOR 103 SC in maize

Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba.***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}	E_rC_{50}
(µg/L)		91 000	96 700	21 570	37.8
AF		100	100	10	10
RAC (µg/L)		910	967	2 157	3.78
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**				

Step 1					
	2.04	0,002	0,002	0,001	0,540

Table 9.7-12: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for DFP-ASTCA for each organism group based on FOCUS Steps 1 calculations for the use of MEZOFLOR 103 SC in maize

Test species		***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba.***
Endpoint (µg/L)		EC_{50} 30	E_rC_{50} 96 000	E_rC_{50} 100 000
AF		100	10	10
RAC (µg/L)		0.3	9 600	10 000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	0.26	0,867	0,000	0,000

Table 9.7-13: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for ASTCA for each organism group based on FOCUS Steps 1 and 2 calculations for the use of MEZOFLOR 103 SC in maize

Test species		***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba.***
Endpoint (µg/L)		EC_{50} 30	E_rC_{50} 9 200	E_rC_{50} 10 200
AF		100	10	10
RAC (µg/L)		0.3	920	1 200
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	0.56	**1.867**	0.001	0.000
Step 2				

N-Europe	0.05	0.167	0.000	0.000
S-Europe	0.09	0.300	0.000	0.000

Table 9.7-14: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for TSA for each organism group based on FOCUS Steps 1 calculations for the use of MEZOFLOR 103 SC in maize

Test species		***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba.***
Endpoint (µg/L)		EC_{50} 30	E_rC_{50} 94 000	E_rC_{50} 100 000
AF		100	10	10
RAC (µg/L)		0.3	9 400	10 000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	0.08	0.267	0.000	0.000

Table 9.7-15: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for TSA for each organism group based on FOCUS Steps 1 calculations for the use of MEZOFLOR 103 SC in maize

Test species		***Pseudokirchn. subcapitata***	***Lemna gibba.***
Endpoint (µg/L)		E_rC_{50} 100 000	E_rC_{50} 100 000
AF		10	10
RAC (µg/L)		10 000	10 000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**		
Step 1			
	0.49	0.000	0.000

Table 9.7-16: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for 5-OH ASTP for each organism group based on FOCUS Steps 1 calculations for the use of MEZOFLOR 103 SC in maize

Test species		***Pseudokirchn. subcapitata***	***Lemna gibba.***
Endpoint (µg/L)		E_rC_{50} 100 000	E_rC_{50} 100 000
AF		10	10
RAC (µg/L)		10 000	10 000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**		
Step 1			
	0.22	0.000	0.000

Table 9.7-17: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for ASTP for each organism group based on FOCUS Steps 1 calculations for the use of MEZOFLOR 103 SC in maize

Test species		***Pseudokirchn. subcapitata***	***Lemna gibba.***
Endpoint (µg/L)		E_rC_{50} 100 000	E_rC_{50} 88 000
AF		10	10
RAC (µg/L)		10 000	8 800
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**		
Step 1			
	0.17	0.000	0.000

The combined risk assessment for mixture MEZOFLOR 103 SC

Following the dilution and spraying of the formulated product, much of the formulation constituents are likely to be lost by volatilisation. Therefore, shortly after application of a formulated product, aquatic organisms are mainly exposed to the active substance present in the formulation.

According to the new EFSA Scientific Opinion (EFSA, 2013) measured and calculated mixture toxicity should be compared to determine synergistic, additive or antagonistic effects of the formulation.

In the following the concentration addition (CA) model is used as proposed by EFSA.

To determine the respective formulation effect, EFSA proposed to calculate the model deviation ratio (MDR), which divides the calculated mixture toxicity ($LC_{50}/EC_{50\ mix\text{-}CA}$) by the measured mixture toxicity ($LC_{50}/EC_{50\ MEZOFLOR\ 103\ SC}$).

Ecotox studies are biological test systems which underlie a certain natural biological variability when repeating a study. Hence, a threshold has to be defined when an increased/decreased mixture toxicity effect cannot be seen as only additive any longer. EFSA proposes a factor of 5, *i.e.* if the MDR is between 0.2 and 5 the observed and calculated mixture toxicities are considered in agreement.

Active susbtance / species	Test system	Endpoint (mg a.s./L)
Mesotrione		
Fish	96 h LC_{50}	120
Daphnia magna	48 h EC_{50}	622
Algae	72 h E_rC_{50}	13
Lemna gibba	7 d E_rC_{50}	0.0077
Florasulam		
Fish	96 h LC_{50}	100
Daphnia magna	48 h EC_{50}	292
Algae	72 h E_rC_{50}	0.00894
Lemna gibba	14 d E_rC_{50}	0.00118

Table 9.7-18 EC_{50} for the mixture of active substances for *Daphnia magna*.

Test substance	Concentration of active substance in formulation (g/L)	Fraction of active substance in the formulation mixture[a]	EC_{50} mg a.s./L	Fraction of active substance/ ErC_{50} for the active substance	EC_{50} mix (mg a.s./L)
mezotrione	100	0.97	622	0.0015	625
florasulam	3	0.03	292	0.0001	
Total	103			0.0016	

Table 9.7-19 EC_{50} for the mixture of active substances for *Fisch*.

Test substance	Concentration of active substance in formulation (g/L)	Fraction of active substance in the formulation mixture[a]	EC_{50} mg a.s./L	Fraction of active substance/ ErC_{50} for the active substance	EC_{50} mix (mg a.s./L)
mezotrione	100	0.97	120	0.008	120
florasulam	3	0.03	100	0.0003	
Total	103			0.0083	

Table 9.7-18 EC_{50} for the mixture of active substances for *Algae*.

Test substance	Concentration of active substance in formulation (g/L)	Fraction of active substance in the formulation mixture[a]	EC_{50} mg a.s./L	Fraction of active substance/ ErC_{50} for the active substance	EC_{50} mix (mg a.s./L)
mezotrione	100	0.97	13	0.075	0.29
florasulam	3	0.03	0.00892	3.363	
Total	103			3.44	

Table 9.7-19 EC_{50} for the mixture of active substances for *Lemna gibba*

Test substance	Concentration of active substance in formulation (g/L)	Fraction of active substance in the formulation mixture[a]	EC_{50} mg a.s./L	Fraction of active substance/ ErC_{50} for the active substance	EC_{50} mix (mg a.s./L)
mezotrione	100	0.97	0.0077	126	0.007
florasulam	3	0.03	0.00118	25.4	
Total	103			151.4	

Table 9.7-20 Summary of results obtained in the studies with the formulated product MEZOFLOR 103 SC and comparison of calculated and measured mixture toxicity.

Test species	Endpoint and Test system	Measured toxicity for MEZOFLOR 103 SC (mg/L)	Measured toxicity of MEZOFLOR 103 SC recalculated to mg a.s./L	Calculated toxicity of MEZOFLOR 103 SC	Model deviation ratio (MDR)
Daphnia magna	EC_{50} 48h acute	100	10.3	625	60.67
Lemna gibba	ErC_{50} 7d	2.5	0.258	0.007	0.027
Fish	LC_{50} acute	100	10.3	120	11.65
Algae	E_rC_{50} 72h	45.6	4.73	0.29	0.061

The mixture toxicity of the formulation was re-calculated based on the nominal contents of mesotrione (100 g/L) and florasulam (3 g/L) within the formulation.

The calculated MDR values are above 5 for *fish* and *Daphnia magna* organism. However, since the LC50 and EC50 were estimated based on the limit tests, these values are not reliable for using them in mixture toxicity.

In case of *Algae* and *Lemna gibba* MDR is <0.2 indicating further investigation of antagonism.

Table 9.7-21 PEC_{mix} EC_{50} for the mixture of active substances for *Lemna gibba*

Test substance	Concentration of active substance in formulation (ug/L)	Fraction of active substance in the formulation mixture[a]	EC_{50} mg a.s./L	Fraction of active substance/ ErC_{50} for the active substance	PEC_{mix} ErC_{50} (mg a.s./L)
mezotrione	35.62	0.97	0.0077	126	0.007
florasulam	1.27	0.03	0.00118	25.4	
Total	36.9			151.4	

Table 9.7-22 PEC_{mix} EC_{50} for the mixture of active substances for *Algae*

Test substance	Concentration of active substance in formulation (g/L)	Fraction of active substance in the formulation mixture[a]	EC_{50} mg a.s./L	Fraction of active substance/ ErC_{50} for the active substance	PEC_{mix} ErC_{50} (mg a.s./L)
mezotrione	35.62	0.97	13	0.075	0.29
florasulam	1.27	0.03	0.00892	3.363	
Total	36.9			3.44	

Table 9.7-23 Comparison of the calculated endpoints for mix MEZOFLOR 103 SC and predicted endpoints using the PEC_{mix}.

Test species	Endpoint mix for formulaton (mg a.s./L)	PEC mix endpoint (mg a.s./L)	Factor Endpoint for formulation/Pec mix endpoint
Lemna	0.007	0.007	1
Algae	0.29	0.29	1

[a] The mixture toxicity of the formulation was re-calculated based on the nominal contents of mesotrione (100 g/L) and florasulam (3 g/Kg) within the formulation.

[b] The mixture toxicity of the formulation was re-calculated based on the mixture composition at the PEC_{mix} for mesotrione (5.29 mg/L at Step 2 N-Europe) and florasulam (0.06 mg/L at Step 2 for N-Europe).

The calculated factors are at range 0.8-1.2 for each organism indicating that the mixture composition in the formulation study giving the measured mixture **toxicity is similar to the mixture composition at the PEC_{mix}.** Therefore, ETR_{mix} is calculated according to EFSA GD, 2013 and presented below:

Table 9.7-24 ETR mix with STEP4 PEC_{sw} with 4 meter buffer zone for mesotrione for each organism as the worst case.

Nozzel reduction	No-spray buffer (m)	PECsw mg/L	ETRmix= PECmix/ECxppp		Trigger	
	Vegetated filter strip (m)	4 meter	Algae	*Lemna gibba*	Algae	*Lemna gibba*
0%	R1	0.0002287	< 0.1	< 0.1	**0.10**	**0. 10**
	R2	0.0003118	< 0.1	< 0.1		
	R3	0.0003273	< 0.1	< 0.1		
	R4	0.0002324	< 0.1	< 0.1		

9.7.4 Overall conclusions

Taking into consideration risk mitigation calculations for MEZOFLOR 103 SC use in maize, following risk mitigation measures should be applied:

- **4 m buffer non-spray zone with 4 meter vegetated filter strip.**

zRMS comment: The evaluation of the risk for aquatic 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" (EFSA Journal 2013;11(7):3290).

Conclusion from the risk assessment based on the active substance mesotrione:

The PECsw/sed calculations for mesotrione have been approved for applications proposed in GAP. PECsw and PECsed calculations were carried out according to the FOCUS recommendations. The Applicant has been used FOCUS models: STEPS1-2 and Step 3. PECsw/sed were also carried out at Step 4 according to FOCUS L&M Guidance. The Applicant used the geometric mean value. In opinion of the zRMS this is acceptable, as being in line with current requirements concerning selection of Kfoc to be used for modelling purposes.

PECsw/sed are acceptable to describe predicted environmental concentrations of mesotrione and its metabolites in surface water and sediment and are appropriate to be used for the subsequent risk assessment for aquatic and sediment organisms.

MS should identify risk reduction measures at the national level.

Taking into consideration risk mitigation calculations for **MEZOFLOR 103 SC** use in maize, following risk mitigation measures should be applied:
- **4 m buffer non-spray zone with 4 meter vegetated filter strip.**

Conclusion from the risk assessment based on the active substance florasulam:

The calculations of PECsw/sed for florasulam and its metabolites submitted by Applicant have been accept-ed. All input parameters for active substances and its metabolites are in line EFSA conclusion 2015;13(1): 3984. PECsw values were calculated in Step 1 and 2 for active substances and their metabolites for proposed uses in GAP. No further calculation was needed.

The combined risk assessment for mixture **MEZOFLOR 103 SC** was accepted by zRMS.

Conclusion from the risk assessment based on the formulated product:
Taking into consideration risk mitigation calculations for **MEZOFLOR 103 SC** use in maize, following risk mitigation measures should be applied:
- **4 m buffer non-spray zone with 4 meter vegetated filter strip.**

Final risk mitigation measures should be considered at MSs level.

9.8 Effects on bees (KCP 10.3.1)

9.8.1 Toxicity data

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

Effects on bees of MEZOFLOR 103 SC were not evaluated as part of the EU assessment of mesotrione and florasulam. New data submitted with this application are listed in **Błąd! Nie można odnaleźć źródła odwołania.** and summarised in Appendix 2.

Table 9.8-1: Endpoints and effect values relevant for the risk assessment for bees

Species	Substance	Exposure System	Results	Reference
Apis mellifera	Mesotrione	Oral	LD_{50} > 11 µg/bee	EFSA Journal 2016;14(3):4419
Apis mellifera	Mesotrione	Contact	LD_{50} > 100 µg/bee	EFSA Journal 2016;14(3):4419
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

Species	Substance	Exposure System	Results	Reference
Apis mellifera	MEZOFLOR 103 SC	Oral	LD_{50} > 200 µg/bee	Kulec-Płoszczyca E. /2021/ B-17-21
Apis mellifera	MEZOFLOR 103 SC	Contact	LD_{50} > 200 µg/bee	Kulec-Płoszczyca E. /2021/ B-18-21
Apis mellifera	MEZOFLOR 103 SC	Chronic oral	LDD_{50} > 15.8 µg/bee	Kulec-Płoszczyca E. /2021/ B-16-21
Apis mellifera	MEZOFLOR 103 SC	Larval chronic test	NOED >100 µg/larva	Orzechowska U. /2021/ 0030/0017/E
Bombus terrestris	MEZOFLOR 103 SC	Oral	LD_{50} > 200 µg/bee	Kulec-Płoszczyca E. /2021/ B-19-21
Bombus terrestris	MEZOFLOR 103 SC	Contact	LD_{50} > 200 µg/bee	Kulec-Płoszczyca E. /2021/ B-20-21

9.8.2 Justification for new endpoints

Not relevant.

9.8.3 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).

9.8.3.1 Hazard quotients for bees

Table 9.8-2: First-tier assessment of the risk for bees due to the use of mesotrione, florasulam and MEZOFLOR 103 SC in maize

Intended use	Maize		
Active substance	Mesotrione		
Application rate (g/ha)	1 × 125		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	11	125	11.4
Contact toxicity	100		1.25
Intended use	Maize		
Active substance	Florasulam		
Application rate (g/ha)	1 × 3.75		
Test design	**LD_{50} (lab.)** (µg/bee)	**Single application rate** (g/ha)	**Q_{HO}, Q_{HC}** criterion: $Q_H \leq 50$
Oral toxicity	100	3.75	0.0375
Contact toxicity	100		0.0375

Product **Application rate (g/ha)**	MEZOFLOR 103 SC 1 × 1328		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	200	1328	6.6
Contact toxicity	200		6.6

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

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

Not relevant.

9.8.4 Effects on bumble bees

Table 9.8-3: First-tier assessment of the risk for bees due to the use of MEZOFLOR 103 SC in maize

Product **Application rate (g/ha)**	MEZOFLOR 103 SC 1 × 1328		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	200	1328	6.6
Contact toxicity	200		6.6

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

9.8.5 Effects on solitary bees

Not relevant.

9.8.6 Overall conclusions

The HQ values are lower than the trigger of 50, indicating low risk to bees and bumblebees from application of MEZOFLOR 103 SC. Calculation conducted for MEZOFLOR 103 SC regarding to the oral and contact toxicity also confirm no risk for bees and bumblebees due to the use that formulation: achieved values are lower than 50.
Therefore a low risk to bees is expected from the application of MEZOFLOR 103 SC following application according to the proposed GAP.

zRMS comments:

The HQ values are lower than the trigger of 50, indicating low risk to bees from following application of **MEZOFLOR 103 SC.** In addition, the chronic study for adult bees and a study effects on honey bee development and other honey bee life stages have been submitted by Applicant. The studies were accepted by RMS. The risk assessment based on this studies should be considered when GD for Bees, 2013 is implemented at EU level. Final decision should be taken into account at MSs level.

9.9 Effects on arthropods other than bees (KCP 10.3.2)

9.9.1 Toxicity data

Studies on the toxicity to arthropods other than bees 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 arthropods of MEZOFLOR 103 SC were not evaluated as part of the EU assessment of mesotrione and 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 non-target arthropods

Species	Substance	Exposure System	Results	Reference
Typhlodromus pyri (protonymphs)	Florasulam	Laboratory test glass plates (2D)	LR_{50} > 15 g/ha	EFSA Journal 2015; 13(1):3984
Aphidius rhopalosiphi (adults)	Florasulam	Laboratory test glass plates (2D)	LR_{50} > 15 g/ha	EFSA Journal 2015; 13(1):3984
Typhlodromus pyri (protonymphs)	MEZOFLOR 103 SC	Laboratory test glass plates (2D)	LR_{50} >1.062 kg/ha* ER_{50} > 1.062 kg/ha*	Kulec-Płoszczyca E. /2021/ B-14-21
Aphidius rhopalosiphi (adults)	MEZOFLOR 103 SC	Laboratory test glass plates (2D)	LR_{50} > 1.062 kg/ha* ER_{50} > 1.062 kg/ha*	Kulec-Płoszczyca E. /2021/ B-15-21

* recalculated using density 1.062 g/L

zRMS comment:

zRMS agrees with the toxicity endpoints proposal by the Applicant.

9.9.2 Justification for new endpoints

Not relevant.

9.9.3 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.

Table 9.9-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of MEZOFLOR 103 SC in maize

Intended use	Maize
Active substance/product	MEZOFLOR 103 SC
Application rate (g/ha)	1 × 1328
MAF	1

Test species **Tier I**	**LR_{50} (lab.)** **(g/ha)**	**$PER_{in\text{-}field}$** **(g/ha)**	**$HQ_{in\text{-}field}$** **criterion: HQ ≤ 2**
Typhlodromus pyri	1062	1328	1.25
Aphidius rhopalosiphi	1062		1.25

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.

zRMS comments: zRMS agrees with the Applicant's assessment with the in-field risk to non-target arthropods from the proposed use of **MEZOFLOR 103 SC** above. A low risk is demonstrated to the 2 standard first tier.

9.9.3.1 Risk assessment for off-field exposure

Table 9.9-3: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of MEZOTRION 103 SC in maize

Intended use	Maize
Active substance/product	MEZOFLOR 103 SC
Application rate (g/ha)	1 × 1328
MAF	1
vdf	10 (Tier 1)

Test species Tier I	**LR_{50} (lab.) (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	1062	0.0277	3.67	10	0.035
Aphidius rhopalosiphi	1062				0.035

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.

zRMS comments: zRMS agrees with the Applicant's assessment with the off-field risk to non-target arthropods from the proposed use of **MEZOFLOR 103 SC** above. A low risk is demonstrated to the 2 standard first tier.

9.9.3.2 Additional higher-tier risk assessment

Not relevant.

9.9.3.3 Risk mitigation measures

No risk mitigation needed.

9.9.4 Overall conclusions

According to results of studies for for *Typhlodromus pyri* and *Aphidius rhopalosiphi* and risk assessment, using MEZOFLOR 103 SC in a dose of 1.25 L/ha poses no risk for non-target arthropods.

zRMS comments: Agreed.

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

9.10.1 Toxicity data

Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with mesotrione and florasulam and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of MEZOFLOR 10 SC were not evaluated as part of the EU assessment of mesotrione and florasulam. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Table 9.10-1: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna)

Species	Substance	Exposure System	Results	Reference
Eisenia fetida	Florasulam	Mixed into substrate / 56 d, chronic	NOEC = 0.203 mg/kg dw	EFSA Journal 2015; 13(1):3984
Eisenia fetida	5-OH-florasulam	Mixed into substrate / 56 d, chronic	NOEC = 0.14mg/kg dw	EFSA Journal 2015; 13(1):3984
Eisenia fetida	DFP-ASTCA	Mixed into substrate / 56 d, chronic	NOEC = 0.0304 mg/kg dw	EFSA Journal 2015; 13(1):3984
Eisenia fetida	ASTCA	Mixed into substrate / 56 d, chronic	NOEC = 1.0 mg/kg dw	EFSA Journal 2015; 13(1):3984
Eisenia fetida	TSA	Mixed into substrate / 56 d, chronic	NOEC = 10.0 mg/kg dw	EFSA Journal 2015; 13(1):3984
Eisenia fetida	MNBA	Mixed with soil using quartz sand 56 d, chronic 5% peat content	NOEC = 1050 mg/kg dw	EFSA Journal 2016;14(3):4419
Eisenia fetida	AMBA	Mixed with soil using quartz sand 56 d, chronic 5% peat content	NOEC = 1050 mg/kg dw	EFSA Journal 2016;14(3):4419
Eisenia fetida	MEZOFLOR 103 SC	Mixed into substrate 56 d, chronic 10 % peat content	EC_{10} = 7.08 mg/kg dw	Pieczka P. /2021/ G-63-20
Hypoaspis aculeifer	5-OH-florasulam	chronic	NOEC = 1.25 mg/kg d.w. soil	EFSA Journal 2015; 13(1):3984
Hypoaspis aculeifer	DFP-ASTCA	chronic	NOEC = 10 mg/kg d.w.soil	EFSA Journal 2015; 13(1):3984
Hypoaspis aculeifer	ASTCA	chronic	NOEC = 100 mg/kg d.w.soil	EFSA Journal 2015; 13(1):3984
Hypoaspis aculeifer	TSA	chronic	NOEC = 50 mg/kg d.w.soil	EFSA Journal 2015; 13(1):3984
Hypoaspis aculeifer	MEZOFLOR 103 SC	Mixed into substrate	$NOEC_{reprod.} \geq 1000$	Górska M. /2024

Species	Substance	Exposure System	Results	Reference
		14 d, chronic	mg/kg d.w. soil	/G-45-24
Folsomia candida	5-OH-florasulam	Chronic 28 days	NOEC = 2.5 mg/kg d.w. soil	EFSA Journal 2015; 13(1):3984
Folsomia candida	DFP-ASTCA	Chronic 28 days	NOEC = 10 mg/kg d.w.soil	EFSA Journal 2015; 13(1):3984
Folsomia candida	ASTCA	Chronic 28 days	NOEC = 12.5 mg/kg d.w.soil	EFSA Journal 2015; 13(1):3984
Folsomia candida	TSA	Chronic 28 days	NOEC = 50 mg/kg d.w.soil	EFSA Journal 2015; 13(1):3984
Folsomia candida	MEZOFLOR 103 SC	Mixed into substrate 28 d, chronic	$NOEC_{reprod.} \geq 1000$ mg/kg d.w. soil	Czarnynoga M. /2024/ G-44-24

9.10.2 Justification for new endpoints

During the study G-63-20 no significant changes in reproduction was observed in test item concentration of 10 mg/kg dw. EC_{10} was calculated as 7.08 mg/kg dw (Amendment no 1 to the final report) and it should be used for risk assessment as a worst case. In the final report , the obtained EC_{10} value is out of the range of tested test item concentrations, and was obtained by extrapolation. Sine according to OECD 54, ecotoxicological endpoint should be interpolated and not extrapolated, to get the most reliable values, the amendment with new statistical analysis was performed. Moreover, the new analysis is much more precise. According to *Outcome of the Pesticides Peer Review Meeting on general recurring in ecotoxicology (EFSA Supporting publication 2019:EN-1673)* normalised width of confidence interval is much lower and goodness of fit is better. Moreover, laboratory test represents the worst case, and it can be assumed, that real impact on the earthworm might be a bit lower. Therefore using EC_{10} value of 7.08 mg/kg dw is reasonable.

9.10.3 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).

9.10.3.1 First-tier risk assessment

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.3. According to the assessment of environmental-fate data, multi-annual accumulation in soil do not need to be considered for mesotrione and florasulam, but have to be considered for AMBA, metabolite of mesotrione.

Table 9.10-2: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of MEZOFLOR 103 SC in maize

Intended use	Maize

Chronic effects on earthworms			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Florasulam	0.203	0.004	51
5-OH-florasulam	0.14	0.003	47
DFP-ASTCA	0.0304	0.001	30
ASTCA	1.0	0.001	1000
TSA	10.0	0.000	> 1000
Mesotrione	1000	0.125	8000
MNBA	1050	0.052	> 20 000
AMBA	1050	0.035	30 000
MEZOFLOR 103 SC	7.08 (EC_{10})	1.328	5.33
Chronic effects on non-target soil organisms - *Hypoaspis aculeifer*			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
5-OH-florasulam	1.25	0.003	417
DFP-ASTCA	10	0.001	10000
ASTCA	100	0.001	100000
TSA	50	0.000	> 1000
MEZOFLOR 103 SC	1000	1.328	753
Chronic effects on non-target soil organisms - *Folsomia candida*			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
5-OH-florasulam	2.5	0.003	834
DFP-ASTCA	10	0.001	10000
ASTCA	12.5	0.001	12500
TSA	50	0.000	> 1000
MEZOFLOR 103 SC	1000	1.328	753

TER values shown in bold fall below the relevant trigger.

9.10.3.2 Higher-tier risk assessment

Not relevant.

9.10.4 Overall conclusions

An estimation of risk indicate low risk for earthworms, and other non-target soil organisms. Calculations conducted due to the influence **MEZOFLOR 103 SC** due to the long-term toxicity and reproductive did not indicate any hazardous properties and danger.

zRMS comment: Agreed.

Risk assessment for earthworms

Risk for earthworms for formulation **MEZOFLOR 103 SC** is low. No additional calculations for earthworms are needed.

Risk assessment for macroorganisms other than earthworms
Risk for *Folsomia candida* and *Hypoaspis aculeifer* for formulation **MEZOFLOR 103 SC** is low. No additional calculations for earthworms are needed.

All the long-term TER values are much higher than the trigger value of 5, indicating that **MEZOFLOR 103 SC** poses low acute risk to earthworms and macroorganisms other than earthworms (*Folsomia candida, Hypoaspis aculeifer*) when applied according to the proposed use rates (maize).

The studies for formulation of MEZOFLOR 103 SC for earthworms and *Folsomia candida* and *Hypoaspis aculeifer* with risk assessment was accepted by zRMS only provisionally. The toxicity endpoints were based on nominal concentration. At the end on the studies concentration of substances active fell under 80% of nominal. The TWA or geometric mean measured concentration should be calculated over the duration of the test and used if the concentration falls under 80% of nominal. The Applicant should complete the calculation the toxicity endpoints based on geometric mean measured concentration with risk assessment.

According to Regulation 284/2013 the acute risk assessment for earthworms for mesotrione and metabolite MNBA is not needed.

The Applicant notes that the active substances mesotrione and florasulam are substances with a low half-life in soil. Below, Applicant summarized, the data validated in Section B8 of the registration dossier, and the data available in the Pesticide Properties Database for mesotrione and florasulam regarding their degradation time in soil.

Active substance	DT_{50} (days) (dRR, Section B8)	DT_{90}* (lab at 20 °C)	DT_{90}* (field)	Notes*
MESOTRIONE	27.9 – pH < 7 5.4 – pH > 7 4 – worst case	46.0	57	EU dossier (2016): lab studies DT_{50} range 4.0-44.3 days, DT_{90} range 14.3-78.9 days, Soils=19; Field study DT_{50} range 3-7 days, DT_{90} range 36-78 days;
FLORASULAM	1.55	6.57	40.5	EU dossier lab studies: DT_{50} range 0.58-4.29 days, DT_{90} range 1.92-14.24 days, field studies DT_{50} range 2-18 days, DT_{90} range 23-61 days

* Data from Pesticide Properties Database :https://sitem.herts.ac.uk/aeru/ppdb/en/Reports/442.htm

Based on the above data, it is clear that the loss of active substances in the studies coded as: G-63-20; G-45-24 and G-44-24 is due to their natural degradation process in the soil.

If the active substances are absent in the soil, there can be no toxicity increase due to their action, at most the toxicity due to the action of metabolites can be calculated, if such studies are required or available at European level. Risk assessment for the metabolites of florasulame and mesotrione has been presented in

table 9.10-2 in Section B9 of dRR. Endpoints for metabolites derived from soil studies have been validated at Europen level.

In the opinion of the Applicant, recalculation of endpoints in experiments lasting according to

- OECD 222: 56 days,
- OECD 232: 28 days
- OECD 226: 14 days

based on the geometric mean of the actual active substance content at the beginning, middle and at the end of the experiment, will artificially underestimate the endpoints.

In the opinion of the Applicant, analytical measurement values after 28 or 56 days, when the content of the active substance is at the level of 6.2% (mesotrione, **Table 10.9.4-1**), and 0% (florasulam, **Table 10.9.4-2**), can not be taken into account because the toxicity caused by these substances is likely to be lacking on a par with day 0 when the content of the active substance is at the level of 84.8% (mesostrione, **Table 10.9.4-1**), 92.7% (florasulam, **Table 10.9.4-2**), especially if we are dealing with substances that degrade easily in the soil.

It is likely that in the presented by the Applicant soil studies, the greatest toxicity was caused by the active substances in the first weeks after the product was applied into the soil. It could of course translate into a later chronic effect. However, we cannot erroneously assume that an active substance content of 84% of the nominal value and 6% of the nominal value causes the same toxic effect, what we assume by averaging these results. Such a procedure results in artificially low endpoints and is not required in OECD procedure for soils organism.

This type of approach may be appropriate for substances deposited in the soil, whose DT_{50} is longer than the duration of the experiment, but for unknown reasons the analytical test will show a recovery value of less than 80% and the fate of these substances in soil is unknown. For substances that readily degrade in soil, it would be reasonable to test the toxicity of their degradation products if required.

Table 10.9.4-1 Results from analysis of mesotrione in test sample in study coded as G-63-20

Date of analysis	Concentration of mesotrione [mg/kg]	Concentration determined in particular replicates in dry weight [mg/kg]			Average [mg/kg]	SD [mg/kg]	RSD [%]	Recovery [%]
		1	2	3				
day 0 (16.04.2021)	control	ND	ND	ND	ND	-	-	-
	95.70	79.69	79.70	84.08	81.16	2.53	3.1	84.8
day 28 (14.05.2021)	control	ND	ND	ND	ND	-	-	-
	95.70	70.87	70.86	71.01	70.91	0.08	0.1	74.1
day 56 (11.06.2021)	control	ND	ND	ND	ND	-	-	-
	95.70	5.96	5.97	5.88	5.94	0.05	0.8	6.2

Table 10.9.4-2 Results from analysis of florasulame in test sample in study coded as G-63-20

Date of analysis	Concentration of florasulam [mg/kg]	Concentration determined in particular replicates In dry weight [mg/kg]			Average [mg/kg]	SD [mg/kg]	RSD [%]	Recovery [%]
		1	2	3				
day 0 (16.04.2021)	control	ND	ND	ND	ND	--	--	--
	2.80	2.61	2.61	2.57	2.60	0.02	0.9	92.7
day 28 (14.05.2021)	control	ND	ND	ND	ND	--	--	--
	2.80	1.73	1.71	1.74	1.73	0.02	0.9	61.7
day 56 (11.06.2021)	control	ND	ND	ND	ND	--	--	--
	2.80	ND	ND	ND	ND	--	--	--

The Applicant would also like to point out that:

1) According to the specificity of the experiment performed in line with OECD guideline 222, exposure of adult organisms occurs during the first 28 days (4 weeks), after which the adult earthworms are removed from the experimental system, therefore it can be assumed that the highest exposure to the active substance occurs during the first 4 weeks (28 days) of the experiment (what can translate into a chronic effect, eg. number of folding cocoons), all the more so, the use of the geometric mean of the analytical measurements, taking into account the measurement of the concentration of active substances, on day 56, for the calculation of the endpoints appears to be unfounded.
2) The OECD guidance for none of the above tests, mentions the need to recalculate endpoints depending on the concentration of active substance tested, e.g. when it falls below 80%. Such provisions are found in selected methodologies for aquatic organisms, but then another methods are also available to replace e.g. water and keep the concentration of the active substance constant to ensure that endpoints are set at exposure to a given value of the active substance.
3) A decrease in the concentration of an active substance in an experimental system, caused by the decomposition of this substance (documented low DT_{50}, DT_{90} in soil, confirmed by analytical tests) should, by definition, cause a decrease in toxicity caused by this particular substance.

   Therefore, the endpoints should be increased by the calculated (in the attached documentation) recovery factor.

   Even if an increase in toxicity would be observed in a given experiment, it would not be due to the action of the active substance, which is no longer present in the system because it has been degraded, but to the toxicity of one of its degradation metabolites.

   In conclusion, in the opinion of the Applicant, when dealing with non-persistent substances, it is not possible to determine the geometric mean of all 3 measurement points and underestimate the endpoints, because this would be tantamount to the fact that the decomposition of the active sub-

stance in its normal time in the soil, generates more toxicity caused by these substances and not by its metabolites, which seems to make no physical sense.
Therefore, the Applicant has used endpoints based on the nominal value of the active substances in the calculation. In the dRR submitted for evaluation, the applicant submitted a risk assessment based on a nominal value which is the worst case calculation.

In the opinion of the Applicant, the optional determination of new endpoints should be based on the use of the recovery factor, determined in the amendments to the test reports, according to the assumption that the loss of active substances in the soil, due to their decomposition after 56, 28 or 14 days, causes a reduction in the toxicity of the formulation resulting from the action of these substances, and consequently an increase in the value of the endpoints (**Tables 10.9.4-3, 10.9.4-4, 10.9.4-5**). The presented endpoints are not the worst case, that's why the risk assessment for the soil organisms are not recalculated for this endpoints.

Tabel 3. The endpoint values increased by the recovery factor 0.351 in the study coded as: G-63-20

Parameter	Value [mg test item/kg dry weight of artificial soil]	Value [mg of mesotrione/kg dry weight of artificial soil]
EC_{10}	7.08 (4.81 – 9.71)	9.57 (6.50 – 13.12)
EC_{20}	21.56 (16.47 – 27.27)	29.13 (22.25 – 36.84)
EC_{50}	181.44 (143.59 – 237.29)	245.13 (193.99 – 320.58)

Tabel 4. The endpoint values increased by the recovery factor 0.714 in the study coded as: G-44-24

Endpoint	Value [mg of the test item/kg dry weight of the artificial soil]	Endpoint values corrected by the recovery factor [mg of the test item/kg dry weight of the artificial soil]
LC_{10}	> 1000.0	> 1714.0
LC_{20}	> 1000.0	> 1714.0
LC_{50}	> 1000.0	> 1714.0
NOEC (survival)	≥ 1000.0	≥ 1714.0
EC_{10}	> 1000.0	> 1714.0
EC_{20}	> 1000.0	> 1714.0
EC_{50}	> 1000.0	> 1714.0

Tabel 5. The endpoint values increased by the recovery factor 0.837 in the study coded as: G-45-24

Endpoint	Value [mg of the test item/kg dry weight of the artificial soil]	Endpoint values corrected by the recovery factor [mg of the test item/kg dry weight of the artificial soil]
EC_{10}	>1000.0	>1837.40
EC_{20}	>1000.0	>1837.40
EC_{50}	>1000.0	>1837.40
NOEC (reproduction)	≥1000.0	≥1837.40
LC_{10}	>1000.0	>1837.40
LC_{20}	>1000.0	>1837.40
LC_{50}	>1000.0	>1837.40
NOEC (survival)	≥1000.0	≥1837.40

zRMS comment:

The toxicity endpoints were based on nominal concentration. At the end on the studies concentration of substances active fell under 80% of nominal. The TWA or geometric mean measured concentration should be calculated over the duration of the test and used if the concentration falls under 80% of nominal. The Applicant should complete the calculation the toxicity endpoints based on geometric mean measured concentration with risk assessment. The new data with toxicity endpoints recalculation and justification that in this case the best solution is to use endpoints based on nominal concentration in risk assessment for soil organisms was provided by Applicant. The recalculation should be treated as additional source information. However, the justification that in this case the best solution is to use endpoints based on nominal concentration in risk assessment for soil organisms was accepted by zRMS. In this case, when the concentration values fall below the limit of quantification of the analytical method, it is not possible to determine reliable toxicity endpoints based on geometrically measured concentrations. In addition, it can be noted that a decrease in the content of the active substance in the experimental system, caused by the decomposition of this substance (documented low DT_{50}, DT_{90} in soil, confirmed by analytical tests) usually causes a decrease in the toxicity caused by this substance. In the case of unstable substances, precise specification of reliable endpoints based on the measured geometric mean is not possible, especially when the concentration values fall below the limit of quantification of the analytical method. In order to reliably measure the decrease in such rapidly dispersing compounds, more time points of sampling at much shorter intervals (even within the first day) and a much higher number of repetitions may be necessary. Detailed technical guidance on the design of ecotoxicological studies is needed before analytical measurements are routinely included in ecotoxicological studies involving soil invertebrates. Therefore, the revision and validation of the technical guidelines (OECD 222, 232, 226; 2016 a, b, c) is necessary to achieve greater clarity. The study of soil organisms in ecotoxicology differs significantly from, for example, routine aquatic testing procedures, therefore the implementation of such appropriate analysis in the relevant tests cannot be easily adapted or transferred. The risk assessment for soil organisms, in this case, based on toxicity endpoints based on nominal concentration was accepted by zRMS. **This should be considered at the level of Member States.**

9.11 Effects on soil microbial activity (KCP 10.5)

9.11.1 Toxicity data

Studies on effects soil microorganisms have been carried out with mesotrione, florasulam and their rele-

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

Effects on soil microorganisms of MEZOTRON 103 SC were not evaluated as part of the EU assessment of mesotrione and florasulam. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Table 9.11-1: Endpoints and effect values relevant for the risk assessment for soil microorganisms

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	AMBA	28 d, aerobic soil type	Nitrate formation rate 1.13 mg/kg soil dw below ± 25 %	EFSA Journal 2016;14(3):4419
N-mineralisation	MNBA	28 d, aerobic soil type	Nitrate formation 1.13 mg/kg soil dw below ± 25 %	EFSA Journal 2016;14(3):4419
N-mineralisation	Florasulam	100 d, aerobic soil type	Nitrate formation 0.05 mg/kg soil dw below ± 25 %	EFSA Journal 2015; 13(1):3984
N-mineralisation	5-OH-florasulam	100 d, aerobic soil type	Nitrate formation 0.036 mg/kg soil dw below ± 25 %	EFSA Journal 2015; 13(1):3984
N-mineralisation	DFP-ASTCA	100 d, aerobic soil type	Nitrate formation 0.0076 mg/kg soil dw below ± 25 %	EFSA Journal 2015; 13(1):3984
N-mineralisation	ASTCA	100 d, aerobic soil type	Nitrate formation 1.0 mg/kg soil dw below ± 25 %	EFSA Journal 2015; 13(1):3984
N-mineralisation	TSA	100 d, aerobic soil type	Nitrate formation 0.05 mg/kg soil dw below ± 25 %	EFSA Journal 2015; 13(1):3984
N-mineralisation	MEZOFLOR 103 SC	42 d, aerobic soil type	Nitrate formation rate 7.08 mg of the test item/kg soil dw below ± 25 %	Wołany M. /2021/ G-64-20

9.11.1.1 Justification for new endpoints

Not relevant.

9.11.2 Risk assessment

The evaluation of the risk for soil microorganisms was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).
The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.3 and were already used in the risk assessment for earthworms and other

non-target soil organisms (meso- and macrofauna) (see 9.10).

Table 9.11-2: Assessment of the risk for effects on soil micro-organisms due to the use of MEZOFLOR 103 SC in maize

Intended use	Maize		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
AMBA	1.13 (at 28 d)	0.035 (plateau)	yes
MNBA	1.13 (at 28 d)	0.052	yes
Florasulam	0.05 (at 100 d)	0.004	yes
5-OH-florasulam	0.036 (at 100 d)	0.003	yes
DFP-ASTCA	0.0076 (at 100 d)	0.001	yes
ASTCA	1.0 (at 100 d)	0.001	yes
TSA	0.05 (at 100 d)	0.000	yes
MEZOFLOR 103 SC	7.08 (at 42 d)	1.328	yes

9.11.3 Overall conclusions

On the basis of results it was assessed that MEZOFLOR103 SC in considered applications does not pose unacceptable risk to soil microorganisms.

zRMS comments:

The risk assessment for soil micro-organism after exposure of **MEZOFLOR 103 SC** has been accepted by the zRMS. The effects on the nitrogen transformations are acceptable (<25%) at concentration which is higher than the maximum relevant PECs for the maximum application rate of **MEZOFLOR 103 SC**. The results indicate no adverse effect on nitrogen transformation even at soil concentrations well higher than the ones expected following application of **MEZOFLOR 103 SC.**

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

9.12.1 Toxicity data

Effects on non-target terrestrial plants of MEZOFLOR 103 SC were not evaluated as part of the EU assessment of mesotrione or florasulam. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Table 9.12-1: Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants

Species	**Substance**	**Exposure System**	**Results**	**Reference**
Pisum sativum. $_d$ *Brassica oleracea* $_d$ *Daucus carota* $_d$ *Allium cepa* $_m$ *Lolium perenne* $_m$ *Avena sativa* $_m$	MEZOFLOR 103 SC	21 d Seedling emergence	$ER_{50\ plant\ weight}$ = 118.21 g/ha $ER_{50\ plant\ weight}$ = 68.24 g/ha $ER_{50\ plant\ length}$ = 366.29 g/ha $ER_{50\ plant\ length}$ = 520.07 g/ha $ER_{50\ plant\ weight}$ > 1000 g/ha $ER_{50\ plant\ weight}$ > 1000 g/ha	Wołany M. /2021/ G-66-20

Species	Substance	Exposure System	Results	Reference
Pisum sativum. $_d$ *Brassica oleracea* $_d$ *Daucus carota* $_d$ *Allium cepa* $_m$ *Lolium perenne* $_m$ *Avena sativa* $_m$	MEZOFLOR 103 SC	21 d Vegetative vigour	$ER_{50\ plant\ weight}$ = 37.13 g/ha $ER_{50\ plant\ weight}$ = 13.67 g/ha $ER_{50\ plant\ weight}$ = 169.28 g/ha $ER_{50\ plant\ weight}$ = 19.12 g/ha $ER_{50\ plant\ weight}$ > 1000 g/ha $ER_{50\ plant\ weight}$ > 1000 g/ha	Pieczka P. /2021/ G-65-20

m: monocotyledonous; d: dicotyledonous

9.12.2 Justification for new endpoints

Not relevant.

9.12.3 Risk assessment

9.12.3.1 Tier-1 risk assessment (based screening data)

Not relevant.

9.12.3.2 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 9.12-2: Assessment of the risk for non-target plants due to the use of MEZOFLOR 103 SC in maize

Intended use	Maize
Active substance/product	MEZOFLOR 103 SC
Application rate (g/ha)	1 × 1328
MAF	1

Test species	**ER_{50} (g/ha)**		**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**TER criterion: TER ≥ 5**
Brassica oleracea	Vegetative vigour	13.67	2.77	36.8	**0.37**
Brassica oleracea	Seedling emergence	68.24	2.77	36.8	**1.85**

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

9.12.3.3 Higher-tier risk assessment

Not relevant.

9.12.3.4 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 9.12-3: Risk assessment for non-target terrestrial plants due to the use of MEZOFLOR 103 SC in maize considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles) for the most sensitive species *Brassica oleracea* based on 21 d Vegetative vigour and Seedling emergence

Intended use		Maize			
Active substance/product		MEZOFLOR 103 SC			
Application rate (g/ha)		1 × 1328			
MAF		1			
Buffer strip (m)	**Drift rate (%)**	**$PER_{off-field}$ (g/ha)**	**$PER_{off-field}$ 50 % drift red. (g/ha)**	**$PER_{off-field}$ 75 % drift red. (g/ha)**	**$PER_{off-field}$ 90 % drift red. (g/ha)**
1	2.77	36.8	18.4	9.2	3.68
5	0.57	7.6	3.8	1.9	0.76
10	0.29	3.9	1.9	0.96	0.39
15	0.2	2.7	1.3	0.66	0.27
Brassica oleracea- vegetative vigour					
Toxicity value ER_{50} = 13.67 g/ha		**TER** **criterion: TER ≥ 5**			
1		0.37	0.74	1.5	3.7
5		1.8	3.6	**7.2**	**18**
10		3.5	**7.0**	**14.0**	**35**
15		**5.1**	**10**	**20**	**51**
Brassica oleracea- Seedling emergence					
Toxicity value ER_{50} = 68.24 g/ha		TER criterion: TER ≥ 5			
1		1.9	3.7	**7.1**	**18.5**
5		**8.9**	**17.9**	35.9	89.7

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

9.12.4 Overall conclusions

Taking into consideration risk mitigation calculations for MEZOFLOR 103 SC – use in maize, following risk mitigation measures should be applied for vegetative vigour:

- **5 m buffer zone and 75 % drift reduction nozzle,**
- **10 m buffer zone and 50 % drift reduction nozzle,**

- **15 m buffer zone**

Taking into consideration risk mitigation calculations for MEZOFLOR 103 SC – use in maize, following risk mitigation measures should be applied for: seedling emergence

- **5 m buffer zone and 50 % drift reduction nozzle,**
- **1 m buffer zone and 75 % drift reduction nozzle,**
- **5 m buffer zone**

Conclusion: as worst case, risk mitigation measures for vegetative vigour was applied.

zRMS comment:

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002). It is restricted to off-field situations, as non-target plants are non-crop plants located outside the treated area. The deterministic risk based on the ER_{50} = 13.67 g/ha value *(Brassica oleracea)* from vegetative vigour test and $PER_{off\text{-}field}$, indicated needs for further refinement.
The risk following mitigation measures are proposed: **MEZOFLOR 103 SC** achieve the acceptability criteria TER ≥ 5 with applying:
- 5 m buffer zone and 75 % drift reduction nozzle,
- 10 m buffer zone and 50 % drift reduction nozzle,
- 15 m buffer zone.

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

Not relevant.

9.14 Monitoring data (KCP 10.8)

9.15 Classification and Labelling

MEZOFLOR 103 SC was classified and labeled according to REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.

For classification of MEZOFLOR 103 SC results of acute studies for water living organisms were used, as well as mixtures classification method.

Acute Category 1: Since studies results shows that for each type of organism (fish, invertebrates, algae, macrophytes), LC_{50}/EC_{50} is higher than 1 mg of product per liter. Therefore, product should not be classified as Aquatic Acute 1.

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

CLASSIFICATION	
Hazard classes, categories:	Aquatic Chronic 1
LABELLING	

Hazard pictograms:	 GHS09
Signal word:	Warning
Hazard statements:	H410 – Very toxic to aquatic life with long lasting effects
Precautionary statements:	P273 – Avoid release to the environment. P391 - Collect spillage. P501 - Dispose of contents/container to an approved waste disposal plant.

Standard phrases under Regulation (EU) No 547/2011

SP 1	Do not contaminate water with the product or its container (Do not clean application equipment near surface water/Avoid contamination via drains from farmyards and roads).
SPe 3	To protect aquatic organisms respect a: **- 4 m buffer non-spray zone with 4 meter vegetated filter strip.** To protect non-target terrestrial plants respect a: **- 5 m buffer zone and 75 % drift reduction nozzle,** **- 10 m buffer zone and 50 % drift reduction nozzle,** **- 15 m buffer zone**

zRMS comment: Agreed.

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	Czarnecka Małgorzata	2021	MEZOFLOR 103 SC *Daphnia magna*, Acute Immobilisation Test Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Study code: W-56-20 GLP, Unpublished	N	Synthos AGRO Sp z o.o.
KCP 10.2	Czarnecka Małgorzata	2021	MEZOFLOR 103 SC Lemna gibba CPCC 310, Growth inhibition test Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Study code: W-59-20 GLP, Unpublished	N	Synthos AGRO Sp z o.o.
KCP 10.2	Nierzędska Ewa	2021	MEZOFLOR 103 SC *Raphidocelis subcapitata* SAG 61.81 (formerly *Pseudokirchneriella subcapitata*), Growth inhibition test Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Study code: W-57-20 GLP, Unpublished	N	Synthos AGRO Sp z o.o.
KCP 10.2	Nierzędska Ewa	2021	MEZOFLOR 103 SC *Navicula pelliculosa* SAG 1050-3, Growth inhibition test Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Study code: W-58-20 GLP, Unpublished	N	Synthos AGRO Sp z o.o.
KCP 10.2	[redacted]	[redacted]	MEZOFLOR 103 SC Rainbow trout, Acute Toxicity Testing [redacted] GLP, Unpublished	Y	Synthos AGRO Sp z o.o.
KCP 10.3.1.1.1	Kulec-Płoszczyca Elżbieta	2021	MEZOFLOR 103 SC Honeybees (*Apis mellifera* L.), Acute Oral Toxicity Test Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Study code: B-17-21 GLP, Unpublished	N	Synthos AGRO Sp z o.o.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.1.1.2	Kulec-Płoszczyca Elżbieta	2021	MEZOFLOR 103 SC Honeybees (*Apis mellifera* L.), Acute Contact Toxicity Test Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Study code: B-18-21 GLP, Unpublished	N	Synthos AGRO Sp z o.o.
KCP 10.3.1.1.3	Kulec-Płoszczyca Elżbieta	2021	MEZOFLOR 103 SC Bumblebees (Bombus spp.), Acute Oral Toxicity Test Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Study code: B-19-21 GLP, Unpublished	N	Synthos AGRO Sp z o.o.
KCP 10.3.1.1.4	Kulec-Płoszczyca Elżbieta	2021	MEZOFLOR 103 SC Bumblebees (Bombus spp.),, Acute Contact Toxicity Test Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Study code: B-20-21 GLP, Unpublished	N	Synthos AGRO Sp z o.o.
KCP 10.3.1.2	Kulec-Płoszczyca Elżbieta	2021	MEZOFLOR 103 SC Honeybees (*Apis mellifera* L.), Chronic Oral Toxicity Test Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Study code: B-16-21 GLP, Unpublished	N	Synthos AGRO Sp z o.o.
KCP 10.1.2	Grimm T., Dietzen C., von Blanckenhagen F.	2013	„Generic field study on small mammals focal species and wood mouse (Apodemus sylvaticus) PT in maize fields in Germany". Rifcon GmbH. Oxon unpublished Report No.: R12225 GLP, Unpublished	Y	Oxon Italia, S.p.A.,
KCP 10.3.1.3	Orzechowska Urszula	2021	Honey Bee Larval Toxicity Test following Repeated Exposure to the test item MEZOFLOR 103 SC according to OECD GD 239 ENV/JM/MONO(2016)34 SORBOLAB Research Laboratory LLC Study code: 0030/0017/E GLP, Unpubished	N	Synthos AGRO Sp z o.o.
KCP 10.3.2	Kulec-Płoszczyca Elżbieta	2021	A laboratory test for evaluating the effects of MEZOFLOR 103 SC on the predatory mite, *Typhlodromus pyri* (Sch.) Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Study code: B-14-21 GLP, Unpublished	N	Synthos AGRO Sp z o.o.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.2	Kulec-Płoszczyca Elżbieta	2021	A laboratory test for evaluating the effects of MEZOFLOR 103 SC on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani - Perez) Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Study code: B-15-21 GLP, Unpublished	N	Synthos AGRO Sp z o.o.
KCP 10.4	Pieczka Paweł	2021	MEZOFLOR 103 SC Earthworm reproduction test (Eisenia andrei) Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Study code: G-63-20 GLP, Unpublished	N	Synthos AGRO Sp z o.o.
KCP 10.4	Pieczka Paweł	2021	MEZOFLOR 103 SC Earthworm reproduction test (Eisenia andrei), AMENDMENT NO. 2 TO THE FINAL REPORT Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Study code: G-63-20 GLP, Unpublished	N	Synthos AGRO Sp z o.o.
KCP 10.4.2	Czrnynoga Magdalena	2024	MEZOFLOR 103 SC Collembolan (*Folsomia candida*) Reproduction Test, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Study code: G-44-24 GLP, Unpublished	N	Synthos AGRO Sp z o.o.
KCP 10.4.2	Czrnynoga Magdalena	2024	MEZOFLOR 103 SC Collembolan (*Folsomia candida*) Reproduction Test, AMENDMENT NO. 1 TO THE FINAL REPORT Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Study code: G-44-24 GLP, Unpublished	N	Synthos AGRO Sp z o.o.
KCP 10.4.2	Górka Małgorzata	2024	MEZOFLOR 103 SC Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Study code: G-45-24 GLP, Unpublished	N	Synthos AGRO Sp z o.o.
KCP 10.4.2	Górka Małgorzata	2024	MEZOFLOR 103 SC Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test, AMENDMENT NO. 1 TO THE FINAL REPORT	N	Synthos AGRO Sp z

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Study code: G-45-24 GLP, Unpublished		o.o.
KCP 10.5	Wołany Magdalena	2021	MEZOFLOR 103 SC Soil Microorganisms: Nitrogen Transformation Test Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Study code: G-64-20 GLP, Unpublished	N	Synthos AGRO Sp z o.o.
KCP 10.6	Pieczka Paweł	2021	MEZOFLOR 103 SC Terrestrial Plant Test: Vegetative Vigour Test Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Study code: G-65-20 GLP, Unpublished	N	Synthos AGRO Sp z o.o.
KCP 10.6	Pieczka Paweł	2024	MEZOFLOR 103 SC Terrestrial Plant Test: Vegetative Vigour Test- AMENDMENT NO. 1 TO THE FINAL REPORT Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Study code: G-65-20 GLP, Unpublished	N	Synthos AGRO Sp z o.o.
KCP 10.6	Wołany Magdalena	2021	MEZOFLOR 103 SC Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Study code: G-66-20 GLP, Unpublished	N	Synthos AGRO Sp z o.o.
KCP 10.6	Wołany Magdalena	2024	MEZOFLOR 103 SC Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test- AMENDMENT NO. 1 TO THE FINAL REPORT Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Study code: G-66-20 GLP, Unpublished	N	Synthos AGRO Sp z o.o.

List of data submitted in support of the evaluation

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	**Vertebrate study** Y/N	Owner
KCP 10.1.2.2	Schneider, E.	2016	Determination of mesotrione and its metabolite (MNBA) residues in maize following treatment with Mesotrione 100 SC under field conditions in Northern and Southern France in 2015. ANADIAG, Report No. B5116 GLP, unpublished	**N**	Globachem NV
KCP 10.1.2.2	Hazlerigg C., Garrat J.	2016	A kinetic analysis of the dissipation of mesotrione in maize Report No. E2016-13 Enviresearch Non GLP, unpublished	**N**	Globachem NV
KCP 10.1.2.2	Katzschner I., Grimm T.	2019	Generic monitoring of European hares to determine proportion of time spent foraging in early maize in Central Europe Report No. R1740045 RIFCON GmbH GLP, Unpublished	**Y**	Syngenta

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

Data point	**Author(s)**	**Year**	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
KCP 10.1	[redacted]	[redacted]	Generic field monitoring of birds and mammals on maize and beet fields in Austria Report no.: WFC/FS 017 GLP	Y	---
KCP 10.1.1	[redacted]	[redacted]	XDE-570 An acute Toxicity Study with the Japanese Quail	Y	Dow AgroScience
KCP 10.1.1	[redacted]	[redacted]	XDE-570 A reproduction study with the Mallard (*Anas platyrhynchos*)	Y	Dow AgroScience
KCP	[redacted]	[redacted]	Acute oral toxicity of mesotrione to *Colinus virginianus*	Y	---

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
10.1.1			GLP, not published		
KCP 10.1.1	---	[redacted]	Reproductive toxicity of mesotrione to *Anas platyrhynchos* GLP, not published	Y	---
KCP 10.1.1	[redacted]	[redacted]	ZA1296 - Statistical Re-analysis: Effects on reproduction in mallard duck (Anas platyrhynchos) Syngenta [redacted] This is CONFIDENTIAL INFORMATION	Y	Syngenta
KCP 10.1.2	---	---	Two generation reproductive toxicity in the rat GLP, not published	Y	---
KCP 10.1.2	[redacted]	[redacted]	ZA1296: Acute oral toxicity to the rat GLP, not published	Y	Syngenta
KCP 10.1.2	[redacted]	[redacted]	Acute oral toxicity to the rat GLP, not published	Y	---
KCP 10.1.2	[redacted]	[redacted]	ZA 1296: Multigeneration study in the rat GLP, not published	Y	Syngenta
KCP 10.2	[redacted]	[redacted]	Acute toxicity of mesotrione to *Oncorhynchus mykiss* GLP, not published	Y	Syngenta
KCP 10.2	[redacted]	[redacted]	Evaluation of the Acute Toxicity of XDE-570 Herbicide to the Rainbow Trout, Oncorhynchus mykiss Walbaum. DECO-ES-2940 DECO-ES-2940 GLP, not published	Y	Dow AgroScience
KCP 10.2	[redacted]	[redacted]	Chronic toxicity of mesotrione to *Pinephales promelas* GLP, not published	Y	Syngenta
KCP 10.2	[redacted]	[redacted]	Acute toxicity of MNBA (97.1% purity) to *Oncorhynchus mykiss* GLP, not published	Y	Syngenta

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
KCP 10.2	[redacted]	[redacted]	Acute toxicity of AMBA (99% purity) to *Oncorhynchus mykiss* GLP, not published	Y	Syngenta
KCP 10.2	[redacted]	[redacted]	Florasulam technical: an early life-stage toxicity test with the fathead minnow (Pimephales promelas). Dow AgroSciences, Study Number 101334. GLP, unpublished	Y	Dow AgroScience
KCP 10.2	Dark R.	2013	ZA1296 - Statistical Re-analysis: Toxicity to the Green Alga Selenastrum capricornutum Syngenta tecsolve, North Ascot, United Kingdom, ZA1296/0214/1 Not GLP, not published Syngenta File No ZA1296_10173	N	Syngenta
KCP 10.2	Dark R.	2013a	MNBA - Statistical Re-analysis: Toxicity to the Green Alga Selenastrum capricornutum Syngenta tecsolve, North Ascot, United Kingdom, ZA1296/0533/1 Not GLP, not published Syngenta File No CA3511_10008	N	Syngenta
KCP 10.2	Dark R.	2013b	AMBA - Statistical Re-analysis: Toxicity to the Green Alga Selenastrum capricornutum Syngenta tecsolve, North Ascot, United Kingdom, AMBA/0220/1 Not GLP, not published Syngenta File No R044276_10005	N	Syngenta
KCP 10.2	Gentle & Hamer	1995	Acute toxicity of mesotrione to *Daphnia magna* GLP, not published	N	Syngenta
KCP 10.2	Hancock G.A.	2007	5-Hydroxy-florasulam: growth inhibition test with the aquatic plant duckweed, *Lemna gibba* GLP, unpublished	N	Dow AgroScience
KCP 10.2	Kelly C.R.	1997	XDE-570: Toxicity to the sediment dwelling fhase of the midge *Chirinomus riparius*, GLP, unpublished	N	Dow AgroScience
KCP 10.2	Kent & Shillaber	1997	Acute toxicity of MNBA (97.1% purity) to *Daphnia magna*	N	Syngenta

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
			GLP, not published		
KCP 10.2	Kirk H.D. et al.	1995	Evaluation of the acute toxicity of XDE-570 herbicide to the daphnid, Daphnia magna STRAUS GLP, unpublished	N	Dow AgroScience
KCP 10.2	Kirk H.D. et al.	1995	Evaluation of the acute toxicity of 5-hydroxy XDE-570 herbicide to the daphnid, Daphnia magna STRAUS GLP, unpublished	N	Dow AgroScience
KCP 10.2	Kirk H.D. et al.	1996	Evaluation of the chronic toxicity of XDE-570 herbicide to the daphnid, Daphnia magna STRAUS, GLP, unpublished	N	Dow AgroScience
KCP 10.2	Kirk H.D.	1998	Toxicity of metabolites of XDE-570 to *Daphnia magna*. GLP, unpublished	N	Dow AgroScience
KCP 10.2	Kirk H.D. et al.	2000	5-(Aminosulfonyl)-1H-1,2,4-triazole-3-carboxylic acid (florasulam M4 metabolite): growth inhibition test with the freshwater aquatic plant, duckweed, *Lemna gibba* L. GLP, unpublished	N	Dow AgroScience
KCP 10.2	Kirk H.D. et al.	2000	5-(Aminosulfonyl)-1H-1,2,4-triazole-3-carboxylic acid (florasulam M4 metabolite): growth inhibition test with the freshwater green alga, *Selenastrum capricornutum* PRINTZ GLP, unpublished	N	Dow AgroScience
KCP 10.2	Liedtke A.	2013	ZA1296 - Statistical Re-analysis: Chronic Toxicity to Daphnia magna Syngenta Harlan Laboratories Ltd., Itingen, Switzerland, D79284 Not GLP, not published Syngenta File No ZA1296_10163	N	Syngenta
KCP 10.2	Liedtke A.	2013a	ZA1296 - Statistical Re-analysis: Toxicity to Lemna gibba Syngenta Harlan Laboratories Ltd., Itingen, Switzerland, D83053 Not GLP, not published	N	Syngenta

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
			Syngenta File No ZA1296_10164		
KCP 10.2	Liedtke, A.	2013b	R44276 - Toxicity to the aquatic higher plant *Lemna gibba* in a 7-day growth inhibition test. Report Number D55614. Harlan Laboratories Ltd., Itingen, Switzerland GLP, not published	N	Syngenta
KCP 10.2	Liedtke, A.	2013c	R169649 - Toxicity to the aquatic higher plant *Lemna gibba* in a 7-day growth inhibition test. Report Number D55592. Harlan Laboratories Ltd., Itingen, Switzerland. (	N	Syngenta
KCP 10.2	Liedtke, A.	2013d	SYN546974 - Toxicity to the aquatic higher plant Lemna gibba in a 7-day growth inhibition test Syngenta Harlan Laboratories Ltd., Itingen, Switzerland, D77394 GLP, not published	N	Syngenta
KCP 10.2	Magor & Gore	1998	Acute toxicity of AMBA (99% purity) to *Daphnia magna* GLP, not published	N	Syngenta
KCP 10.2	Milazzo D.P.	1995	The toxicity of XDE-570 herbicide to aquatic plant, *Lemna gibba L.* G-3 GLP, unpublished	N	Dow AgroScience
KCP 10.2	Milazzo D.P., et al.	1995	XDE-570 herbicide: The toxicity to the green alga, *Selenastrum capricornutum* Printz GLP, unpublished	N	Dow AgroScience
KCP 10.2	Morris *et al.*	1996	Chronic toxicity of mesotrione to *Daphnia magna* GLP, not published	N	Syngenta
KCP 10.2	Porch J.R.	2011	Florasulam (TPSA metabolite): a 7-day static-renewal toxicity test with duckweed (*Lemna gibba* G3) GLP, unpublished	N	Dow AgroScience
KCP 10.2	Porch, J.R., et al.	2011	TPSA metabolite of florasulam: a 96-hour toxicity test with the freshwater alga (*Pseudokirchneriella subcapitata*). GLP, unpublished	N	Dow AgroScience
KCP 10.2	Rebstock M.	2011	5-OH-ASTP metabolite of florasulam (X12251401): growth inhibition test with the unicellular green alga, *Pseudokirchneriella subcapitata.* GLP, unpublished	N	Dow AgroScience

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	Rebstock M.	2011	ASTP metabolite of florasulam (X516274): growth inhibition test with the unicellular green alga, *Pseudokirchneriella subcapitata.* GLP, unpublished	N	Dow AgroScience
KCP 10.2	Rebstock M.	2011	DFP-ASTCA metabolite of florasulam (X12239339): growth inhibition test with the freshwater aquatic plant, duckweed, *Lemna gibba* GLP, unpublished	N	Dow AgroScience
KCP 10.2	Rebstock M.	2011	5-OH-ASTP metabolite of florasulam (X12251401): growth inhibition test with the freshwater aquatic plant, duckweed, *Lemna gibba* GLP, unpublished	N	Dow AgroScience
KCP 10.2	Rebstock M.	2011	TSA metabolite of florasulam (X634074): growth inhibition test with the freshwater aquatic plant, duckweed, *Lemna gibba* GLP, unpublished	N	Dow AgroScience
KCP 10.2	Rebstock M.	2011	ASTP metabolite of florasulam (X516274): growth inhibition test with the freshwater aquatic plant, duckweed, *Lemna gibba* GLP, unpublished	N	Dow AgroScience
KCP 10.2	Rebstock M.	2011	DFP-ASTCA metabolite of florasulam (X12239339): growth inhibition test with the unicellular green alga, *Pseudokirchneriella subcapitata* GLP, unpublished	N	Dow AgroScience
KCP 10.2	Rebstock M.	2011	TSA metabolite of florasulam (X634074): growth inhibition test with the unicellular green alga, *Pseudokirchneriella subcapitata*. GLP, unpublished	N	Dow AgroScience
KCP 10.2	Shillabeer, kent & Smith	1997	Chronic toxicity of mesotrione to *Pseudokirchneriella subcapitata* GLP, not published	N	Syngenta
KCP 10.2	Smith, Magor & Shillabeer	1998c	Chronic toxicity of AMBA (99% purity) to *Pseudokirchneriella subcapitata* GLP, not published	N	Syngenta
KCP 10.2	Smyth *et al.*	1997c	Chronic toxicity of MNBA (97.1% purity) to *Pseudokirchneriella subcapitata*	N	Syngenta

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
			GLP, not published		
KCP 10.2	Smyth *et al.*	1997d	Chronic toxicity of mesotrione to *Lemna gibba* GLP, not published	N	Syngenta
KCP 10.2	Taylor S., Taylor J.	2013b	ZA1296 - Statistical Re-analysis: Chronic toxicity to fathead minnow (Pimephales promelas) embryos and larvae Syngenta Cambridge Environmental Assessments, United Kingdom, CEA.1043 Not GLP, not published Syngenta File No ZA1296_10151	Y	Syngenta
KCP 10.2	Ward T.J. et al.	1995	XDE-570: Acute toxicity to the grass shrimp, *Palaemonetes pungo* GLP, unpublished	N	Dow AgroScience
KCP 10.3.1	Beech, P.	1996	A Determination of the Oral LD50s for XDE 570 against the Honey Bee, Apis mellifera Agrochemical Evaluation Unit, Department of Biology, The University, Southampton, UK DOW-96-3 GHE-P-6705 GLP/GEP (Y/N) Y Published (Y/N) N	N	Dow AgroScience
KCP 10.3.1	Jackson, D. & Gough, H.J.	1995	ZA 1296: Acute Contact and Oral Toxicity to the Honey Bees (Apis mellifera) of Technical Material GLP, not published	N	Syngenta
KCP 10.3.1	Palmer, SJ	1994	XDE-570: An Acute Contact Study with the Honey Bee Wildlife International Ltd, Easton, Maryland, USA 103-407 DECO-ES-2819 GLP/GEP (Y/N) Y Published (Y/N) N	N	Dow AgroScience
KCP 10.3.2	Austin, H.M.	1997	A Laboratory Study to Evaluate the Effects of XDE-570 on the Predatory Mite, Typhlodromus pyri Ecotox Limited, Tavistock, Devon, UK ER-96-31 GHE-P-6706 GLP/GEP (Y/N) Y Published (Y/N) N	N	Dow AgroScience
KCP 10.3.2	Austin, H.M.	1997	A Laboratory Study to Evaluate the Effects of XDE-570 on the Parasitic Wasp, Aphidius rhopalosiphi Ecotox Limited, Tavistock, Devon, UK ER-96-34 GHE-P-6707 GLP/GEP (Y/N) Y	N	Dow AgroScience

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**
			Published (Y/N) N		
KCP 10.4	Bembridge, J.D. & Jackson, D.	1996	ZA 1296: Toxicity of Technical Material to the Earthworm Eisenia fetida in an Artificial Soil Test. Zeneca Agrochemicals Report No: RJ2225B. DP 59893.	N	Syngenta
KCP 10.4	Friedrich S,	2013a	R169649 – Sublethal Toxicity to the Earthworm Eisenia fetida in Artificial Soil with 5 % Peat, Report Number 13 10 48 086 S. BioChem agrar Labor für biologische und chemische Analytik GmbH, Kupferstraße 6 04827 Gerichshain, Germany (Syngenta file No. CA3511_10002).	N	Syngenta
KCP 10.4	Friedrich S.	2013b	R44276 – Sublethal Toxicity to the Earthworm Eisenia fetida in Artificial Soil with 5% Peat, Report Number 13 10 48 111 S. BioChem agrar Labor für biologische und chemische Analytik GmbH, Kupferstraße 6, 04827 Gerichshain, Germany. (Syngenta file No. R044276_10002).	N	Syngenta
KCP 10.4	Lührs, U.	2008b	Effects of ASTCA metabolite of florasulam on reproduction and growth of earthworms Eisenia fetida in artificial soil Institut fiir Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 080038 (Accession Number) 2001599 GLP/GEP (Y/N): Y Published (Y/N): N	N	Dow AgroScience
KCP 10.4	Witte, B.	2010b	Effects of 5-hydroxy-florasulam on reproduction and growth of earthworms Eisenia fetida in artificial soil Institut fiir Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 101340 (Accession Number) 2006605 GLP/GEP (Y/N): Y Published (Y/N): N	N	Dow AgroScience
KCP 10.4	Witte, B.	2011a	Effects of DFP-ASTCA metabolite of florasulam on reproduction and growth of earthworms Eisenia fetida in artificial soil Institut fiir Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 101341	N	Dow AgroScience

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**
			(Accession Number) 2009374 GLP/GEP (Y/N): Y Published (Y/N): N		
KCP 10.4	Witte, B.	2011b	Effects of TSA metabolite of florasulam on reproduction and growth of earthworms Eisenia fetida in artificial soil Institut fiir Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 110132 (Accession Number) 2009730 GLP/GEP (Y/N): Y Published (Y/N): N	N	Dow AgroScience
KCP 10.5	Feil, N.	2008	Effects of ASTCA metabolite of florasulam on the activity of the soil microflora in the laboratory. Institut fiir Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 080039 (Accession Number) 2000205 GLP/GEP (Y/N): Y Published (Y/N): N	N	Dow AgroScience
KCP 10.5	Feil, N.	2010	Effects of 5-hydroxy-florasulam on the activity of the soil microflora in the laboratory Institut fiir Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 101342 (Accession NNumber) 2007411 GLP/GEP (Y/N): Y Published (Y/N): N	N	Dow AgroScience
KCP 10.5	Feil, N.	2011a	Effects of DFP-ASTCA metabolite of florasulam on the activity of the soil microflora in the laboratory. Institut fiir Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 101343 (Accession Number) 2009901 GLP/GEP (Y/N): Y Published (Y/N): N	N	Dow AgroScience
KCP 10.5	Feil, N.	2011b	Effects of TSA metabolite of florasulam on the activity of the soil microflora in the laboratory Institut fiir Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 110143	N	Dow AgroScience

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**
			(Accession Number) 2010747 GLP/GEP (Y/N): Y Published (Y/N): N		
KCP 10.5	Schulz L,	2013b	R169649 and R44276 – Effects on the Activity of Soil Microflora (Nitrogen and Carbon Transformation Tests), Report Number 12 10 48 045 C/N. BioChem agrar, Labor für biologische und chemische Analytik GmbH, Kupferstraâe 6, 04827 Gerichshain, Germany (Syngenta file No. CA3511_10000).	N	Syngenta

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of the new studies

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

Study 1

Refinement of PT value and focal species

zRMS comment: The PT value of 0.139 for wood mouse was accepted at the EU level during mesotrione evaluation. The focal species for maize at early BBCH growth stages such as wood mouse and brown hare were accepted by zRMS

Report	„Generic field study on small mammals focal species and wood mouse (*Apodemus sylvaticus*) PT in maize fields in Germany". [redacted]
Guideline(s):	No official test guideline(s) available at present
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No
Conclusion:	The study aimed at monitoring the abundance of small mammals species in general in early growth stage in maize fields in Germany and of wood mice (Apodemus sylvaticus) in particular, since available data indicate that this may be an appropriate focal species for this stage. Confirmatory data for field study are available in Part C

zRMS comment: The f_{TWA} value used to refine the risk to mammals was calculated based on the residue level decrease study for the active substance mesotrione in corn (DT_{50}= 14h), authored by Schneider E. from 2016: "Determination of mesotrione and its metabolite (MNBA) residue In maize following treatment with Mesotrione 100 SC under field conditions in northern and southern France in 2015, together with the report by Hazlerigg C.: "A kinetic analysis of the dissipation of mesotrione in maize". The Applicant does not have the full report: "A kinetic analysis of the dissipation of mesotrione in maize". The risk calculation used unprotected data contained in the TEMSA SC product documentation (mesotrione, 100g/L), in which the report was described in detail. Authorization of the TEMSA SC product was renewed in Poland in accordance with the decision (MRiRW Permit No. R-190/2015 of 29.10.2015 renewed by MRiRW Decision No. R – 25 /2021o of 23.06.2021) of 23.06.2021. These studies were presented in connection with the need to refine the risk assessment for two mammalian species: *Apodemus sylvaticus* and *Lepus europaeus*, the data were necessary to obtain an acceptable risk assessment for the product TEMSA SC (mesotrione, 75-125 g/ha), in the process of renewal of the authorization in Poland.

The possibility of using these residue studies to refine risk assessments should be considered at Member State level.

Normally a DT_{50} of 10 days is assumed in the birds and mammals risk assessment as a default value. For this product **MEZOFLOR 103 SC** however, a lower DT_{50} could be expected based on five plant residue trials that were conducted in Europe (see dR Part B7 for a description of these studies). A kinetic analysis of the dissipation of mesotrione in maize was conducted by enviresearch and a report of this study was submitted to support this refinement of the DT_{50} of mesotrione on plants (Hazlerigg & Garratt, 2016).

FOCUS (2006, 2014) degradation kinetics guidance was applied to calculate DT_{50} endpoints for mesotrione modelling from residues measured in five plant residue trials in Europe. The data were described reasonably well by either SFO kinetics or bi-phasic FOMC kinetics and acceptable endpoints were derived for all five studies.

The calculated DT_{50} values and statistics for the decline of mesotrione in maize are shown in the table below. The DT_{50} values ranged from 10.1 to 21.9 hours. The final DT_{50} recommended for modelling is the geomean of 14 hours. The DT_{50} = 14 hours as geometric mean was also proposed by Applicant. However, according to the harmonization arrangements for Poland, when the tests include 4 - 9 locations - maximum values can be used DT_{50}. The worst case is DT_{50} = 21.9 hours and this value should be used in risk assessment. Estimated new f_{TWA} = 0.063 based on residue decline study will be used as a risk refinement for reproductive risk to mammals in post-emergence use. MAFm * TWA (refined DT_{50}) = 0.063 should be used in risk assessment. The registration dossier included 5 studies on the dynamics of residue depletion in maize (4 in the N-EU zone and 1 in the S-EU zone), on the basis of which the DT_{50} value was determined. In the opinion of RMS, one study of the southern zone residues (B5116 EF1) undermines the validity of using the geometric mean and should be excluded from the analysis. It is most reasonable to use the maximum value can be used DT_{50}.

Trial	Site	European area	Crop, variety	Trial type
B5116 AN1	Seebach, Alsace, France	North	Maize, Karedas	Decline curve
B5116 MA1	Donnelay, Lorraine, France	North	Maize, P3184	Decline curve
B5116 BM1	Thorée les Pins, Pays de la Loire, France	North	Maize, P9074	Decline curve
B5116 ND1	Hérin, Nord Pas de Calais, France	North	Maize, Ramses	Decline curve
B5116 EF1	Saint-Livrade, Aquitaine, France	South	Maize, Roberi	Decline curve

Table: Summary of fitted parameters for the decline of mesotrione.

Study	Kinetic model	t-test	χ2-error	Visual fit	DT50 (hours)
B5116 AN1	FOMC	n/a	7.93	Good	13.3 *
B5116 MA1	SFO	Pass	6.92	Good	21.9
B5116 BM1	SFO	Pass	14.3	Medium	10.1
B5116 ND1	FOMC	n/a	6.74	Good	15.2 *
B5116 EF1	FOMC	n/a	10.9	Good	12.1 *
Geomean of all trials					**14.0**

* Pseudo first-order DT_{50} calculated as FOMC DT_{90}/3.32 (FOCUS 2006, 2014)

Estimated new f_{TWA} = 0.063 based on residue decline study will be used as a risk refinement for reproductive risk to mammals in post-emergence use. The worst case is DT_{50} = 21.9 hours. Refinement of DT_{50} should be considered at MSs level.

Study 2

Report	Determination of mesotrione and its metabolite (MNBA) residues in maize following treatment with Mesotrione 100 SC under field conditions in Northern and Southern France in 2015. Schneider, E., 2016, Study No B5116
Guideline(s):	Yes, OECD 509, SANCO 7525/VI/95 rev. 10.3, SANCO/825/00 rev.9.1, SANCO/3029/99 rev.4
Deviations:	Yes, the weight of 1 analytical sample and 7 spare samples was below 0.4 kg as required by the study plan. The deviation has no impact on the study as more as 12 plants were collected for each sample. Moreover the weight of the analytical sample is close to 0.4 kg and spare samples should not be analysed
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No
Materials and methods	*Field phase*: 4 residue trials were carried out in Northern Europe (Northern France) and 1 residue trial was carried out in Southern Europe (Southern France). In each trial, one plot was treated once with Mesotrione 100 g/L SC at the application rate of 1.5 L/ha (150 g/ha mesotrione). The growth stage of the maize at application was BBCH 14-16. The application volume was 200-400 L/ha. A second plot remained untreated. Only at 1 trial site minimal rainfall occurred during the study, while no rainfall was recorded at the other 4 trial sites for the duration of the study.
Results and discussions	Residues in control samples were non-detectable or below the limit of quantification. The residue results in whole plant for mesotrione and MNBA in the treated specimens are summarized below:

Trial No.	Analyte	Residues (mg/kg) 0 HAA	1 HAA	2 HAA	4 HAA	7 HAA	21 HAA	28 HAA	49 HAA	3 DAA
B5116 AN1	Mesotrione	27.08 * 34.46 **	35.04	28.97	22.43	18.38	10.87	5.67	2.90	1.13
	MNBA	0.06* 0.06**	0.21	0.37	0.63	0.82	0.81	0.51	0.44	0.22
B5116 MA1	Mesotrione	20.21* 30.17**	21.25	20.12	18.97	19.32	13.56	7.96	4.27	1.15
	MNBA	0.02* 0.02**	0.09	0.09	0.19	0.42	0.64	0.43	0.27	0.11
B5116 BM1	Mesotrione	32.17* 30.06**	35.60	25.08	23.37	18.11	11.20	4.32	0.76	0.39
	MNBA	0.04* 0.03**	0.23	0.29	0.79	0.82	1.27	0.62	0.31	0.25
B5116 ND1	Mesotrione	16.42* 19.16**	15.41	13.14	11.85	8.17	5.13	3.07	1.71	0.38
	MNBA	0.02* 0.02**	0.07	0.17	0.42	0.58	0.45	0.54	0.50	0.21
B5116 EF1	Mesotrione	24.31* 34.02**	27.85	25.42	18.05	10.02	7.81	6.55	2.57	1.03
	MNBA	0.03* 0.03**	0.26	0.45	0.54	0.60	0.61	0.51	0.37	0.22

* Analytical sample, the results of these samples were not used to calculate the mesotrione half-life.
** Retain sample, the results of these samples were not used to calculate the mesotrione half-life.
HAA: Hour after application
DAA: Days after application
LOQ = 0.01 mg/kg

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Active ingredient:	**Mesotrione**	Producer of commercial product:	GLOBACHEM NV
Crop/crop group:	Maize/Maize		
Responsible body for reporting:	ANADIAG, 16 rue Ampère		
(name, address):	67500 HAGUENAU, France		
Country:	Northern France	Indoor/Glasshouse/Outdoor:	Outdoor
Content of active substance (g/kg or g/L):	100 g/L	Other a.s. in formulation:	-
Formulation (e.g. WP):	SC	(common name and content):	
Commercial product (name):	**Mesotrione 100 SC**	Residues calculated as:	mg/kg mesotrione and MNBA

1	2	3	4	5			6	7	8	9	10	11	12
Report-No ; Location including Postal Code	**Commodity /Variety** (a)	**Date of 1) Planting 2) Flowering 3) Harvest** (b)	**Method of treatment** (c)	**Application rate per treatment (actual)** g a.s./ha	Water (L/ha)	g a.s./hL	**Dates of treatment(s) or No. of treatment(s) and last date** (d)	**Growth stage at last treatment or date** (e)	**Portion analysed** (a)	**Mesotrione residues (mg/kg)**	**MNBA residues (mg/kg)**	**PHI** (f)	**Remarks** (g)
B5116 AN1 Seebach 67160 Northern France	Maize / Karedas	1) 16/04/2015 2) n.r. 3) not recorded	Medium volume spraying Overall spraying	159.0	318	50.0	03/06/2015	14-16	Whole plants	35.04	0.21	1 hour	LOQ = 0.01 mg/kg
									Whole plants	28.97	0.37	2 hours	
									Whole plants	22.43	0.63	4 hours	
									Whole plants	18.38	0.82	7 hours	
									Whole plants	10.87	0.81	21 hours	
									Whole plants	5.67	0.51	28 hours	
									Whole plants	2.90	0.44	49 hours	
									Whole plants	1.13	0.22	3 days	

Remarks:
(a) According to EEC and Codex Classification (both) should be used
(b) Only if relevant
(c) High or low volume spraying, spreading, dusting etc
(d) Year must be indicated
(e) BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4
(f) Minimum number of days after last application (Label pre-harvest interval, PHI, underline)
(g) Remarks may include: Climatic conditions; Reference to analytical method and information on which metabolites are included
n.r.: not recorded

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Active ingredient:	**Mesotrione**	Producer of commercial product:	GLOBACHEM NV
Crop/crop group:	Maize/Maize		
Responsible body for reporting:	ANADIAG, 16 rue Ampère		
(name, address):	67500 HAGUENAU, France		
Country:	Northern France	Indoor/Glasshouse/Outdoor:	Outdoor
Content of active substance (g/kg or g/L):	100 g/L	Other a.s. in formulation:	-
Formulation (e.g. WP):	SC	(common name and content):	
Commercial product (name):	**Mesotrione 100 SC**	Residues calculated as:	mg/kg mesotrione and MNBA

1	2	3	4	5			6	7	8	9	10	11	12
Report-No ; Location including Postal Code	**Commodity /Variety** (a)	**Date of 1) Planting 2) Flowering 3) Harvest** (b)	**Method of treatment** (c)	**Application rate per treatment (actual)** g a.s./ha	Water (L/ha)	g a.s./hL	**Dates of treatment(s) or No. of treatment(s) and last date** (d)	**Growth stage at last treatment or date** (e)	**Portion analysed** (a)	**Mesotrione residues (mg/kg)**	**MNBA residues (mg/kg)**	**PHI** (f)	**Remarks** (g)
B5116 MA1 Donnelay 57810 Northern France	Maize / P3184	1) 13/04/2015 2) 10/07/2015 to 15/07/2015 3) 26/08/2015	Medium volume spraying Overall spraying	142.5	285	50.0	02/08/2015	16	Whole plants	21.25	0.09	1 hour	LOQ = 0.01 mg/kg
									Whole plants	20.12	0.09	2 hours	
									Whole plants	18.97	0.19	4 hours	
									Whole plants	19.32	0.42	7 hours	
									Whole plants	13.56	0.64	21 hours	
									Whole plants	7.96	0.43	28 hours	
									Whole plants	4.27	0.27	49 hours	
									Whole plants	1.15	0.11	3 days	

Remarks:
(a) According to EEC and Codex Classification (both) should be used
(b) Only if relevant
(c) High or low volume spraying, spreading, dusting etc
(d) Year must be indicated
(e) BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4
(f) Minimum number of days after last application (Label pre-harvest interval, PHI, underline)
(g) Remarks may include: Climatic conditions; Reference to analytical method and information on which metabolites are included
n.r.: not recorded

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Active ingredient:	**Mesotrione**	Producer of commercial product:	GLOBACHEM NV
Crop/crop group:	Maize/Maize		
Responsible body for reporting:	ANADIAG, 16 rue Ampère		
(name, address):	67500 HAGUENAU, France		
Country:	Northern France	Indoor/Glasshouse/Outdoor:	Outdoor
Content of active substance (g/kg or g/L):	100 g/L	Other a.s. in formulation:	-
Formulation (e.g. WP):	SC	(common name and content):	
Commercial product (name):	**Mesotrione 100 SC**	Residues calculated as:	mg/kg mesotrione and MNBA

1	2	3	4	5			6	7	8	9	10	11	12
Report-No ; Location including Postal Code	**Commodity /Variety**	**Date of 1) Planting 2) Flowering 3) Harvest**	**Method of treatment**	**Application rate per treatment (actual)**			**Dates of treatment(s) or No. of treatment(s) and last date**	**Growth stage at last treatment or date**	**Portion analysed**	**Mesotrione residues (mg/kg)**	**MNBA residues (mg/kg)**	**PHI**	**Remarks**
				g a.s./ha	**Water (L/ha)**	**g a.s./hL**							
	(a)	(b)	(c)				(d)	(e)	(a)			(f)	(g)
B5116 BM1 Thorée les Pins 72800 Northern France	Maize / P9074	1) 20/04/2015 2) n.r. 3) n.r.	Medium volume spraying Overall spraying	142.8	238	60.0	30/05/2015	15-16	Whole plants	35.60	0.23	1 hour	LOQ = 0.01 mg/kg
									Whole plants	25.08	0.29	2 hours	
									Whole plants	23.37	0.79	4 hours	
									Whole plants	18.11	0.82	7 hours	
									Whole plants	11.20	1.27	21 hours	
									Whole plants	4.32	0.62	28 hours	
									Whole plants	0.76	0.31	49 hours	
									Whole plants	0.39	0.25	3 days	

Remarks:
(a) According to EEC and Codex Classification (both) should be used
(b) Only if relevant
(c) High or low volume spraying, spreading, dusting etc
(d) Year must be indicated
(e) BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4
(f) Minimum number of days after last application (Label pre-harvest interval, PHI, underline)
(g) Remarks may include: Climatic conditions; Reference to analytical method and information on which metabolites are included
n.r.: not recorded

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Active ingredient:	**Mesotrione**	Producer of commercial product:	GLOBACHEM NV
Crop/crop group:	Maize/Maize		
Responsible body for reporting:	ANADIAG, 16 rue Ampère		
(name, address):	67500 HAGUENAU, France		
Country:	Northern France	Indoor/Glasshouse/Outdoor:	Outdoor
Content of active substance (g/kg or g/L):	100 g/L	Other a.s. in formulation:	-
Formulation (e.g. WP):	SC	(common name and content):	
Commercial product (name):	**Mesotrione 100 SC**	Residues calculated as:	mg/kg mesotrione and MNBA

1	2	3	4	5			6	7	8	9	10	11	12
Report-No ; Location including Postal Code	**Commodity /Variety**	**Date of 1) Planting 2) Flowering 3) Harvest**	**Method of treatment**	**Application rate per treatment (actual)**			**Dates of treatment(s) or No. of treatment(s) and last date**	**Growth stage at last treatment or date**	**Portion analysed**	**Mesotrione residues (mg/kg)**	**MNBA residues (mg/kg)**	**PHI**	**Remarks**
				g a.s./ha	**Water (L/ha)**	**g a.s./hL**							
	(a)	(b)	(c)				(d)	(e)	(a)			(f)	(g)
B5116 ND1 Hérin 59195 Northern France	Maize / Ramses	1) 12/04/2015 2) n.r. 3) n.r.	Medium volume spraying Overall spraying	149.3	199	75.0	24/06/2015	16	Whole plants	15.41	0.07	1 hour	LOQ = 0.01 mg/kg
									Whole plants	13.14	0.17	2 hours	
									Whole plants	11.85	0.42	4 hours	
									Whole plants	8.17	0.58	7 hours	
									Whole plants	5.13	0.45	21 hours	
									Whole plants	3.07	0.54	28 hours	
									Whole plants	1.71	0.50	49 hours	
									Whole plants	0.38	0.2'	3 days	

Remarks:
(a) According to EEC and Codex Classification (both) should be used
(b) Only if relevant
(c) High or low volume spraying, spreading, dusting etc
(d) Year must be indicated
(e) BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4
(f) Minimum number of days after last application (Label pre-harvest interval, PHI, underline)
(g) Remarks may include: Climatic conditions; Reference to analytical method and information on which metabolites are included
n.r.: not recorded

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Active ingredient:	**Mesotrione**	Producer of commercial product:	GLOBACHEM NV
Crop/crop group:	Maize/Maize		
Responsible body for reporting:	ANADIAG, 16 rue Ampère		
(name, address):	67500 HAGUENAU, France		
Country:	Southern France	Indoor/Glasshouse/Outdoor:	Outdoor
Content of active substance (g/kg or g/L):	100 g/L	Other a.s. in formulation:	-
Formulation (e.g. WP):	SC	(common name and content):	
Commercial product (name):	**Mesotrione 100 SC**	Residues calculated as:	mg/kg mesotrione and MNBA

1	2	3	4	5			6	7	8	9	10	11	12
Report-No ; Location including Postal Code	**Commodity /Variety**	**Date of 1) Planting 2) Flowering 3) Harvest**	**Method of treatment**	**Application rate per treatment (actual)**			**Dates of treatment(s) or No. of treatment(s) and last date**	**Growth stage at last treatment or date**	**Portion analysed**	**Mesotrione residues (mg/kg)**	**MNBA residues (mg/kg)**	**PHI**	**Remarks**
				g a.s./ha	**Water (L/ha)**	**g a.s./hL**							
	(a)	(b)	(c)				(d)	(e)	(a)			(f)	(g)
B5116 EF1 Sainte-Livrade 47110 Southern France	Maize / Roberi	1) 14/04/2015 2) n.r. 3) n.r.	Medium volume spraying Overall spraying	150.0	300	50.0	26/05/2015	16	Whole plants	27.85	0.26	1 hour	LOQ = 0.01 mg/kg
									Whole plants	25.42	0.45	2 hours	
									Whole plants	18.05	0.54	4 hours	
									Whole plants	10.02	0.60	7 hours	
									Whole plants	7.81	0.61	21 hours	
									Whole plants	6.55	0.51	28 hours	
									Whole plants	2.57	0.37	49 hours	
									Whole plants	1.03	0.22	3 days	

Remarks:
(a) According to EEC and Codex Classification (both) should be used
(b) Only if relevant
(c) High or low volume spraying, spreading, dusting etc
(d) Year must be indicated
(e) BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4
(f) Minimum number of days after last application (Label pre-harvest interval, PHI, underline)
(g) Remarks may include: Climatic conditions; Reference to analytical method and information on which metabolites are included
n.r.: not recorded

Conclusion:	The data from the residue trials can be used to determine the DT_{50} of mesotrione in maize (see KCP 10.1.2.2 in section B9).

Study 3

Report	A kinetic analysis of the dissipation of mesotrione in maize, Hazlerigg C. & Garrat J, 2016, E2016-13
Guideline(s):	Yes, FOCUS guidance
Deviations:	No
GLP:	/
Acceptability:	Yes
Duplication (if vertebrate study)	/
Materials and methods	Residue trials were undertaken and reported by Schneider (2016) at 5 sites in France. Mesotrione 100 g/L was applied as a foliar spray to maize and the whole plant was analysed at intervals. An overview of the trials and the measured residues are given in the Appendix of this study and are also described in dRR Part B7 (and the relevant study report (Schneider (2016)). FOCUS (2006, 2014) degradation kinetics guidance was applied to calculate DT_{50} endpoints for mesotrione modelling from residues measured in five plant residue trials in Europe. The data were described reasonably well by either SFO kinetics or bi-phasic FOMC kinetics and acceptable endpoints were derived for all five studies
Results and discussions	The calculated DT_{50} values and statistics for the decline of mesotrione in maize are shown in Table A 4. The DT_{50} values ranged from 10.1 to 21.9 hours. The final DT_{50} recommended for modelling is the geomean of 14 hours:

Table A 6: Summary of fitted parameters for the decline of mesotrione

Study	Kinetic model	t-test	χ2-error	Visual fit	DT_{50} (hours)
B5116 AN1	FOMC	n/a	7.93	Good	13.3 *
B5116 MA1	SFO	Pass	6.92	Good	21.9
B5116 BM1	SFO	Pass	14.3	Medium	10.1
B5116 ND1	FOMC	n/a	6.74	Good	15.2 *
B5116 EF1	FOMC	n/a	10.9	Good	12.1 *
Geomean of all trials					**14.0**

* pseudo first-order DT_{50} calculated as FOMC DT90/3.32 (FOCUS 2006, 2014)

Conclusion	FOCUS (2006, 2014) guidance was applied to calculate DT_{50} endpoints for mesotrione modelling from residues measured in four plant residue trials in Europe. The data were described reasonably well by either SFO kinetics or bi-phasic FOMC kinetics. The DT_{50} values ranged from 10.1 to 21.9 hours with a geomean of 14 hours. This refined DT_{50} of 14 hours (instead of the default value of 10 days) was used in the higher tier risk assessment for the reproductive risk to mammals.

zRMS comment: zRMS agrees with the refined PT value (PT = 0.139) for wood mouse and PT value (PT = 0.62) for brown hare.

The refinement PT value for wood mouse and brown hare should be considered by MSs level. Study 4

Report	Generic monitoring of European hares to determine proportion of time spent foraging in early maize in Central Europe, Grimm T., Katzchner (2019), Syngenta Ltd., RIFCON GmbH, Report No. R1740045
Guideline(s):	No official test guideline available. Study was conducted under consideration of the EFSA Guidance Document Risk Assessment for Birds and Mammals (2009)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	/

Materials and methods

Test Material	No substance was tested.
Test organisms	
Species:	European brown hare (*Lepus europaeus*)
Crop:	Maize, BBCH 00-19
Test design	
Replication:	5 study sites
Duration of study:	3 months

Study Design and Methods
Experimental dates: April - June 2018
Study sites
The study was conducted in five study sites in Central Europe in two main areas for maize growing (with on average 36 % of the landscape surface within the home ranges of the investigated hares comprising maize). Three study sites were located in Lower Saxony (Germany), near Holtrop, Rastede and Burhafe, and two study sites were located in the administrative county Győr-Moson-Sopron (Hungary), one near Szany and one close to Bösarkany. The proportion of maize of the total landscape surface even exceeded 45% (in the study site Rastede, Germany) and 50 % (in the study site Bösarkany, Hungary) which represents the highest coverage of maize on regional scale known in Central Europe. The crop cover map of maize in Europe (with proportion of maize >20%) together with test sites location (marked with black circles) is presented on figure below.

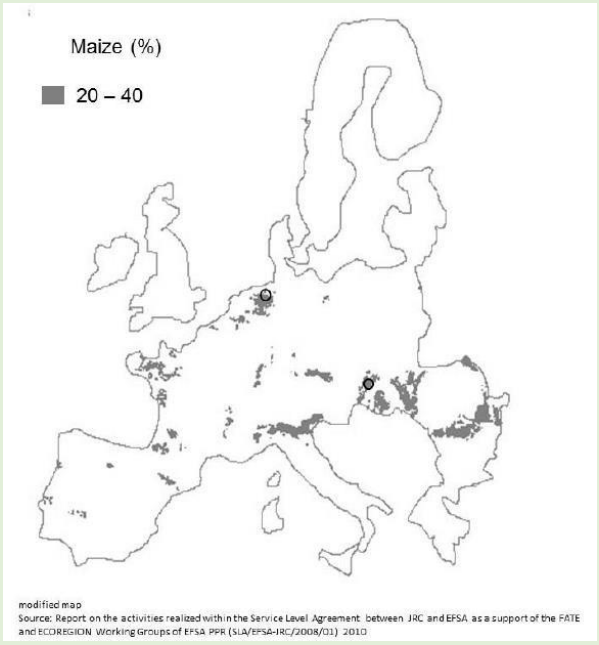


Figure: Crop cover map of maize in Europe and location of the study sites (the map presents all areas in Europe in which the proportion of maize cover of the total land surface is more than 20%)

The vegetation status of maize in the study sites was recorded using BBCH growth stage scale. The study sites, comprising the area around the trapping locations of the tagged animals and the positions in which they were recorded during single checks and 24h telemetry, were mapped. Surveys of drilled maize fields before the emergence of maize plants showed that virtually no weeds occurred on these fields, indicating that pre-emergence maize fields are likely to be unattractive habitats for hares due to their lack of food and cover.

Trapping

The majority of animals were trapped and fitted with radio tags at the beginning of the Field Phase (i.e. before the drilling of maize). All tracked hares were captured either on future maize fields (i.e. fields to become maize fields later once drilled), already drilled maize fields or nearby in off-crop structures around such maize fields.

Hares were trapped using series of nets. The animals were chased into these nets by beaters walking towards the net line.

Each trapped animal was sexed, weighed and equipped with a radio tag (Biotrack Ltd., UK; www.biotrack.co.uk) and released at the trapping site.

Radio-tracking

For radio-tracking, animals were located with Yagi antennas according to two different approaches: single check telemetry and 24h telemetry.

For single check telemetry each animal was located once at the beginning (after all hares were tagged) and the end of the entire Field Phase (when 24h telemetry was finished) in order to survey its presence in the respective study site during the entire Field Phase.

During 24h telemetry sessions the animal was radio-tracked continuously for 24 hours by two observers, locating the animal from two different positions, which allowed triangulating the animal's exact position. Each change of habitat (if possible) and/or each change of behaviour (i.e. active/inactive) was recorded with time and bearing angle to the signal of the animal. The 24h telemetry sessions were conducted when the BBCH growth stages of the maize fields were <20. Main focus was given to the period of emergence until the end of leaf development (i.e. BBCH growth stages 09 to 19).

In order to confirm the animals' behaviour based on the radio signals, animals were observed with binoculars, scopes and night observation devices to get 'visual contact' whenever possible.

Calculation of PT

	For each telemetry session, the proportion of diet obtained in maize fields (PT) was calculated as the proportion of the 'potentially foraging' time the individual hare spent in that crop. Thus, the 'time potentially foraging' is the sum of the time periods covered by behavioural categories when foraging could not be excluded. All instances when the animal was definitely known to be performing non-foraging activities (e.g. resting or fighting) were excluded from PT calculations. A mean PT value (± standard deviation) and 90$_{th}$ percentile values were calculated based on all single PT values. In addition, the total visual contact time and the respective behaviour categories during 24h telemetry were calculated in order to compare behaviour categories based on radio-tracking signals with behaviour confirmed via visual contact.
Results and discussions	In total, radio-tracking sessions of 21 individual hares at five study sites were performed during the early crop development of maize in Central Europe. Radio-tracking sessions were performed from late April until early June 2018. The number of conducted 24h telemetry sessions was 23 (17 in Germany, six in Hungary), since two individuals were radio tracked twice. One session had to be excluded from analysis, as this session was not considered as a 'consumer session' since the animal was never located being 'active' in a maize field during the session, had no maize in the 24h home range, and was not caught on a maize field. Maize fields covered on average approximately 36% of the total landscape surface and 44% of the arable land surface within the 24h home ranges of hares in all study sites. The calculated single PT values ranged from 0.02 to 0.94 resulting in an average of 0.36 (±0.26) and 90$_{th}$ percentile of 0.62. Calculated PT values did not differ substantially between different study sites; mean values were slightly higher in Germany (0.38) than in Hungary (0.31). Drilled maize fields which were not yet emerged were checked in each study site for the occurrence of weeds at the beginning of the Field Phase (except of Szany, where all maize fields, except one, were already emerged). Fields that were still not emerged prior to the start of 24h radio-tracking were checked for weed occurrence again (one field in Holtrop, one field in Szany and two fields in Bösarkany). Each survey showed that no weeds occurred on not yet emerged maize fields in the five study sites. Therefore since hares on those fields could not be foraging, those results were excluded from the PT analysis. This is clear from the photographs included in the report. The total visual contact time during all 24h-telemetry sessions was 68 hours and 39 minutes which reflects 12% of the total radio tracking time of 552 hours. In 49% of the visually observed time, hares were classified as resting. The active behaviour during visual contact was classified as 'foraging behaviour' for 32% and as 'other behaviour' (such as e.g. fighting, running or grooming) for 19%.
Conclusion	This study demonstrated that maize fields, at pre-emergence growth stage, are in general not relevant foraging habitats for hares. This report gives most appropriate, reliable and robust PT values for European hares using maize fields during early growth stages (BBCH growth stages < 20) in Central Europe for the use in wildlife risk assessments according to the recommendations of EFSA (2009). Values were calculated under worst-case assumptions (such as highest maize proportions in the study areas, data evaluation of 'consumers' only and therefore high exposure risk for each individual) and the PT values are considered to be conservative.

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

Study 1

Comments of zRMS:	Study was carried out according to appropriate OECD 202 and all validity criteria were met. **Deviation from the study:** No deviations occurred from the OECD Guideline for the Testing of Chemicals No. 202. The validity criteria: In the definitive test, the validity criteria were met according to the OECD Guideline No. 202 (2004) and EU Method C.2.: - the percentage of immobilization of *Daphnia magna* in the control was 0% (criterion: not more than 10%), - the dissolved oxygen concentrations in the test vessels were within the range of 8.7–9.2 mg/L (criterion: not less than 3 mg/L). At exposure termination, the determined concentration of mesotrione was 99.7% of the nominal concentration, whereas the determined concentration of florasulam was 106.4% of the nominal concentration. Therefore, the concentrations of mesotrione and florasulam were stable under test conditions. **The study is considered acceptable.** **Agreed endpoints:**

Endpoint value [mg/L]	Time of exposure	
	24 h	48 h
EC_{50}	>100	>100

Report	MEZOFLOR 103 SC: Daphnia magna, Acute Immobilisation Test, Czarnecka M., 2021, Study code: W-56-20
Guideline(s):	OECD 202 / EU Method C.2
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Aim of the study
The aim of the study was to demonstrate that the test item concentration causing 50% immobilisation of Daphnia magna, i.e. the EC50 value after 48 h of exposure, is higher than the test item concentration of 100 mg/L (limit test).

Summary:
Immobilisation of Daphnia magna exposed to the test item, MEZOFLOR 103 SC was investigated during

a 48-hour static test. The definitive test was performed with a single test item concentration of 100 mg/L as a limit test. The test was performed in glass beakers of 150 mL capacity, containing 100 mL of either the test item concentration or the control per replicate. Four replicates were used for the test item concentration and the control, each with five Daphnia magna. The Daphnia magna were observed for immobilisation after 24 and 48 h of exposure and any abnormal behaviour or appearance. The Daphnia magna were considered immobile if they showed no ability to swim within 15 seconds after gentle swirling of the test vessel. No immobilisation of Daphnia magna was observed during the period of exposure, neither in the control, nor in the test item concentration of 100 mg/L. The concentrations of mesotrione and florasulam were determined using a high performance liquid chromatography (HPLC) with Diode Array Detection. Samples of the test item concentration of 100 mg/L and the control collected at exposure initiation and at exposure termination were chemically determined. At exposure initiation, the determined concentration of mesotrione was 100.5% of the nominal concentration, whereas the determined concentration of florasulam was 106.5% of the nominal concentration. The results confirm that the test item concentration was prepared correctly. At exposure termination, the determined concentration of mesotrione was 99.7% of the nominal concentration, whereas the determined concentration of florasulam was 106.4% of the nominal concentration. Therefore, the concentrations of mesotrione and florasulam were stable under test conditions. The endpoint value was determined based on the nominal test item concentration.

Material and methods:
Test item: MEZOFLOR 103 SC;
batch no. SNS-H-05-15,
the (determined) content of mesotrione: 101.6 g/L;
the (determined) content of florasulam: 3.0 g/L;
density at 20°C: 1.062 g/mL;
manufacturing date: May 2020,
expiry date: May, 2022.

Test system: Daphnia magna Straus (< 24 h old at exposure initiation); not first brood progeny; neonates collected from a laboratory culture cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna.

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

Nominal test item concentration: 100 mg/L plus the control (limit test).

Test conditions: Temperature: 18.9 – 19.9°C;
pH of the control: 7.09 – 7.57;
dissolved oxygen concentration in the control: 8.9 – 9.2 mg/L;
daily cycle 16 h light : 8 h dark; fluorescent light source;
no feeding;
no aeration;
medium: Elendt M7.

Chemical determinations: The concentrations of mesotrione and florasulam were determined using a high performance liquid chromatography (HPLC) with Diode Array Detection.

Endpoint value: EC50/48 h.

Results: The endpoint value based on nominal test item concentration is given below: **The EC50/48 h is higher than 100 mg/L.**

Study 2

Comments of zRMS:	Study was carried out according to appropriate OECD 201 and all validity criteria were met. Deviation from the study: No deviations occurred from the OECD Test Guidelines No. 201. In opinion zRMS, above deviations did not affect the study results. The validity criteria: - the biomass in the control increased by a factor of 54.2 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 5.7% (criterion: it must not exceed 7%). - the mean coefficient of variation for the section-by-section growth rate in the control culture was 10.6% (criterion: it must not exceed 35%). The study is considered acceptable. At exposure termination, the determined concentrations of mesotrione were in the range of 82.6 – 97.8%, the determined concentrations of florasulam were in the range of 96.5– 106.4% of the nominal concentration. Therefore, the concentrations of mesotrione and florasulam were stable under test conditions. Agreed endpoints:

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

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_rC_{50}	> 50	19.36 (12.59-33.60)	45.89 (33.13-71.45)
E_rC_{20}	52.94 (35.34-135.44)	3.84 (1.42-6.49)	4.14 (2.64-5.75)
E_rC_{10}	20.23 (6.97-30.49)	1.65 (0.37-3.35)	1.18 (0.56-1.95)
LOEC	50	1.85	1.85
NOEC	16.67	0.62	0.62

(-) – 95% confidence interval
Calculations were made according to [9], [SOP/W/68]

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

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_yC_{50}	> 50	5.84 (3.80-8.99)	3.46 (2.71-4.40)
E_yC_{20}	25.68 (13.59-40.73)	1.52 (0.60-2.53)	0.93 (0.58-1.29)
E_yC_{10}	10.32 (2.01-17.62)	0.76 (0.21-1.45)	0.47 (0.24-0.72)
LOEC	50	1.85	1.85
NOEC	16.67	0.62	0.62

(-) – 95% confidence interval
Calculations were made according to [9], [SOP/W/68]

Report	MEZOFLOR 103 SC: *Raphidocelis subcapitata* SAG 61.81 (formerly *Pseudokirchneriella subcapitata*), Growth inhibition test, Nierzędska E., 2021, Study code: W-57-20
Guideline(s):	OECD 201 / EU Method C.3
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Aim of the study

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

Summary:

The growth of the algae Raphidocelis subcapitata SAG 61.81 (formerly Pseudokirchneriella subcapitata)

exposed to the test item, MEZOFLOR 103 SC was investigated during a 72-hour test. The test was performed in glass flasks with a capacity of 250 mL containing 100 mL of either the test item concentration, or the control, per replicate. The initial density of the algae was 1 x 104 cells/mL. The definitive test was performed using the following test item concentrations: 50, 16.67, 5.56, 1.85, 0.62 mg/L (with a spacing factor of 3.0) plus the control. The number of algae cells was determined with an indirect method, which involves a spectrophotometric measurement of the absorbance of algal suspension at 670 nm and converting its value into the number of cells using a standard curve. The absorbance for each treatment was measured after 24, 48 and 72 hours of exposure. The microscopic observations of algae cells morphology were performed at exposure termination. In the test item concentrations of 0.62 and 1.85 mg/L no differences in shape, size and colour of algal cells were reported as compared to the algae cells in the control. In the test item concentration of 5.56 mg/L single bigger and opalescent algae cells were reported as compared to the algae cells in the control. In the test item concentration of 16.67 mg/L single swollen, bigger and opalescent algal cells were reported as compared to the algae cells in the control. In the test item concentration of 50 mg/L almost no cells, single swollen and opalescent algae cells were reported as compared to the algae cells in the control.
The concentrations of mesotrione and florasulam were chemically analysed with a validated high performance liquid chromatography with DAD detection. Samples of each test item concentration and the control were collected at exposure initiation and at exposure termination. At exposure initiation, the determined concentrations of mesotrione were in the range of 86.0 – 98.0% of the nominal concentration, the determined concentrations of florasulam were in the range of 102.8 – 106.2% of the nominal concentration. The results confirm that the test item concentrations were prepared correctly. At exposure termination, the determined concentrations of mesotrione were in the range of 82.6 – 97.8%, the determined concentrations of florasulam were in the range of 96.5 – 106.4% of the nominal concentration. Therefore, the concentrations of mesotrione and florasulam were stable under test conditions. The endpoint values were determined based on nominal test item concentrations.

Materials and methods:

Test item: MEZOFLOR 103 SC;
batch no. SNS-H-05-15,
the (determined) content of mesotrione: 101.6 g/L;
the (determined) content of florasulam: 3.0 g/L,
density at 20°C: 1.062 g/mL;
manufacturing date: May, 2020,
expiry date: May, 2022.

Test system: The unicellular freshwater green algae, *Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata* (Reinsch) Korshikov (*Selenastrum capricornutum* Prinz.) SAG 61.81 cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Ecotoxicology Research Group, Laboratory of Aquatic Organisms Toxicology. The culture was obtained from the Algal Collection at the University of Göttingen, Germany.

Test design: 72 hours of exposure;
three replicates per each test item concentration;
six replicates per the control;
initial algae cell density: 1 x 104 cells/mL.

Nominal test item concentrations: 50, 16.67, 5.56, 1.85, 0.62 mg/L plus the control.

Test conditions: Temperature: 22.2 – 22.8°C;
pH of the control: 7.28 – 7.64;
mean light intensity: 6840 - 7365 lux;
constant illumination and shaking;
medium: AAP.

Chemical determinations: The concentrations of mesotrione and florasulam were determined with the validated high performance liquid chromatographic method with DAD detection.

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, Multiple Sequentially-rejective Welsh-t-test After Bonferroni-Holm.

Endpoint values: ErC50/72 h, EyC50/72 h, NOEC/72 h, LOEC/72 h.

Results:
The endpoint values based on the nominal test item concentrations are given below:

The ErC50/72 h value is 45.89 mg/L (95% confidence interval: 33.13 – 71.45).
The LOEC/72 h value for growth rate is 1.85 mg/L.
The NOEC/72 h value for growth rate is 0.62 mg/L.
The EyC50/72 h value is 3.46 mg/L (95% confidence interval: 2.71 – 4.40).
The LOEC/72 h value for yield is 1.85 mg/L.
The NOEC/72 h value for yield is 0.62 mg/L.

Study 3

Comments of zRMS:	Study was carried out according to appropriate OECD 201 and all validity criteria were met. Deviation from the study: No deviations occurred from the OECD Test Guidelines No. 201 The validity criteria: - the biomass in the control increased by a factor of 96.3 within the 72-hour test period (criterion: at least a 16-fold growth), - the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 0.7% (criterion: it must not exceed 7%). - the mean coefficient of variation for the section-by-section growth rate in the control culture was 31.6% (criterion: it must not exceed 35%). At exposure termination, the determined concentrations of mesotrione were in the range of 90.0 – 99.6%, the determined concentrations of florasulam were in the range of 92.9 – 104.6% of the nominal concentration. Therefore, the concentrations of mesotrione and florasulam were stable under test conditions. **The study is considered acceptable.** **Agreed endpoints:**

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

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_rC_{50}	> 100	> 100	> 100
E_rC_{20}	54.19 (n.d.)	> 100	> 100
E_rC_{10}	n.d.	> 100	> 100 (38.47-1287.99)
LOEC	100	100	100
NOEC	33	33	33

(-) – 95% confidence interval
Calculations were made according to [9], [SOP/W/68]
n.d.- not determined

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

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_yC_{50}	> 100	> 100	> 100
E_yC_{20}	1.60 (n.d.)	> 100	49.62 (0.78 – 77.55)
E_yC_{10}	n.d.	36.15 (12.28-512.56)	29.03 (0.01 - 51.61)
LOEC	100	100	100
NOEC	33	33	33

(-) – 95% confidence interval
Calculations were made according to [9], [SOP/W/68]
n.d.- not determined

Report	MEZOFLOR 103 SC: *Navicula pelliculosa* SAG 1050-3, Growth inhibition test, Growth inhibition test, Nierzędska E., 2021, Study code: W-58-20
Guideline(s):	OECD 201 / EU Method C.3
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Aim of the study:
The aim of the study was to demonstrate that the test item concentrations causing 50% inhibition of growth rate and yield of the diatoms *Navicula pelliculosa* SAG 1050-3 (ErC50 and EyC50 after 72 hours of exposure, respectively) are higher than the highest applied test item concentration, i.e 100 mg/L. The LOEC and NOEC values were also determined.

Summary:
The growth of the diatoms Navicula pelliculosa SAG 1050-3 exposed to the test item, MEZOFLOR 103 SC was investigated during a 72-hour test. The test was performed in glass flasks with a capacity of 250 mL containing 100 mL of either the test item concentration, or the control, per replicate. The initial density of diatoms was 1 x 104 cells/mL. The definitive test was performed using the following test item concentrations: 100, 33, 11, 3.70, 1.23 mg/L (with a spacing factor of 3.0) plus the control.
The number of diatoms cells was determined with an direct method, which involves a microscopic counting cells in a Bürker chamber for each replicate of the test item concentration and the control. The cells number was determined after 24, 48, and 72 h of exposure. The microscopic observations of diatoms cells morphology were performed at exposure termination.

In all test item concentrations no differences in shape, size and colour of diatoms cells were reported as compared to the diatoms cells in the control.
The concentrations of mesotrione and florasulam were chemically analysed with a validated high performance liquid chromatography with DAD detection. Samples of each test item concentration and the control were collected at exposure initiation and at exposure termination.
At exposure initiation, the determined concentrations of mesotrione were in the range of 88.0 – 98.4% of the nominal concentration, the determined concentrations of florasulam were in the range of 92.9 – 103.9% of the nominal concentration. The results confirm that the test item concentrations were prepared correctly.
At exposure termination, the determined concentrations of mesotrione were in the range of 90.0 – 99.6%, the determined concentrations of florasulam were in the range of 92.9 – 104.6% of the nominal concentration. Therefore, the concentrations of mesotrione and florasulam were stable under test conditions.
The endpoint values were determined based on nominal test item concentrations.

Materials and methods:

Test item: MEZOFLOR 103 SC;
batch no. SNS-H-05-15,
the (determined) content of mesotrione: 101.6 g/L;
the (determined) content of florasulam: 3.0 g/L,
density at 20°C: 1.062 g/mL;
manufacturing date: May, 2020,
expiry date: May, 2022. T

Test system: The freshwater diatoms *Navicula pelliculosa* (Bréb.) Hilse specification SAG 1050 – 3, cultivated at The Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Ecotoxicology Research Group, Laboratory of Aquatic Organisms Toxicology. The diatoms were obtained from the Culture Collection of Algae at Göttingen University, Germany.

Test design: 72 hours of exposure; three replicates per each test item concentration; six replicates per the control; initial algae cell density: 1 x 104 cells/mL.

Nominal test item concentrations: 100, 33, 11, 3.70, 1.23 mg/L plus the control.

Test conditions: Temperature: 22.8 – 23.1°C;
pH of the control: 7.53 – 7.71;
mean light intensity: 6496 - 6576 lux;
constant illumination and shaking;
medium: AAP.

Chemical determinations: The concentrations of mesotrione and florasulam were determined with the validated high performance liquid chromatographic method with DAD detection.

Statistics: Probit method calculations and analyses by: Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Multiple Sequentially-rejective U-test After Bonferroni-Holm.

Endpoint values: ErC50/72 h, EyC50/72 h, NOEC/72 h, LOEC/72 h.

Results:

The endpoint values based on the nominal test item concentrations are given below:

The ErC50/72 h value is higher than 100 mg/L.
The LOEC/72 h value for growth rate is 100 mg/L.
The NOEC/72 h value for growth rate is 33 mg/L.
The EyC50/72 h value is higher than 100 mg/L.

The LOEC/72 h value for yield is 100 mg/L.
The NOEC/72 h value for yield is 33 mg/L.

Study 4

Comments of zRMS:	Study was carried out according to appropriate OECD 201 and all validity criteria were met. Deviation from the study: No deviations occurred from the OECD Test Guidelines No. 221. The validity criteria: - the doubling time of frond number in the control was 2.5 days (the factor of frond number in the control between 0 and 7 day was 7.1), - the average specific growth rate in the control between day 0 and day 7 was 0.280 d^{-1} (minimum requirement: higher than 0.275 d^{-1}). In spent samples at renewals and at exposure termination, the determined concentrations of mesotrione were in the range of 81.9 – 111.0% of the nominal concentration and the determined concentrations of florasulam were in the range of 90.2–107.7% of the nominal concentration. Therefore, the concentrations of mesotrione and florasulam were stable under test conditions. The endpoint values were determined based on the nominal test item concentrations **The study is considered acceptable.** **Agreed endpoints:**

Growth rate endpoint values based on nominal test item concentration [mg/L] – definitive test

Endpoint value [mg/L]	Frond number			Dry weight
	0-2 d	0-4 d	0-7 d	0-7 d
E_rC_{10}	0.007 *(0.000 – 0.050)*	0.040 *(0.017 – 0.078)*	0.051 *(0.028 – 0.084)*	0.167 *(0.055 – 0.355)*
E_rC_{20}	0.053 *(0.002 – 0.224)*	0.172 *(0.091 – 0.283)*	0.196 *(0.125 – 0.285)*	0.701 *(0.324 – 1.222)*
E_rC_{50}	2.339 *(0.706 – 9.158)*	2.747 *(1.927 – 3.947)*	2.537 *(1.956 – 3.300)*	10.957 *(7.262 – 17.198)*
LOEC	0.8	0.032	0.032	0.8
NOEC	0.16	0.0064	0.0064	0.16

Calculations according to [9], [SOP/W/68]
(-) - 95% confidence interval

Yield endpoint values based on nominal test item concentration [mg/L] – definitive test

Endpoint value [mg/L]	Frond number			Dry weight
	0-2 d	0-4 d	0-7 d	0-7 d
E_yC_{10}	0.004 *(0.000 – 0.028)*	0.011 *(0.004 – 0.020)*	0.009 *(0.005 – 0.015)*	0.045 *(0.012 – 0.107)*
E_yC_{20}	0.025 *(0.001 – 0.120)*	0.045 *(0.024 – 0.073)*	0.036 *(0.022 – 0.053)*	0.181 *(0.069 – 0.352)*
E_yC_{50}	1.064 *(0.271 – 4.386)*	0.698 *(0.492 – 0.989)*	0.474 *(0.363 – 0.618)*	2.635 *(1.594 – 4.415)*
LOEC	0.8	0.032	≤0.0064	0.8
NOEC	0.16	0.0064	<0.0064	0.16

Calculations according to [9], [SOP/W/68]
(-) - 95% confidence interval

Report	MEZOFLOR 103 SC: *Lemna gibba* CPCC 310, Growth inhibition test, Czarnecka M., 2021, Study code: W-59-20
Guideline(s):	OECD 201 / EU Method C.3
Deviations:	Yes (deviation had no impact on the results)
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Aim of the study:
The aim of the study was to determine the test item concentrations causing 50% inhibition of growth rate and yield of duckweed *Lemna gibba* (Linné) CPCC 310 (ErC50, EyC50 after 7 days of exposure, respectively based on frond number and dry weight). For growth rate and yield, the LOEC and NOEC values after 7 days of exposure based on dry weight were determined. For growth rate, the LOEC and NOEC values after 7 days of exposure based on frond number were also determined.

Summary:
The growth of Lemna gibba exposed to the test item, MEZOFLOR 103 SC, was investigated in a 7 day semi-static test with two renewals. The test was performed in glass crystallizers containing 150 mL of either the test item concentration or the control. The initial frond number in each test item concentration

and the control was nine. The following test item concentrations were used: 100, 20, 4.0, 0.8, 0.16, 0.032, and 0.0064 mg/L plus the control.
The total number of fronds in each test vessel was counted twice during exposure (day 2 and 4) 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.
At exposure termination, in the test item concentrations of 0.0064, 0.032, 0.16 and 100 mg/L, no distinctive changes from the normal development of plants in the control were observed. In the test item concentration of 0.8 mg/L discoloration of young fronds was observed. In the test item concentrations of 0.4 and 20 mg/L deformed fronds were observed.
The concentrations of mesotrione and florasulam were chemically analyzed using a validated liquid chromatographic method with MS/MS detection (LC-MS/MS). Samples of all fresh test item concentrations and the control collected at exposure initiation and all spent test item concentrations and the control collected at the first renewal were chemically determined. Moreover, fresh samples of the highest (100 mg/L) and the lowest test item concentration (0.0064 mg/L) plus the control at both renewals as well as spent samples of the highest and the lowest test item concentration plus the control during the second renewal and at exposure termination were chemically analyzed.
In fresh samples at exposure initiation and at renewals, the determined concentrations of mesotrione were in the range of 91.2 – 114.7% of the nominal concentration and the determined concentrations of florasulam were in the range of 88.9 – 117.3% of the nominal concentration. The results confirm that the test item concentrations were prepared correctly.
In spent samples at renewals and at exposure termination, the determined concentrations of mesotrione were in the range of 81.9 – 111.0% of the nominal concentration and the determined concentrations of florasulam were in the range of 90.2 – 107.7% of the nominal concentration. Therefore, the concentrations of mesotrione and florasulam were stable under test conditions.
The endpoint values were determined based on the nominal test item concentrations.

Materials and methods:
Material and methods:
Test item: MEZOFLOR 103 SC;
batch no. SNS-H-05-15,
the (determined) content of mesotrione: 101.6 g/L;
the (determined) content of florasulam: 3.0 g/L;
density at 20°C: 1.062 g/mL;
manufacturing date: May 2020,
expiry date: May, 2022.

Test system: Freshwater aquatic plant Lemna gibba L. specification CPCC 310, cultured in the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Ecotoxicology Research Group, Laboratory of Aquatic Organisms Toxicology, stock G3 from Canadian Phycological Culture Centre (CPCC), Department of Biology, University of Waterloo, Ontario, Canada.

Test design: Semi-static system with two renewals (7 days of exposure); three replicates for each test item concentration and six replicates for the control.

Nominal test item concentrations: 100, 20, 4.0, 0.8, 0.16, 0.032, and 0.0064 mg/L plus control

Test conditions: Temperature: 22.9 – 23.3ºC;
pH of the control: 7.54 – 8.99;
light intensity: 7248 – 7525 lux;
constant illumination;
test vessels: glass crystallizers containing 150 mL of each treatment;
initial frond number: 9, i.e. 3 plants per 3 fronds;
medium: 20X AAP.

Chemical determinations: The concentrations of mesotrione and florasulam were determined using the

validated high performance liquid chromatographic method with MS/MS detection.

Statistics: Probit method calculations and analysis by Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Dunnet's Multiple t-test Procedure, Williams Multiple Sequential t-test Procedure.

Endpoint value: ErC50, ErC20, ErC10, EyC50, EyC20, EyC10, LOEC and NOEC, based on frond number and dry weight.

Results:
The endpoint values based on the nominal test item concentrations:
Endpoints based on the frond number:

The ErC50/7 d value is 2.537 mg/L (95% confidence interval 1.956 – 3.300).
The ErC20/7 d value is 0.196 mg/L (95% confidence interval 0.125 – 0.285).
The ErC10/7 d value is 0.051 mg/L (95% confidence interval 0.028 – 0.084).
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 0.474 mg/L (95% confidence interval 0.363 – 0.618).
The EyC20/7 d value is 0.036 mg/L (95% confidence interval 0.022 – 0.053).
The EyC10/7 d value is 0.009 mg/L (95% confidence interval 0.005 – 0.015).
For yield, the NOEC/7 d value is lower than 0.0064 mg/L, whereas LOEC/7 d value is lower or equal to 0.0064 mg/L.

Endpoints based on the dry weight:
The ErC50/7 d value is 10.957 mg/L (95% confidence interval 7.262 – 17.198).
The ErC20/7 d value is 0.701 mg/L (95% confidence interval 0.324 – 1.222).
The ErC10/7 d value is 0.167 mg/L (95% confidence interval 0.055 – 0.355).
The EyC50/7 d value is 2.635 mg/L (95% confidence interval 1.594 – 4.415).
The EyC20/7 d value is 0.181 mg/L (95% confidence interval 0.069 – 0.352).
The EyC10/7 d value is 0.045 mg/L (95% confidence interval 0.012 – 0.107).
For growth rate and yield, the NOEC/7 d value is 0.16 mg//L, whereas the LOEC/7 d value is 0.8 mg/L.

Study 5

Comments of zRMS:	Study was carried out according to appropriate OECD 201 and all validity criteria were met. Deviation from the study: One deviation from the study plan occurred regarding the measurements of subsample of fish before the experiment. The measurements of the subsample were not conducted due to the 3R principles. Dimensions of fish were evaluated based on the length and weight dead fish from the tank. The deviation did not have impact on the results generated in the study. In opinion zRMS, above deviations did not affect the study results. **The validity criteria:** The following validity criteria specified in the OECD Guideline No. 201 were met: - the mortality in the control was 0% at exposure termination (should not exceed 10% or 1 fish if less than 10 fish are used); - dissolved oxygen concentrations were within the range of 91 – 98% of air saturation value (obligatory above 60% of air saturation value). **The study is considered acceptable.** In samples at exposure termination, the determined concentration of florasulam was 101.3% of the nominal concentration.

The determined concentrations of mesotrione was 99.3% of the nominal concentration. Therefore, the concentrations of florasulam and mesotrione were stable under test conditions during 96 h of exposure.

Agreed endpoints:

Endpoint values [mg/L]	Time of exposure			
	24 h	48 h	72 h	96 h
LC_{50}	>100	>100	>100	>100

Report	MEZOFLOR 103 SC: Rainbow trout, Acute Toxicity Testing, [redacted]
Guideline(s):	OECD 201 / EU Method C.3
Deviations:	Yes (deviation had no impact on the results)
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Aim of the study:

The aim of the study was fulfilled because demonstrated that the test item concentration causing 50% mortality of rainbow trout (Oncorhynchus mykiss) i.e. LC50 value after 96 h of exposure was higher than the applied test item concentration for exposure, i.e. 100 mg/L (limit test).

Summary:

The acute toxicity study of the test item MEZOFLOR 103 SC on the rainbow trout was conducted as a static test. Approximately 3 months old rainbow trout (Oncorhynchus mykiss Walb.) were exposed to the one test item concentration of 100 mg/L plus control for 96 h (limit test). The test vessels were glass aquaria with a capacity of 10 L. There was one replicate of the test item concentration and the control. Seven fish were introduced into each aquarium. The fish were observed for mortality and intoxication symptoms after 2.5, 5.5, 24, 28, 48, 52, 72, 76 and 96 h of exposure (twice a day).

In the control and in the test item concentration of 100 mg/L no mortality of fish was observed during exposure.

The concentrations of florasulam and mesotrione were chemically determined using a validated liquid chromatographic method with Diode Array Detection (DAD). The test item concentration and the control were chemically determined at exposure initiation and at exposure termination.

In samples at exposure initiation, the determined concentration of florasulam was 102.8% of the nominal concentration, whereas the determined concentration of mesotrione was 98.3% of the nominal concentration. The results confirm that the test item concentrations were prepared correctly.

In samples at exposure termination, the determined concentration of florasulam was 101.3% of the nominal concentration, whereas the determined concentration of mesotrione was of 99.3% of the nominal concentration. Therefore, the concentrations of florasulam and mesotrione were stable under test conditions during 96 h of exposure.

The endpoint values were estimated based on the nominal test item concentrations.

Material and methods:

Test item:

MEZOFLOR 103 SC;
batch no. SNS-H-05-15;
content of florasulam (determined): 3.0 g/L;
content of mesotrione (determined): 101.6 g/L,

density:1.062 g/mL;
manufacturing date: 05.2020;
expiry date: 05.2022.

Test system:
Rainbow trout (Oncorhynchus mykiss Walb.),
age: approximately 3 months,
average weight: 0.52 g ± 0.07 g,
average body length: 4.11 cm ± 0.13 cm (excluding control),
supplier: 'The Culture of Salmonidae Fish in Zawoja', Poland.

Test design: Static system (96 h of exposure), one replicate of the test item concentration and control, seven fish in each aquarium, the ratio of fish weight per volume (10 L) was 0.36 g/L.

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

Test conditions:
Temperature of water in the control: 10.9 – 11.4°C;
pH of the control: 7.94 – 8.12;
dissolved oxygen concentration in the test item concentrations and the control: 91 – 98% ASV;
lighting daily cycle: 16 h light : 8 h dark;
no feeding;
constant aeration,
mean light illumination: 840 lx at exposure initiation.

Chemical determinations: The concentrations of florasulam and mesotrione were determined using a validated high performance liquid chromatographic method with DAD detection.

Endpoint values: LC50, LOEC and NOEC.

Results:
The endpoint value estimated on the basis of the nominal test item concentration: The LC50 value after 96 h of exposure is **higher than 100 mg/L**.

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

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

Comments of zRMS:	The study was accepted by zRMS. Study was carried out according to appropriate OECD 213 and all validity criteria were met.

Deviation from the study: None.

The validity criteria:

TEST VALIDITY CRITERIA

The following validity criteria were met during the test:

- the mortality for the control was 3.3% at the end of the experiment (criterion: it must not exceed 10%).
- the LD_{50}/24 h of the reference item (dimethoate) was 0.17 µg a.i./bee (criterion: 0.10 – 0.35 µg a.i./bee).

Agreed endpoints:

Dose [µg/bee]	Number of tested bees [no.]	Mortality after 48 h after the beginning of the treatment			LD_{50} [µg/bee]
		Total			
		[no.]	[%]	[%][a]	
0.0 (Control)	30	1	3.3	–	> 200.0
12.5	30	1	3.3	0.0	
25.0	30	2	6.7	3.4	
50.0	30	4	13.3	10.3	
100.0	30	6	20.0	17.2	
200.0	30	8	26.7	24.1	

[a]: mortality corrected according to the Abbott formula

Report	MEZOFLOR 103 SC: Honeybees (*Apis mellifera* L.), Acute Oral Toxicity Test, Kulec-Płoszczyca E., 2021, Study code: B-17-21
Guideline(s):	OECD 213 / EU Method C.16.
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Aim of the study

The aims of the study were to determine the acute oral toxicity of MEZOFLOR 103 SC a laboratory method to adult worker honeybees and to calculate the LD50 values with, if possible or to demonstrate that the LD50 value is higher than the highest dose used in the test.

Summary:

The acute oral toxicity study of MEZOFLOR 103 SC was conducted to determine the LD50. Five doses of the test item were used. These included: 12.5, 25.0, 50.0, 100.0 and 200.0 µg/honeybee. The range of doses was selected on the basis of the preliminary non-GLP range-finding test results. Each group of 10 bees (3 replicates containing 10 bees each) was fed with 100 µL of 50% sucrose solution, containing the test item at the doses mentioned above, using a micropipette. During the entire experiment, the insects were caged in groups of 10. The recommended reference item, i.e. dimethoate was used to verify the sen-

sitivity of the honeybees and the precision of the test procedure. After the administration, the insects were observed for mortality and other signs of toxicity. These observations were made 4, 24 and 48 hours after the beginning of the treatment. The acute oral toxicity test finished after the 48-hour observation.

Material and methods:

Test item: MEZOFLOR 103 SC
content: 101.6 g/L of mesotrione (CAS No. 104206-82-8)
3.0 g/L of florasulam (CAS No. 145701-23-1)
batch no.: SNS-H-05-15
production date: 05.2020
expiry date: 05.2022

Biological test system: the honeybee, Apis mellifera L., strain: carnica
– age: approximately 3 weeks
– source: an apiary at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna [SOP/B/14],

Test design: the test item: – exposure duration: 48 hours
– number of doses: 5 doses and a control
– number of replicates: 3 replicates
– number of bees: 10 bees/replicate

the reference item: – exposure duration: 24 hours
– number of doses: 3 doses
– number of replicates: 3 replicates
– number of bees: 10 bees/replicate

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

Reference item doses: 0.1, 0.2 and 0.4 µg a.i./bee

Test conditions: – temperature: 24.5 – 25.0°C
– relative air humidity: 65 – 68%

Place: dark room

Statistical analysis: Probit analysis using linear max. likelihood regression

Endpoints: – honeybee mortality after 24 and 48 hours of the exposure,
– the oral LD50/24 h of the reference item (dimethoate).

Results: The acute oral toxicity study of the test item, MEZOFLOR 103 SC on honeybees (*Apis mellifera L.*) in the laboratory test are summarized below.

Dose [µg/bee]	Number of tested bees [no.]	Mortality after 48 h after the beginning of the treatment			LD_{50} [µg/bee]
		Total			
		[no.]	[%]	[%][a]	
0.0 (Control)	30	1	**3.3**	**–**	**> 200.0**
12.5	30	1	**3.3**	**0.0**	
25.0	30	2	**6.7**	**3.4**	
50.0	30	4	**13.3**	**10.3**	
100.0	30	6	**20.0**	**17.2**	
200.0	30	8	**26.7**	**24.1**	

[a]: mortality corrected according to the Abbott formula

Conclusions: The median lethal doses LD50/24 h and LD50/48 h are higher than the highest dose used in the test, i.e. 200.0 µg/honeybee.

Comments of zRMS:	The study was accepted by zRMS. Study was carried out according to appropriate OECD 214 and all validity criteria were met.

Deviation from the study: According to the Guideline No. 214/ EU Method C.17., the honeybees may be anesthetized with carbon dioxide for application of the test item. Anesthesia was replaced with mechanical immobilisation [[SOP/B/48]. This method was described in the Study Plan and the SOP/B/48. The mentioned deviation had not effect on the results of the study.

The validity criteria:

TEST VALIDITY CRITERIA

The following validity criteria were met during the test:

- the mortality for the control was 6.7% after 48 h (criterion: it must not exceed 10.0%),
- the LD_{50}/24 h of the reference item (dimethoate) was 0.28 µg a.i./bee (criterion: 0.10 – 0.30 µg a.i./bee).

Agreed endpoints:

Dose [µg/bee]	Number of tested bees [no.]	Mortality after 48 h of exposure			LD_{50} [µg/bee]
		Total			
		[no.]	[%]	[%] Corr[a]	
0.0 (control)	30	2	6.7	–	> 200.0
12.5	30	0	0.0	-7.1*	
25.0	30	0	0.0	-7.1*	
50.0	30	0	0.0	-7.1*	
100.0	30	0	0.0	-7.1*	
200.0	30	1	3.3	-3.6*	

[a]: mortality corrected according to the Abbott formula

*: the negative value indicates that the mortality in the group treated with the test item was lower than in the control group

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

Report	MEZOFLOR 103 SC: Honeybees (*Apis mellifera* L.), Acute Contact Toxicity Test, Kulec-Płoszczyca E., 2021, Study code: B-18-21
Guideline(s):	OECD 214
Deviations:	Yes (deviations has no impact on the results).
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Aim of the study:
The aims of the study were to use a laboratory method to determine the acute contact toxicity of MEZOFLOR 103 SC to adult worker honeybees and to determine the LD50 values or to demonstrate that the LD50 values are higher than the highest dose used in the test.

Summary
Mortality of honeybees, Apis mellifera, exposed to MEZOFLOR 103 SC was investigated during 48-hour test. Five doses of the test item were used. These included: 12.5, 25.0, 50.0, 100.0 and 200.0 µg/honeybee. The range of doses was selected on the basis of the preliminary non-GLP range-finding test results. A microapplicator was used to apply the test item. The volume was 1 µL/bee. During the experiment, the insects were caged in groups of 10. The recommended reference item, i.e. dimethoate was used to verify the sensitivity of the honeybees and the precision of the test procedure. After the application, the insects were observed for mortality and signs of toxicity. These observations were made 4, 24 and 48 hours after the beginning of the treatment. The acute contact toxicity test finished after the 48-hour observation.

Material and methods:
Test item: MEZOFLOR 103 SC
content: 101.6 g/L of mesotrione (CAS No. 104206-82-8)
3.0 g/L of florasulam (CAS No. 145701-23-1)
batch no.: SNS-H-05-15
production date: 05.2020
expiry date: 05.2022

Biological test system: the honeybee, Apis mellifera L., strain: carnica
– age: approximately 3 weeks
– source: an apiary at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna,

Test design: the test item: – exposure duration: 48 hours
– number of doses: 5 doses and one control
– number of replicates: 3 replicates
– number of bees: 10 bees/replicate
the reference item: – exposure duration: 24 hours
– number of doses: 3 doses
– number of replicates: 3 replicates
– number of bees: 10 bees/replicate

Test item doses: 12.5, 25.0, 50.0, 100.0 and 200.0 µg test item/bee and a control (0.0 µg/bee)
Reference item doses: 0.1, 0.2 and 0.4 µg a.i./bee
Test conditions: – temperature: 24.0 – 25.5°C
– relative air humidity: 67 – 69%
Place: dark room
Statistical analysis: Probit analysis using linear max. likelihood regression
Endpoints: – honeybee mortality after 24 and 48 hours of the exposure,
– the contact LD50/24 h of the reference item (dimethoate).

Results: The acute contact toxicity study of the test item, MEZOFLOR 103 SC on honeybees (Apis mellifera L.) in the laboratory test are summarized below.

Dose [µg/bee]	Number of tested bees [no.]	Mortality after 48 h of exposure			LD_{50} [µg/bee]
		Total			
		[no.]	[%]	[%] Corr[a]	
0.0 (control)	30	2	**6.7**	–	**> 200.0**
12.5	30	0	**0.0**	**-7.1***	
25.0	30	0	**0.0**	**-7.1***	
50.0	30	0	**0.0**	**-7.1***	
100.0	30	0	**0.0**	**-7.1***	
200.0	30	1	**3.3**	**-3.6***	

[a]: mortality corrected according to the Abbott formula

*: the negative value indicates that the mortality in the group treated with the test item was lower than in the control group

Conclusions: The median lethal doses LD50/24 h and LD50/48 h are higher than the highest dose used in the test, i.e. 200.0 µg/honeybee.

Comments of zRMS:

The study was accepted by zRMS. Study was carried out according to appropriate OECD 247 and all validity criteria were met.

Deviation from the study: 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. According to the Study Plan the Report should contain a copy of the study plan, however it was decided in the test facility to not include study plans in the reports. This deviations had no impact on the quality, integrity and final results of the study.

The validity criteria:

VALIDITY OF THE STUDY

The following validity criteria were met:

- Mortality of the control groups was 0.0% at the end of the test (criterion: ≤ 10%).
- Mortality in the toxic reference item group (dimethoate) at the end of the test was 86.7% (criterion: ≥ 50%).

Agreed endpoints:

Dose			Number of tested bumble-bees [no.]	Mortality after 48 h		LD_{50}/48 h		
test item [µg/ bumble-bee]	mesotrione [µg a.i. / bumble-bee]	florasulam [µg a.i. / bumble-bee]		[no.]	[%]	[µg/ bumble-bee]	mesotrione [µg a.i. / bumble-bee]	florasulam [µg a.i. / bumble-bee]
Control			50	0	**0.0**	**> 200.0**	**> 19.1**	**> 0.56**
200.0	**19.1**	**0.56**	50	0	**0.0**			
Reference item: dimethoate								
Dose [µg/bumblebee]		**4.0**	30	26	**86.7**	–		

A 2.3.1.1.3 KCP 10.3.1.1.3 Acute oral toxicity to bumblebees

Report	MEZOFLOR 103 SC: Bumblebees (*Bombus* spp.), Acute Oral Toxicity Test, Kulec-Płoszczyca E., 2021, Study code: B-19-21
Guideline(s):	OECD 247
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Aim of the study:
The aims of the study were to determine the acute oral toxicity of MEZOFLOR 103 SC to bumblebees (Bombus spp.) with a laboratory method and to demonstrate that the median lethal dose, i.e. the LD50 at the end of exposure, is higher than the dose used in the test, i.e. 200.0 µg test item/bumblebee (limit test).

Summary:
The study was conducted to determine the acute oral toxicity of MEZOFLOR 103 SC to bumblebees (Bombus spp.) with a laboratory method and to demonstrate, that the median lethal dose, i.e. the LD50 at the end of exposure, is higher than the dose used in the test (limit test). One dose of the test item, i.e. 200.0 µg test item/bumblebee, plus the control and one dose of the reference item were used. The design of the definitive test was selected on the basis of the non-GLP preliminary test results. The bumblebees were exposed to the test item distributed in a 50% aqueous sucrose solution. The insects were selected for the exposure in terms of their sizes. The treated diet was provided in calibrated pipettes. Each pipette contained 40 µL of the sucrose solution with the test item at the tested dose. The insects were kept individually in isolators. The sensitivity of the test bumblebees was verified using a reference item, i.e. dimethoate at the dose of 4.0 µg/bumblebee. The insects were observed for mortality and other signs of toxicity 4-5, 24 and 48 hours after the test/ reference item administration. The acute oral toxicity test finished after the 48-hour observation.

Material and methods:

Test item: MEZOFLOR 103 SC
content: 101.6 g/L of mesotrione (CAS No. 104206-82-8)
3.0 g/L of florasulam (CAS No. 145701-23-1)
batch no.: SNS-H-05-15
production date: 05.2020
expiry date: 05.2022

Biological test system: species: bumblebee, Bombus spp.
source: Koppert Polska sp. z o.o. (a commercial supplier)
age: adult worker bumblebees

Experimental design: − a control (50% sucrose solution w/v)
number of replicates: 50;
number of insects: 1 insect/replicate;
− test item:
number of doses: 1,
number of replicates: 50;
number of insects: 1 insect/replicate;
− the reference item:
number of doses: 1,

number of replicates: 30;
number of insects: 1 insect/replicate

Dose of the test item: 200.0 µg test item/bumblebee
Dose of the reference item: 4.0 µg/bumblebee
Exposure duration: 48 hours
Test conditions: temperature: 23.0 – 25.0°C (required: 25 ± 2°C)
relative air humidity: 63 – 66% (required: 60 ± 20%)
place: a dark climate room
Endpoints: − bumblebee mortality after 48 hours of exposure,
− LD50 after 48 hours of exposure
Statistical method: statistical analysis was not needed due to the lack of mortality.

Results: The median lethal doses (LD50/24 h, LD50/48 h) are higher than the dose used in the test, i.e. > 200.0 µg test item/bumblebee, i.e. > 19.1 µg mesotrione/bumblebee and > 0.56 µg florasulam/bumblebee.

Dose			Number of tested bumble-bees [no.]	Mortality after 48 h		LD_{50}/48 h		
test item [µg/ bumble-bee]	mesotrione [µg a.i. / bumble-bee]	florasulam [µg a.i. / bumble-bee]		[no.]	[%]	[µg/ bumble-bee]	mesotrione [µg a.i. / bumble-bee]	florasulam [µg a.i. / bumble-bee]
Control			50	0	**0.0**	**> 200.0**	**> 19.1**	**> 0.56**
200.0	**19.1**	**0.56**	50	0	**0.0**			
Reference item: dimethoate								
Dose [µg/bumblebee]		**4.0**	30	26	**86.7**	–		

Results of chemical determinations:
At exposure initiation, in the fresh test item sample, the concentration of mesotrione was 98.5% and the concentration of florasulam was 105.6% of the nominal concentration. The results confirm that the test item concentration was prepared correctly.

Comments of zRMS:	The study was accepted by zRMS. Study was carried out according to appropriate OECD 246 and all validity criteria were met. Deviation from the study: According to the OECD Guideline No. 246 the bumble-bees 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. According to the Study Plan the Report should contain a copy of the study plan, however it was decided in the test facility to not include study plans in the reports. This deviations had no impact on the quality, integrity and final results of the study. **The validity criteria:** **VALIDITY OF THE STUDY** The following validity criteria were met: – Mortality of the control groups was 0.0% at the end of the test (criterion: ≤ 10%). – Mortality in the toxic reference item group (dimethoate) at the end of the test was 73.3% (criterion: ≥ 50%). **Agreed endpoints:**

Dose			Number of tested bumble-bees [no.]	Mortality after 48 h		LD_{50}/48 h		
test item [µg/ bumble-bee]	mesotrione [µg a.i. / bumble-bee]	florasulam [µg a.i. / bumble-bee]		[no.]	[%]	[µg/ bumble-bee]	mesotrione [µg a.i. / bumble-bee]	florasulam [µg a.i. / bumble-bee]
Control			50	0	0.0	> 200.0	> 19.1	> 0.56
Control + 1% surfactant			50	0	0.0			
200.0	19.1	0.56	50	0	0.0			
Reference item: dimethoate								
Dose [µg/bumblebee]		10.0	30	22	73.3	–		

A 2.3.1.1.4 KCP 10.3.1.1.4 Acute contact toxicity to bumblebees

Report	MEZOFLOR 103 SC: Bumblebees (*Bombus* spp.), Acute Contact Toxicity Test, Kulec-Płoszczyca E., 2021, Study code: B-20-21
Guideline(s):	OECD 246
Deviations:	Yes (deviations has no impact on the results).
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Aim of the study:
The aims of the study were to determine the acute contact toxicity of MEZOFLOR 103 SC to bumblebees (Bombus spp.) with a laboratory method and to demonstrate that the median lethal dose, i.e. the LD50 at the end of exposure, is higher than the dose used in the test, i.e. 200.0 µg test item/bumblebee (limit test).

Summary:
The study was conducted to determine the acute contact toxicity of MEZOFLOR 103 SC to bumblebees (Bombus spp.) with a laboratory method and to demonstrate, that the median lethal dose, i.e. the LD50 at the end of exposure, is higher than the dose used in the test (limit test). One dose of the test item, i.e. 200.0 µg test item/bumblebee, plus the controls and one dose of the reference item were used. The design of the definitive test was selected on the basis of the non-GLP preliminary test results. The bumblebees were exposed to the test item diluted in distilled water with surfactant Triton X-100 and applied to the dorsal part of the thorax, using a microapplicator. The volume was 2 µL/bumblebee. The insects were selected for the exposure in terms of their sizes. After that, the insects were kept individually in isolators. The sensitivity of the test bumblebees was verified using a reference item, i.e. dimethoate at the dose of 10.0 µg/bumblebee. The insects were observed for mortality and other signs of toxicity 4-5, 24 and 48 hours after the test/ reference item administration. The acute contact toxicity test finished after the 48-hour observation.

Material and methods:
Test item: MEZOFLOR 103 SC

content: 101.6 g/L of mesotrione (CAS No. 104206-82-8)
3.0 g/L of florasulam (CAS No. 145701-23-1)
batch no.: SNS-H-05-15
production date: 05.2020
expiry date: 05.2022

Biological test system: species: bumblebee, Bombus spp.
source: Koppert Polska sp. z o.o. (a commercial supplier)
age: adult worker bumblebees

Experimental design: − a control (distilled water)
number of replicates: 50;
number of insects: 1 insect/replicate;
− control with surfactant (distilled water with 1% of Triton(R) X-100)
number of replicates: 50;
number of insects: 1 insect/replicate;
− test item: number of doses: 1,
number of replicates: 50;
number of insects: 1 insect/replicate;
− the reference item:
number of doses: 1,
number of replicates: 30;
number of insects: 1 insect/replicate

Dose of the test item: 200.0 µg test item/bumblebee

Dose of the reference item: 10.0 µg/bumblebee

Exposure duration: 48 hours

Test conditions: temperature: 23.5 – 26˚C
relative air humidity: 63 – 67%
place: a dark climate room

Endpoints: − bumblebee mortality after 48 hours of exposure,
− LD50 after 48 hours of exposure

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

Results: The median lethal doses (LD50/24 h, LD50/48 h) are higher than the dose used in the test, i.e. > 200.0 µg test item/bumblebee, i.e. > 19.1 µg mesotrione/bumblebee and > 0.56 µg florasulam/bumblebee.

Dose			Number of tested bumble-bees [no.]	Mortality after 48 h		LD_{50}/48 h		
test item [µg/ bumble-bee]	mesotrione [µg a.i. / bumble-bee]	florasulam [µg a.i. / bumble-bee]		[no.]	[%]	[µg/ bumble-bee]	mesotrione [µg a.i. / bumble-bee]	florasulam [µg a.i. / bumble-bee]
Control			50	0	0.0	> 200.0	> 19.1	> 0.56
Control + 1% surfactant			50	0	0.0			
200.0	19.1	0.56	50	0	0.0			
Reference item: dimethoate								
Dose [µg/bumblebee]		10.0	30	22	73.3	–		

Results of chemical determinations

At exposure initiation, in the fresh test item sample, the concentration of mesotrione was 90.7% and the concentration of florasulam was 101.5% of the nominal concentration. The results confirm that the test item concentration was prepared correctly.

Comments of zRMS: The study was accepted by zRMS. Study was carried out according to appropriate OECD 245 and all validity criteria were met.

Deviation from the study: none.

The validity criteria:

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 0.0% (criterion: it must not exceed 15%) [1].
- After 10 days of exposure corrected mortality of the honeybees exposed to the reference item at the concentration of 0.8 mg/kg (0.016 µg/bee/day) was 63.3% (criterion: it must be ≥ 50% on day 10 of exposure).

Agreed endpoints:

The effects of MEZOFLOR 103 SC on mortality of honeybees are summarized below:

Nominal test item concentration/ dose		Ingested[a] dose	Number of tested bees	Total mortality		LC_{50}	LDD_{50}
[µg/30 mg/day] [µg/bee/day]	[mg/kg]	[µg/bee/day]	[no]	No.	[%]	[mg/kg]	[µg/bee/day]
MEZOFLOR 103 SC							
0.0 (Control)			50	0	0.0	> 666.7	> 15.8
20.0	666.7	15.8	50	0	0.0		
Dimethoate (reference item)							
0.024	0.8	0.016	30	19	63.3	not determined	

[a]: ingested doses (dietary doses) were calculated on the basis of the concentrations of the test item / reference item and average sucrose solution consumption

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

Report	MEZOFLOR 103 SC: Honeybees (*Apis mellifera* L.), Chronic Oral Toxicity Test, Kulec-Płoszczyca E., 2021, Study code: B-16-21
Guideline(s):	OECD Guideline No. 245 (2017)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Aim of the study:

The aims of the study were to determine the chronic oral toxicity of the test item, MEZOFLOR 103 SC to honeybees (Apis mellifera L.) and to demonstrate that the median lethal concentration, i.e. the LC50 and median lethal dietary dose, i.e. LDD50 are higher than the test item concentration used for exposure (limit test).

Summary:
The mortality of honeybees exposed to MEZOFLOR 103 SC was investigated during 10-days chronic oral toxicity test. The design of the definitive test was selected on the basis of the preliminary range-finding non-GLP test results. One dose of the test item was used (limit test). The nominal concentration was 666.7 mg/kg of diet (corresponding to the nominal dose of 20.0 µg/30 mg/day). Daily dose, consumed by the bees in the group treated with the test item at the nominal concentration of 666.7 mg/kg (20 µg/30 mg/day) was 15.8 µg/bee/day (dietary dose). Daily dose was calculated on the basis of average consumption of a treated 50% sucrose solution and the nominal dose used for the treatment. Each group of bees (5 replicates/group; 10 bees/replicate) was fed with 2 mL of a 50% sucrose solution containing the test item at the concentration of 666.7 mg/kg or 50% sucrose solution alone (control group) for 10 days. Dimethoate, which is a recommended reference item, was used to verify the sensitivity of the bees and the precision of the test procedure. The group treated with the reference item (3 replicates per 10 bees) was fed the with 2 mL of a 50% sucrose solution containing reference item at the nominal concentration of 0.8 mg/kg (corresponding to the nominal dose of 0.024 µg/30 mg). Daily weighed feeders were used. During the experiment, the insects were caged in groups of 10. Daily dose, consumed by the bees in the group treated with the reference item at the nominal concentration of 0.8 mg/kg (0.024 µg/30 mg/day) was 0.016 µg/bee/day (dietary dose). The insects were observed for mortality and behavioral abnormalities (signs of intoxication) at daily intervals up to 10 days of exposure. Average consumption of a 50% sucrose solution in the control group was 26.35 mg/bee/day. Average consumption in the group treated with the test item at the concentration of 666.7 mg/kg was 23.74 mg/bee/day. Average consumption of a 50% sucrose solution containing the reference item at the concentration of 0.8 mg/kg was 20.25 mg/bee/day.

Material and methods:

Test item: MEZOFLOR 103 SC
content: 101.6 g/L of mesotrione (CAS No. 104206-82-8)
3.0 g/L of florasulam (CAS No. 145701-23-1)
batch no.: SNS-H-05-15
production date: 05.2020
expiry date: 05.2022

Biological test system:
species: the honeybee, Apis mellifera L.;
strain: carnica, source: an apiary at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna;
age: freshly emerged worker honeybees (max. 2 days old) from the same queen-right colony

Experimental design:
− the test item:
number of concentrations: 1 and the control
number of replicates: 5
number of insects: 10 bees/replicate
− the reference item:
number of concentrations: 1
number of replicates: 3
number of insects: 10 bees/replicate
exposure duration: 10 days

Nominal concentration of the test item: 666.7 mg/kg
Nominal dose of the test item: 20.0 µg/bee/day
Test item dietary dose: 15.8 µg/bee/day
Nominal concentration of the reference item (dimethoate): 0.8 mg/kg
Nominal dose of the reference item (dimethoate): 0.024 µg/bee/day
Reference item dietary dose: 0.016 µg/bee/day
Test conditions: temperature: 33.2 – 35.0 ºC;
relative humidity: 50.6 – 69.6%;
Statistical method: statistical analysis was not needed due to the lack of mortality.
Endpoints: honeybee mortality after 10 days of exposure

Results: The validity criterion concerning mortality was met, because mortality in the control was 0.0% after 10 days of exposure [1]. The percentage of mortality of the honeybees exposed to the test item, at the concentration of 666.7 mg/kg (dietary dose 15.8 µg/bee/day) at exposure termination (after 10 days), was 0.0%. On the basis of the obtained mortality results the LC50 is higher than 666.7 mg/kg, and the LDD50 value is higher than 15.8 µg/bee/day. The validity criterion concerning mortality of the honeybees exposed to the reference item, dimethoate was met, because mortality was equal to 63.3% after 10 days of exposure. The results obtained in the reference item group showed that the insects were sensitive to dimethoate.
The effects of MEZOFLOR 103 SC on mortality of honeybees are summarized below:

Nominal test item concentration/ dose		Ingested[a] dose [µg/bee/day]	Number of tested bees [no]	Total mortality		LC_{50} [mg/kg]	LDD_{50} [µg/bee/day]
[µg/30 mg/day] [µg/bee/day]	[mg/kg]			No.	[%]		
MEZOFLOR 103 SC							
0.0 (Control)			50	0	**0.0**	**> 666.7**	**> 15.8**
20.0	**666.7**	**15.8**	50	0	**0.0**		
Dimethoate (reference item)							
0.024	**0.8**	**0.016**	30	19	**63.3**	not determined	

[a]: ingested doses (dietary doses) were calculated on the basis of the concentrations of the test item / reference item and average sucrose solution consumption

Chemical determination:
The concentrations of mesotrione and florasulam were chemically determined using the validated high performance liquid chromatographic method with DAD detection. Fresh samples of the test item concentration and the control were chemically analyzed at test initiation and at the end of the maximum storage period (i.e. after 4 days). At exposure initiation, in the fresh sample of the test item of 666.7 mg/kg, the determined concentration of mesotrione was 99.1% and the concentration of florasulam was 103.6% 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 mesotrione was 101.7% and the concentration of florasulam was 106.7% of nominal concentration. Based on the results of chemical analyses, the concentration of mesotrione and florasulam were stable under storage conditions.

Comments of zRMS:	The study was accepted by zRMS. Study was carried out according to appropriate OECD GD 239 and all validity criteria were met. Deviation from the study: The following concentrations of the test item were used in the stability study: 50 g of the test item/L and 0.05 g of the test item/L (in the Study plan stated incorrectly: 50 g of the test item/L and 0.5 g of the test item/L). In the study of the stability of temperature was recorded once a day with a thermometer min-max. In course of the range-finding and definitive test, periodic decreases of temperature (required: 34-35°C) and humidity (required: 50-100%) occurred. It resulted from daily feedings and observations. These drops were short-termed, did not affect the condition of the test system. **The validity criteria:** The test met the validity criteria (acc. to OECD GD 239 OECD GD 239 ENV/JM/MONO(2016)34): ❖ in control cumulative larval mortality from D3 to D8 was 5.6% (required: ≤15%),

- in control the adults emergence rate on D22 was 86.1% (required: ≥70%).

Agreed endpoints:

Final results of the study

Parameter	Concentration [mg of test item/kg of food]	Parameter	Dose [µg of test item/larva]
LC_{10}	n.d.** (n.d.– n.d.)*	LD_{10}	n.d.*** (n.d.– n.d.)*
LC_{20}	n.d.** (n.d.– n.d.)*	LD_{20}	n.d.*** (n.d.– n.d.)*
LC_{50}	**n.d.**** (n.d.– n.d.)*	LD_{50}	**n.d.***** (n.d.– n.d.)*
NOEC	n.d.**	NOED	n.d.***
LOEC	n.d.**	LOED	n.d.***

* upper and lower confidence limits (95%) given in the brackets
LC_{10} test item concentration causing mortality of 10% population
LC_{20} test item concentration causing mortality of 20% population
LC_{50} test item concentration causing mortality of 50% population
NOEC the highest test item concentration not causing statistically significant differences in relations to the control
LOEC the lowestt test item concentration causing statistically significant differences in relations to the control
LD_{10} test item dose causing mortality of 10% population
LD_{20} test item dose causing mortality of 20% population
LD_{50} test item dose causing mortality of 50% population
NOED the highest test item dose not causing statistically significant differences in relations to the control
LOED the lowest test item dose causing statistically significant differences in relations to the control
n.d. impossible to determine due to mathematical reasons
** **based on the analysis of the results, the value was determined to be >650 mg of the test item/kg of food**
*** **based on the analysis of the results, the value was determined to be >100 µg of the test item/larva**

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

Report	Honey Bee Larval Toxicity Test following Repeated Exposure to the test item ME-ZOFLOR 103 SC according to OECD GD 239 ENV/JM/MONO(2016)34
Guideline(s):	OECD GD 239 ENV/JM/MONO(2016)34
Deviations:	Yes (The deviations had no effect of the test resul)t
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Yes. The deviations had no effect of the test results

Aim of the study:
The assessment test of the test item MEZOFLOR 103 SC toxicity on Honey Bee larvae (*Apis mellifera* L.) was conducted. The aim of the study was determination of the concentration causing 50% mortality of population (LC50 value) and the dose causing mortality of 50% of the population after 22 days (LD50 value). Values NOEC and NOED were estimated for the emerged bees on day 22

Test design

- *stability test:* tested concentrations and control in one replicate
- *range-finding, definitive, reference test:* tested concentrations and control in one replicate; 36 larvae per replicate

Test cages
- *stability test:* volumetric flask of 100 mL volume
- *range-finding, definitive, reference test:* 48-well breeding plates with queen-cell cups placed in the dissector and placed in incubator; from day 15 of the test – transparent plastic boxes placed in test room

Exposition time 4 days (from day 3 to day 6)

Duration of the test
- *stability test:* 72 hours
- *range-finding, definitive, reference test:* 22 days

Tested concentrations (doses)
- *stability test:*
control (0 g of test item/L of solution)
0.05 g of test item/L of solution, corresponding to 0.65 mg of test item/kg of food
50 g of test item/L of solution, corresponding to 650 mg of test item/kg of food
- *range-finding test:*
control (0 mg of test item/kg of food)
0.65 mg of test item/kg of food, corresponding to 0.1 µg of test item/larva
6.5 mg of test item/kg of food, corresponding to 1 µg of test item /larva
65 mg of test item/kg of food, corresponding to 10 µg of test item /larva
650 mg of test item/kg of food, corresponding to 100 µg of test item /larva
- *definitive test (limit test*):
control (0 mg of test item/kg of food)
650 mg of test item/kg of food, corresponding to 100 µg of test item/larva
- *reference test:*
control - 0 mg of reference item/kg of food
control (with acetone) - 0 mg of reference item/kg of food
fenoxycarb 0.32 mg/kg of food, corresponding to 49.28 µg/larva

Test conditions
- *stability test:* average temperature 6.109°C (minimal temperature 4.4°C; maximal temperature 7.3°C); darkness
- *range-finding test:* average temperature 34.218°C (minimal temperature 33.0°C; maximal temperature 34.5°C); average humidity: 85.343% (minimal humidity 45.4%; maximal humidity 99.9%); darkness
- *definitive test and reference test:* average temperature 34.178°C (minimal temperature 33.3°C; maximal temperature 34.7°C); average humidity: 83.447% (minimal humidity 59.6%; maximal humidity 99.9%); darkness.

Final results
In course of the test, the test item has not shown apitoxic effect in mortality of following developmental stages of bees after 22 days of the test. At the end of the study, the concentration and the dose causing 10%, 20% and 50% mortality of the population in the test (LC10, LC20, LC50 and LD10, LD20, LD50 values) were estimated, as well as NOEC and NOED values were estimated at 22 day. The final results of the experiment are presented in Table 1.

Table 1. Final results of the study

Parameter	Concentration [mg of test item/kg of food]	Parameter	Dose [µg of test item/larva]
LC_{10}	n.d.** (n.d.– n.d.)*	LD_{10}	n.d.*** (n.d.– n.d.)*
LC_{20}	n.d.** (n.d.– n.d.)*	LD_{20}	n.d.*** (n.d.– n.d.)*
LC_{50}	**n.d.**** (n.d.– n.d.)*	LD_{50}	**n.d.***** (n.d.– n.d.)*
NOEC	n.d.**	NOED	n.d.***
LOEC	n.d.**	LOED	n.d.***

* upper and lower confidence limits (95%) given in the brackets
LC_{10} test item concentration causing mortality of 10% population
LC_{20} test item concentration causing mortality of 20% population
LC_{50} test item concentration causing mortality of 50% population
NOEC the highest test item concentration not causing statistically significant differences in relations to the control
LOEC the lowestt test item concentration causing statistically significant differences in relations to the control
LD_{10} test item dose causing mortality of 10% population
LD_{20} test item dose causing mortality of 20% population
LD_{50} test item dose causing mortality of 50% population
NOED the highest test item dose not causing statistically significant differences in relations to the control
LOED the lowest test item dose causing statistically significant differences in relations to the control
n.d. impossible to determine due to mathematical reasons
** **based on the analysis of the results, the value was determined to be >650 mg of the test item/kg of food**
*** **based on the analysis of the results, the value was determined to be >100 µg of the test item/larva**

Chemical determination:
Concentrations of the active substances mesotrione and floraulam in aqueous solutions used to preparing food for larvae were determined at the beginning of exposure (D3) and at end of exposure (D6). Determined concentrations of active substances were recalculated to concentration of the test item MEZOFLOR 103 SC.
During whole exposure period (D3 – D6) concentration of the test item was in the range of 80-120% of nominal concentration 50g/L. Result are presented in the table below.

Table 26. Results of analytical measurements of the active substances – mesotrione and florasulam in aqueous solution of the test item - definitive test

Date of analysis	Determination of samples by ECO Laboratory	Determination of samples by FA Laboratory	Mesotrione concentration read from the calibration curve [mg/L]	Florasulam concentration read from the calibration curve [mg/L]	Dilution factor n	Mesotrione concentration taking into account the dilution [mg/L]	Florasulam concentration taking into account the dilution [mg/L]	Mesotrione mean concentration [mg/L]	Florasulam mean concentration [mg/L]	Mean test item concentration [mg/L]	Difference between determined and nominal concentration [%]
28.06.2021	kontrola	361/2021 1	0.00000	0.00000	1	0.00000	0.00000	0.00000	0.00000	0.00	na.
		361/2021 2	0.00000	0.00000		0.00000	0.00000				
	50 g/l	362/2021 1	8.18640	0.29263	500	4093.20000	146.31500	4099.09500	146.86750	46538.83	-6.92
		362/2021 2	8.20998	0.29484		4104.99000	147.42000				
01.07.2021	kontrola 72 h	405/2021 1	0.00000	0.00000	1	0.00000	0.00000	0.00000	0.00000	0.00	na.
		405/2021 2	0.00000	0.00000		0.00000	0.00000				
	50 g/l 72 h	406/2021 1	8.34056	0.31300	500	4170.28000	156.50000	4179.96250	156.86750	48681.54	-2.64
		406/2021 2	8.37929	0.31447		4189.64500	157.23500				

na. not applicable

Comments of zRMS:	The study was accepted by zRMS. Study was carried out according to according to the ESCORT 1 (Barrett K. L. et al., 1994) and the ESCORT 2 (Candolfi M. P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Blümel S. et al., 2000) and all validity criteria were met. Deviation from the study: The study was conducted according to the guidelines developed by the IOBC, BART and EPPO Joint initiative, SOP/B/23 and other procedures related to the study and the Study Plan. However, in the experimental part of the study the following deviation from the guidelines occurred: 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 validity criteria:

TEST VALIDITY CRITERIA

The following validity criteria were met during the study [1]:

- mortality of the control group was 8.3% on day 7 of exposure (criterion: a maximum of 20%),
- corrected mortality of the mites exposed to the reference item at the rate of 4.0 g/ha was 90.9% on day 7 of exposure (criterion: from 50 to 100%),
- the mean number of eggs per female in the control group was 5.8 (required: ≥ 4 eggs per female).

Agreed endpoints:

Results:

The effects of MEZOFLOR 103 SC on mortality and reproduction of *Typhlodromus pyri* in the definitive test are summarized below.

Study group [application rate]	Parameter (endpoint)						
	Mortality (dead + escape mites)			Reproduction			
Test item [L/ha]	Total [%]	Corr.[a] [%]	LR_{50} [L/ha]	Test item [L/ha]	Mean number of eggs per female (Rr) [no.]	Repro-duction reduction (Pr) [%]	ER_{50} [L/ha]
Control	8.3	–	–	Control	5.8	–	–
MEZOFLOR 103 SC							
0.11	13.3	5.5	> 1.0	0.11	5.9	(-0.6)*	> 1.0
0.33	16.7	9.1		0.33+	4.4	25.3	
1.0+	28.3	21.8		1.0+	3.7	36.8	
$NOER_{mortality}$ [L/ha]			0.33	$NOER_{reproduction}$ [L/ha]			0.11
Reference item							
[g/ha]	Total [%]	Corr.[a] [%]	Dimethoate				
4.0	91.7	90.9	not assessed				

[a]: mortality corrected according to the Abbott formula [12]

+: statisticaly significant differences [10], [SOP/B/67]

*: negative value indicates that mean number eggs per female was higher than in the control

A 2.3.2 KCP 10.3.2 - Effects on arthropods other than bees Study 1

Report	A laboratory test for evaluating the effects of MEZOFLOR 103 SC on the predatory mite, *Typhlodromus pyri* (Sch.) Kulec-Płoszczyca E.., 2021, Study code: B-14-21
Guideline(s):	ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Blümel S. et al., 2000)

Deviations:	Yes (deviations had no impact on test results
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Aim of the study:
The aim of the study was to determine the impact of MEZOFLOR 103 SC on mortality and reproduction of the predatory mite, *Typhlodromus pyri* under laboratory conditions. The endpoint of this test was mortality of the mites after 7 days of the treatment and the reproduction reduction (Pr) after 14 days of the treatment.

Summary
The laboratory test involved the evaluation of the effects of the test item, MEZOFLOR 103 SC, on mortality and reproduction of the predatory mite, *Typhlodromus pyri* (Sch.). On the basis of the results of the preliminary range-finding test, it was decided to use three rates of the test item in the definitive test. These were 0.11, 0.33 and 1.0 L/ha. The mites, T. pyri at the protonymphal stage (24 hours old) were exposed to the test item applied to artificial discs. The mites were fed with pine pollen (Pinus sp.) and two spotted spider mite (T. urticae) eggs. Mortality observations were made after 7 days of the treatment. To verify the sensitivity of the mites and the precision of the test procedure, an insecticide, dimethoate was used as a reference item. The rate of the reference item was 4.0 g/ha. The control group was treated with distilled water.

Material and methods:

Test item:	MEZOFLOR 103 SC active substances: 101.6 g/L of mesotrione [CAS: 104206-82-8] 3.0 g/L of florasulam [CAS: 145701-23-1] batch number: SNS-H-05-15 production date: 05.2020 expiry date: 05.2022
Biological test system:	the predatory mite, *Typhlodromus pyri* (Sch.) (Acari: Phytoseiidae) age: 24-hour-old protonymphs source: a laboratory culture at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Ecotoxicology Research Group; the culture was obtained from commercial breeder.
Experimental design:	5 study groups: – a control group (0.0 L/ha) – MEZOFLOR 103 SC at the rate of 0.11 L/ha – MEZOFLOR 103 SC at the rate of 0.33 L/ha – MEZOFLOR 103 SC at the rate of 1.0 L/ha – Dimethoate at the rate of 4.0 g/ha number of replicates: 3 replicates/group number of mites in each replicate: 20
Test conditions:	– temperature: 23.5 – 26.5ºC; – relative air humidity: 59 – 78% – photoperiod: 16 hours light : 8 hours dark – light intensity: 764 lux
Statistical analysis:	Logit analysis using max. likelihood regression, Step-down Cochran-Armitage test procedure, Probit analysis using linear weighted regression, Shapiro Wilk's Test on Normal Distribution, Levene's Test on Variance Homogenity (with Residuals), Williams Multiple Sequential t-test Procedure, Chi2 2x2 Table Test with Bonferroni Correction
Endpoints:	– mite mortality after 7 days of the treatment

– LR50 and NOERmortality
– reproduction reduction (Pr) after 14 days of the treatment
– ER50 and NOERreproduction

Results: The effects of MEZOFLOR 103 SC on mortality and reproduction of *Typhlodromus pyri* in the definitive test are summarized below.

Study group [application rate]	Parameter (endpoint)						
	Mortality (dead + escape mites)			Reproduction			
Test item [L/ha]	Total [%]	Corr.[a] [%]	LR_{50} [L/ha]	Test item [L/ha]	Mean number of eggs per female (Rr) [no.]	Repro-duction reduction (Pr) [%]	ER_{50} [L/ha]
Control	8.3	–	–	Control	5.8	–	–
MEZOFLOR 103 SC							
0.11	13.3	5.5	> 1.0	0.11	5.9	(-0.6)*	> 1.0
0.33	16.7	9.1		0.33+	4.4	25.3	
1.0+	28.3	21.8		1.0+	3.7	36.8	
$NOER_{mortality}$ [L/ha]			0.33	$NOER_{reproduction}$ [L/ha]			0.11
Reference item							
[g/ha]	Total [%]	Corr.[a] [%]		Dimethoate			
4.0	91.7	90.9		not assessed			

[a]: mortality corrected according to the Abbott formula [12]

+: statisticaly significant differences [10], [SOP/B/67]

*: negative value indicates that mean number eggs per female was higher than in the control

Comments of zRMS:	The study was accepted by zRMS. Study was carried out according to the ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Mead-Briggs M.A. et al., 2000) and all validity criteria were met. Deviation from the study: None. **The validity criteria:** **TEST VALIDITY CRITERIA** The following validity criteria were met during the study [3]: – the mortality of the control group after 48 hours was 2.5% (criterion: a maximum of 13.0%), – the Abbott corrected mortality of the reference item group after 24 hours of the treatment was 76.9% (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 23.7 (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).

Agreed endpoints:

Study group	Parameter (endpoints)						
	Mortality after 48 h			Reproduction			
[L/ha]	Total [%]	Corr. [%][a]	LR_{50} [L/ha]	Test item [L/ha]	Mean no. of mummies /female	Fecundity reduction Pr [%]	ER_{50} [L/ha]
Control	2.5	–	–	Control	23.7	–	–
Test item: MEZOFLOR 103 SC							
0.25	2.5	0.0	> 1.0	0.25	22.8	3.7	> 1.0
0.5	5.0	2.6		0.5	21.9	7.3	
1.0	7.5	5.1		1.0	19.9	16.1	
$NOER_{mortality} \geq 1.0$ [L/ha]				$NOER_{fecundity} \geq 1.0$ [L/ha]			
Reference item: dimethoate							
[g/ha]	Mortality after 24 h			Reproduction			
	Total [%]	Corr. [%][a]					
0.12	77.5	76.9		not assessed			

[a]: mortality corrected according to the Abbott formula [10]

Study2

Report	A laboratory test for evaluating the effects of MEZOFLOR 103 SC on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez), Kulec-Płoszczyca E., 2021, Study code: B-15-21
Guideline(s):	ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Mead-Briggs M.A. et al., 2000, Mead-Briggs M.A. et al., 2010)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Aim of the study

The aim of the study was to determine the effect of MEZOFLOR 103 SC on mortality and fecundity of the parasitic wasp, *Aphidius rhopalosiphi*. The endpoint of this test was mortality of the wasps after 48 hours of exposure and fecundity reduction (Pr) 12 days after the oviposition phase.

Summary

The laboratory test involved the evaluation of the effects of the test item, MEZOFLOR 103 SC, on mortality and fecundity of the parasitic wasp, *Aphidius rhopalosiphi*. Three application rates of the test item and a control were used. The rates were 0.25, 0.5 and 1.0 L/ha. The range of rates was selected on the basis of the non-GLP preliminary range-finding test results. Adult wasps were exposed to the test item applied to glass plates. Mortality assessments were made 2, 24 and 48 hours after the introduction of the

wasps to the test arenas. Then, all females which survived 48-hour exposure to MEZOFLOR 103 SC and the ones from the control group were subjected to fecundity assessments. To allow the oviposition, fifteen female wasps from the groups treated with the test item and the control group were individually introduced into fecundity units containing barley plants infested with the aphid, Rhopalosiphum padi. After the 24-hour oviposition, the wasps were removed from the test arenas. After 12 days, the number of mummies (parasitized aphids in which wasp pupae were developing) was recorded. Mortality of the wasps after 48 hours of exposure and the percentage of fecundity reduction (Pr) 12 days after the oviposition were the endpoints. To verify the sensitivity of the wasps and the precision of the test procedure, dimethoate was used as a reference item. The rate of the reference item was 0.12 g/ha. The control group was treated with distilled water.

Materials and methods

Test item: MEZOFLOR 103 SC
- active substances: 101.6 g/L of mesotrione [CAS: 104206-82-8]
  3.0 g/L of florasulam [CAS: 145701-23-1]
- batch number: SNS-H-05-15
- production date: 05.2020
- expiry date: 05.2022

Test system: the parasitic wasp, Aphidius rhopalosiphi (De Stefani-Perez); Hymenoptera: Braconidae, Aphiidinae
– age: imago (24 - 48 hours after emerging from mummies)
– source: a laboratory culture at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Ecotoxicology Research Group; the culture was obtained from commercial breeder.

Test design: testest groups:
– a control group (0.0 L/ha)
– MEZOFLOR 103 SC at the rate of 0.25 L/ha
– MEZOFLOR 103 SC at the rate of 0.5 L/ha
– MEZOFLOR 103 SC at the rate of 1.0 L/ha
– dimethoate at the rate of 0.12 g/ha
number of replicates: 4 replicates/group
number of wasps: 10 wasps/replicate

Test conditions: – temperature: 19 – 22°C
– relative air humidity: 63 – 80%
– photoperiod: 16 hours light: 8 hours dark
– light intensity: mortality assessment and oviposition: 1983 lx
fecundity assessment: 5110 lx

Statistical analysis: Logit analysis using max. likelihood regression, Multiple Sequentiallly-rejective Fisher Test After Bonferroni-Holm, Estimated parameters of the 3-param. normal CDF, Shapiro Wilk's Test on Normal Distribution, Levene's Test on Variance Homogenity (with Residuals), Multiple Sequentiallly-rejective U-test After Bonferroni-Holm

Endpoints: – wasp mortality after 48 hours of exposure
– LR50 and the NOERmortality
– ER50 and the NOERfecundity
– reduction in fecundity (Pr) of surviving female wasps exposed to test item, recorded 12 days after the oviposition period.

Results:

In the definitive test, mortality of the control group, after 48 hours, was 2.5%. After 48 hours of the exposure to MEZOFLOR 103 SC, at the rates of 0.25, 0.5 and 1.0 L/ha, the percentages mortality of A. rhopalosiphi, after Abbott's correction [10], were 0.0, 2.6 and 5.1%, respectively. At the significance level of 0.05, there were no statistically significant difference in mortality between the wasps exposed to the test item at the rates of 0.25, 0.5 and 1.0 L/ha and the control group (Multiple Sequentiallly-rejective Fisher Test After Bonferroni-Holm, p > Alpha*).

Based on the obtained mortality results it can be assumed that **the LR50 is higher than 1.0 L/ha. The**

NOERmortality is higher than or equal to 1.0 L/ha of the test item. Mortality the wasps exposed to dimethoate at the rate of 0.12 g/ha, after 24 hours, after Abbott's correction was 76.9%. Therefore, the validity criterion specified in the method description was met. The results showed that the test organisms were sensitive to dimethoate. The fecundity assessment showed that the mean number of mummies per female in the control group was 23.7. As for the wasps treated with MEZOFLOR 103 SC at the rates of 0.25, 0.5 and 1.0 L/ha the mean number of mummies per female were 22.8, 21.9 and 19.9, respectively. Fecundity reduction (Pr) in the group treated with the test item at the rates 0.25, 0.5 and 1.0 L/ha were 3.7, 7.3 and 16.1%, respectively. At the significance level of 0.05, there were no statistically significant differences in fecundity between the wasps exposed to the test item at all the tested rates, i.e. 0.25, 0.5 and 1.0 L/ha and the control group (Multiple Sequentiallly-rejective U-test After Bonferroni-Holm, p(U) >Alpha(i)). Based on the obtained fecundity results it can be assumed that **the ER50 is higher than 1.0 L/ha of the test item**. **The NOERfecundity is higher than or equal to 1.0 L/ha of the test item.**

The effects of MEZOFLOR 103 SC on mortality and fecundity of Aphidius rhopalosiphi in the laboratory test are summarized below:

Study group	Parameter (endpoints)						
	Mortality after 48 h			Reproduction			
[L/ha]	Total [%]	Corr. [%][a]	LR_{50} [L/ha]	Test item [L/ha]	Mean no. of mummies /female	Fecundity reduction Pr [%]	ER_{50} [L/ha]
Control	2.5	–	–	Control	23.7	–	–
Test item: MEZOFLOR 103 SC							
0.25	2.5	0.0	> 1.0	0.25	22.8	3.7	> 1.0
0.5	5.0	2.6		0.5	21.9	7.3	
1.0	7.5	5.1		1.0	19.9	16.1	
$NOER_{mortality}$ ≥ 1.0 [L/ha]				$NOER_{fecundity}$ ≥ 1.0 [L/ha]			
Reference item: dimethoate							
[g/ha]	Mortality after 24 h			Reproduction			
	Total [%]	Corr. [%][a]					
0.12	77.5	76.9		not assessed			

[a]: mortality corrected according to the Abbott formula [10]

Conclusions:
On the basis of the obtained results it can be concluded that MEZOFLOR 103 SC at the rates of 0.25, 0.5 and 1.0 L/ha has no effect on mortality and fecundity of the wasps.

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

A 2.4.1 KCP 10.4.1 Earthworms

Comments of zRMS:	The study was accepted by zRMS. Study was carried out according to OECD 222 and all validity criteria were met. Deviation from the study: None.

The validity criteria:

VALIDITY CRITERIA

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

- each replicate produced from 99 to 164 juveniles (132.1 mean) at the end of the exposure period (criterion: ≥ 30 juveniles by the end of the experiment),
- the coefficient of variation of reproduction was 17.6% (criterion: ≤ 30%),
- adult mortality over the initial 4 weeks of the experiment was 7.5% (criterion: ≤ 10%).

Agreed toxicity endpoints based on first calculation:

Parameter	Value [mg test item/kg dry weight of artificial soil]	Value [mg of mesotrione/kg dry weight of artificial soil]	Value [mg of florasulam/kg dry weight of artificial soil]
EC_{10}	4.70 (1.31 – 10.05)	0.45 (0.13 – 0.96)	0.01 (0.004 – 0.03)
EC_{20}	15.21 (6.38 – 26.30)	1.46 (0.61 – 2.52)	0.04 (0.02 – 0.07)
EC_{50}	143.88 (97.85 – 221.72)	13.76 (9.36 – 21.21)	0.41 (0.28 – 0.63)
NOEC (reproduction)	10.00	0.96	0.03
LOEC (reproduction)	18.00	1.72	0.05
LC_{50}	>1000.0	>95.67	>2.82
NOEC (survival)	100.00	9.57	0.28
LOEC (survival)	180.00	17.22	0.51

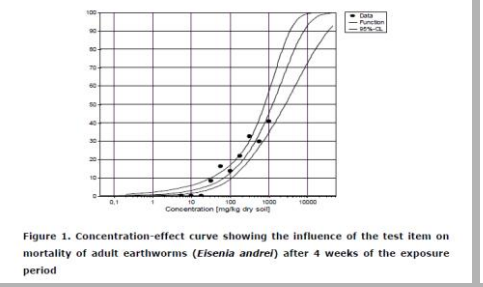


Figure 1. Concentration-effect curve showing the influence of the test item on mortality of adult earthworms (*Eisenia andrei*) after 4 weeks of the exposure period

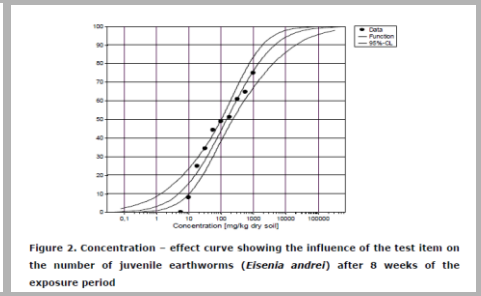


Figure 2. Concentration – effect curve showing the influence of the test item on the number of juvenile earthworms (*Eisenia andrei*) after 8 weeks of the exposure period

EC_{10} value of 4.7 mg/kg dw is not reliable for risk assessment. Justification:

In the final report , the obtained EC_{10} value is out of the range of tested test item concentrations, and was obtained by extrapolation. Sine according to OECD 54, ecotoxicological endpoint should be interpolated and not extrapolated, to get the most reliable values, the amendment with new statistical analysis was performed by Applicant. The calculation was accepted by zRMS. The new analysis is much more precise. According to *Outcome of the Pesticides Peer Review Meeting on general recurring in ecotoxicology (EFSA Supporting publication 2019:EN-1673)* normalised width of confidence interval is much lower and goodness of fit is better. Moreover, laboratory test represents the worst case, and it can be assumed, that real impact on the earthworm might be a bit lower. Therefore using EC_{10} value of 7.08 mg/kg dw is reasonable. During the study no significant changes in reproduction was observed in test item concentration of 10 mg/kg dw. EC_{10} was calculated as

7.08 mg/kg dw (Amendment no 1 to the final report) and it should be used for risk assessment as a worst case.

Agreed toxicity endpoints:

The EC_{10}, EC_{20}, EC_{50} values showing the impact of the test item on reproduction of earthworms obtained by additional statistical analysis are presented in the table given below.

Parameter	Value [mg test item/kg dry weight of artificial soil]	Value [mg of mesotrione/kg dry weight of artificial soil]	Value [mg of florasulam/kg dry weight of artificial soil]
EC_{10}	7.08 (4.81 – 9.71)	0.68 (0.46 – 0.93)	0.02 (0.014 – 0.03)
EC_{20}	21.56 (16.47 – 27.27)	2.06 (1.58 – 2.61)	0.06 (0.05 – 0.08)
EC_{50}	181.44 (143.59 – 237.29)	17.36 (13.74 – 22.70)	0.51 (0.41 – 0.67)

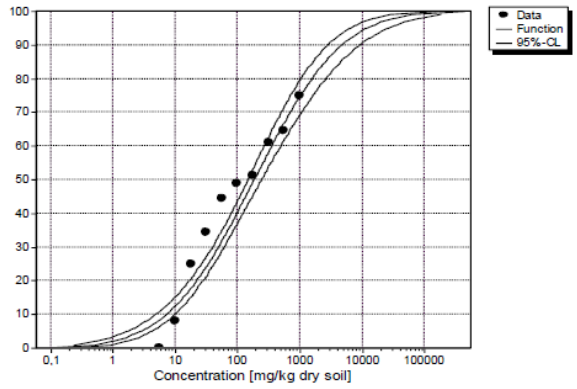


Figure Concentration – effect curve showing the influence of the test item on the number of juvenile earthworms (*Eisenia andrei*) after 8 weeks of the exposure period, determined with additional statistical model.

The studies for formulation of **MEZOFLOR 103 SC** for earthworms with risk assessment was accepted by zRMS only provisionally. The toxicity endpoints were based on nominal concentration. At the end on the studies concentration of substances active – mesotrione and florasulam fell under 80% of nominal. The TWA or geometric mean measured concentration should be calculated over the duration of the test and used if the concentration falls under 80% of nominal. Please complete the calculation the toxicity endpoints based on geometric mean measured concentration.

It should be considered at MSs level.

Report	MEZOFLOR 103 SC Earthworm reproduction test (Eisenia andrei), Pieczka P., 2021, Study code: G-63-20
Guideline(s):	OECD 222
Deviations:	No

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

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

Summary:
The aims of the study were to assess the impact of MEZOFLOR 103 SC on reproduction of the earthworm, *Eisenia andrei* and to determine EC_{10}, EC_{20}, EC_{50} and NOEC. The test item in the form of an aqueous suspension was mixed with a suitable amount of the artificial soil. The concentrations of the test item were: 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0, 320.0, 560.0 and 1000.0 mg/kg dry weight of the artificial soil. Each of them was divided into four replicates. There was also one untreated control group with the deionised water only. Control group was divided into eight replicates. The experiment lasted 8 weeks. After 4 weeks, all of adult earthworms were removed from the test containers and observed. All changes in their behavior and morphology were recorded. The number of earthworms and their body weights were also determined. The impact of the test item on reproduction was evaluated after the additional 4 week period on the basis of the number of juveniles hatched from cocoons during the experiment.

Materials and methods:
Test item: MEZOFLOR 103 SC
batch no.: SNS-H-05-15
Active substances: mesotrione – 101.6 g/L
florasulam – 3.0 g/L
Artificial soil: 10% sphagnum peat, 20% kaolin clay, 70% air-dried quartz sand
Test organism: the earthworm, Eisenia andrei obtained from a standard laboratory culture cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Ecotoxicology Research Group, Laboratory of Soil Organisms Toxicology
Test design: test duration: 8 weeks; number of replicates: 4 replicates/concentration + 8 replicates/control; number of earthworms: 10 earthworms/replicate
Concentrations of the test item: control, 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0, 320.0, 560.0 and 1000.0 mg/kg dry weight of the artificial soil
Test conditions: temperature: 19.0 – 22.0°C;
pH at the beginning of the experiment: 5.68 – 5.95;
pH at the end of the experiment: 5.53 – 5.63;
soil moisture content at the beginning of the experiment: 21.4 – 23.2% (47.9 – 52.0% of the maximum water holding capacity);
soil moisture content at the end of the experiment: 21.1 – 24.0% (47.3 – 53.8% of the maximum water holding capacity);
light-dark cycle: 16h : 8h;
light intensity at the beginning of the experiment: 488.3 – 507.4 lux
light intensity at the end of the experiment: 514.7 – 565.2 lux
Statistical analysis: EC10, EC20, EC50, LC50 – probit analysis using linear max. likelihood regression or Weibull analysis using linear max. likelihood regression NOEC (reproduction) – Shapiro-Wilk's Test on Normal Distribution, Bartlett's Test Procedure on Variance Homogeneity (with Residuals), Williams Multiple Sequential t-test Procedure, NOEC (survival) – Fisher's Exact Binomial Test with Bonferroni Correction LOEC: a values suggested by the ToxRat Professional 2.10 statistical computer software
Endpoint: EC10, EC20, EC50, NOEC, LOEC (reproduction) LC50, NOEC, LOEC (survival).

Results: At concentrations ranging from 5.6 to 1000.0 mg of the test item/kg dry weight of artificial soil, after 4 weeks of exposure to the test item, mortality of the adult earthworms was between 0.0 and 45.0%. As for the control group, mortality of the adult earthworms was equal to 7.5%. 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 95.67 mg of mesotrione + 2.82 mg of florasulam/kg dry weight of the artificial soil). No changes in the appearance (morphology) and behaviour of the living adult earthworms were noticed. After 4 weeks of the exposure period of the test item at the concentrations ranging from 5.6 to 1000.0 mg/kg dry weight of artificial soil, the body weight increase was between -9.2 and 28.2%. As for the control group, the body weight increase was equal to 16.6%.

After 8 weeks of the experiment, the obtained results led to the following conclusions: After the application of the test item at the concentrations ranging from 5.6 to 1000.0 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 33.3 and 137.0 per replicate. The mean number of juveniles in the control group was equal to 132.1 per replicate. After 8 weeks of the experiment, it was concluded that MEZOFLOR 103 SC had a statistically significant impact on reproduction of the earthworms at the concentrations ranging from 18.00 to 1000.0 mg/kg dry weight of the artificial soil. The endpoint values showing the impact of the test item on reproduction and survival of adult earthworms are presented in the table given below.

Parameter	Value [mg test item/kg dry weight of artificial soil]	Value [mg of mesotrione/kg dry weight of artificial soil]	Value [mg of florasulam/kg dry weight of artificial soil]
EC_{10}	4.70 (1.31 – 10.05)	0.45 (0.13 – 0.96)	0.01 (0.004 – 0.03)
EC_{20}	15.21 (6.38 – 26.30)	1.46 (0.61 – 2.52)	0.04 (0.02 – 0.07)
EC_{50}	143.88 (97.85 – 221.72)	13.76 (9.36 – 21.21)	0.41 (0.28 – 0.63)
NOEC (reproduction)	10.00	0.96	0.03
LOEC (reproduction)	18.00	1.72	0.05
LC_{50}	>1000.0	>95.67	>2.82
NOEC (survival)	100.00	9.57	0.28
LOEC (survival)	180.00	17.22	0.51

Chemical analysis:

Concentrations of active substances in soil were determined at the beginning, after four weeks of exposure and at the end of exposure period. Results of analysis are presented in the Tables below:

Table 1. Results from Analysis of mesotrione in test sample

Date of analysis	Concentration of mesotrione [mg/kg]	Concentration determined in particular replicates in dry weight [mg/kg]			Average [mg/kg]	SD [mg/kg]	RSD [%]	Recovery [%]
		1	2	3				
day 0 (16.04.2021)	control	ND	ND	ND	ND	-	-	-
	95.70	79.69	79.70	84.08	81.16	2.53	3.1	84.8
day 28 (14.05.2021)	control	ND	ND	ND	ND	-	-	-
	95.70	70.87	70.86	71.01	70.91	0.08	0.1	74.1
day 56 (11.06.2021)	control	ND	ND	ND	ND	-	-	-
	95.70	5.96	5.97	5.88	5.94	0.05	0.8	6.2

LoQ = 0.2 mg mesotrione/kg
LoD = 0.025 mg mesotrione/kg
- not calculated

Table 3. Results from Analysis of florasulam in test sample

Date of analysis	Concentration of florasulam [mg/kg]	Concentration determined in particular replicates in dry weight [mg/kg]			Average [mg/kg]	SD [mg/kg]	RSD [%]	Recovery [%]
		1	2	3				
day 0 (16.04.2021)	control	ND	ND	ND	ND	--	--	--
	2.80	2.61	2.61	2.57	2.60	0.02	0.9	92.7
day 28 (14.05.2021)	control	ND	ND	ND	ND	--	--	--
	2.80	1.73	1.71	1.74	1.73	0.02	0.9	61.7
day 56 (11.06.2021)	control	ND	ND	ND	ND	--	--	--
	2.80	ND	ND	ND	ND	--	--	--

LoQ = 0.2 mg florasulam/kg
LoD = 0.025 mg florasulam/kg
-- not calculated

Study 1- amendment to the final report

Report	MEZOFLOR 103 SC Earthworm reproduction test (Eisenia andrei), Pieczka P., 2021, Study code: G-63-20, AMENDMENT NO 2. TO THE FINAL REPORT
Guideline(s):	OECD 222
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No
Reason for the amendment no. 1 to the final report	The Amendment No. 2 to the Final Report coded as G-63-20 was prepared on the Sponsor request (e-mail dated on October 15, 2024). The Amendment no. 2 to the Final Report was prepared in order to provide the end-points (EC10, EC20, EC50 LC50, NOEC and LOEC values) corrected by the recovery factor calculated based on the results of the analytical measurements during the study.

The recovery factor was determined by the geometric mean of recovery of the active substances.

Changes in the final report

The Amendment No.2 introduces the supplementation into the content of the point 3.6.7. Observations and evaluation of the Final Report. Additionally the Amendment introduces the Tables 14 - 19.

Endpoint:

EC10, EC20, EC50, NOEC
LC10, LC20, LC50, NOEC

Results:

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Table 14. The mean recovery rates for active substances

Active substance	Nominal concentration of active substance [mg/kg d.w.]	Geometric mean of measured concentrations of active substance [mg/kg d.w.]	Recovery [%]
mesotrione	95.67	32.46	33.92
florasulam	2.82	2.12	75.21

Table 15. The determined recovery factor

Active substance	Product	
	Nominal [mg active substance/kg dry weight of the artificial soil]	Recovered [mg active substance/kg dry weight of the artificial soil]
mesotrione	95.67	32.46
florasulam	2.82	2.12
SUM:	98.49	34.58
Recovery factor*	0.351	

*** Recovery factor = (sum of recovered values)/(sum of nominal values)**

Table 19. The endpoint values increased by the recovery factor

Parameter	Value [mg test item/kg dry weight of artificial soil]	Value [mg of mesotrione/kg dry weight of artificial soil]
EC_{10}	7.08 (4.81 – 9.71)	9.57 (6.50 – 13.12)
EC_{20}	21.56 (16.47 – 27.27)	29.13 (22.25 – 36.84)
EC_{50}	181.44 (143.59 – 237.29)	245.13 (193.99 – 320.58)

zRMS comment:

The new data with toxicity endpoints recalculation and justification that in this case the best solution is to use endpoints based on nominal concentration in risk assessment for soil organisms was provided by Applicant. The recalculation should be treated as additional source information. In this case, when the concentration values fall below the limit of quantification of the analytical method, it is not possible to determine reliable toxicity endpoints based on geometrically measured concentrations. In addition, it can be

noted that a decrease in the content of the active substance in the experimental system, caused by the decomposition of this substance (documented low DT_{50}, DT_{90} in soil, confirmed by analytical tests) usually causes a decrease in the toxicity caused by this substance. In the case of unstable substances, precise specification of reliable endpoints based on the measured geometric mean is not possible, especially when the concentration values fall below the limit of quantification of the analytical method. In order to reliably measure the decrease in such rapidly dispersing compounds, more time points of sampling at much shorter intervals (even within the first day) and a much higher number of repetitions may be necessary. Detailed technical guidance on the design of ecotoxicological studies is needed before analytical measurements are routinely included in ecotoxicological studies involving soil invertebrates. Therefore, the revision and validation of the technical guidelines (OECD 222, 232, 226; 2016 a, b, c) is necessary to achieve greater clarity. The study of soil organisms in ecotoxicology differs significantly from, for example, routine aquatic testing procedures, therefore the implementation of such appropriate analysis in the relevant tests cannot be easily adapted or transferred. The risk assessment for soil organisms, in this case, based on toxicity endpoints based on nominal concentration was accepted by zRMS. **This should be considered at the level of Member States.**

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

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)

Comments of zRMS:	The study was accepted by zRMS. Study was carried out according to OECD 232 and all validity criteria were met. Deviation from the study: Deviation from the OECD Guideline No. 232 (2016): At the end of the test the soil moisture content was determined by drying small sample of the artificial soil in 105°C instead of weighing the test vessels as it is mentioned in OECD Guideline No. 232 (2016) (3.6.6.). Deviation from the OECD Guideline No. 232 and SOP/G/87 According to the OECD Guideline No. 232 and SOP/G/87 eight test concentrations of the test item should be used in order to calculate ER_x and NOER. In this study eleven concentrations was prepared. All above mentioned deviations did not affect the results of the study. **The validity criteria:** **VALIDITY CRITERIA** The results are considered valid because the following criteria were satisfied in the control: • mean adult mortality: 7.5% (criterion: ≤ 20%), • the mean number of juveniles per vessel at the end of the test: 658.3 (criterion: ≥100 juveniles at the end of the test), • the coefficient of variation calculated for the number of juveniles: 12.3% (criterion: ≤ 30%).

Agreed toxicity endpoints:

The endpoint values showing the impact of the test item on the survival of adult collembolans are presented in the Table given below.

Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg of mesotrione/kg dry weight of the artificial soil]	Value [mg of florasulam/kg dry weight of the artificial soil]
LC_{10}	> 1000.0	> 94.25	> 2.83
LC_{20}	> 1000.0	> 94.25	> 2.83
LC_{50}	> 1000.0	> 94.25	> 2.83
NOEC	≥ 1000.0	≥ 94.25	≥ 2.83

The endpoint values showing the impact of the test item on reproduction of *Folsomia candida* are presented in the Table given below.

Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg of mesotrione/kg dry weight of the artificial soil]	Value [mg of florasulam/kg dry weight of the artificial soil]
EC_{10}	> 1000.0	> 94.25	> 2.83
EC_{20}	> 1000.0	> 94.25	> 2.83
EC_{50}	> 1000.0	> 94.25	> 2.83
NOEC	≥ 1000.0	≥ 94.25	≥ 2.83

The studies for formulation of **MEZOFLOR 103 SC** for *Folsomia candida* with risk assessment was accepted by zRMS only provisionally. The toxicity endpoints were based on nominal concentration. At the end on the studies concentration of substances active – mesotrione and florasulam fell under 80% of nominal. The TWA or geometric mean measured concentration should be calculated over the duration of the test and used if the concentration falls under 80% of nominal. Please complete the calculation the toxicity endpoints based on geometric mean measured concentration.

The summary results of determination of active substances of test item in the test samples are shown in table below.

Nominal test item concentration [mg/kg d.w.]	Nominal concentration of florasulam [mg/kg d.w.]	Mean concentration of florasulam determined (n=3) in test samples	
		[mg/kg d.w.]	Recovery ± RSD of nominal concentration [%]
Day 0			
Control	0.0	--	---
1000	2.8	2.882	103 ± 0.7
Day 14			
Control	0.0	--	---
1000	2.8	2.807	100 ± 1.4
Day 28			
Control	0.0	--	---
1000	2.8	1.933	69 ± 4.5

LOQ = 0.2 mg/kg; LOD = 0.025 mg/kg; -- not detected; --- not calculated

Nominal test item concentration [mg/kg d.w.]	Nominal concentration of mesotrione [mg/kg d.w.]	Mean concentration of mesotrione determined (n=3) in test samples	
		[mg/kg d.w.]	Recovery ± RSD of nominal concentration [%]
Day 0			
Control	0.0	--	---
1000	94.3	97.75	104 ± 2.8
Day 14			
Control	0.0	--	---
1000	94.3	62.0	65.7 ± 2.8
Day 28			
Control	0.0	--	---
1000	94.3	49.182	52.2 ± 0.9

LOQ = 0.2 mg/kg; LOD = 0.025 mg/kg; -- not detected; --- not calculated

It should be considered at MSs level.

Study 1

Report	MEZOFLOR 103 SC Collembolan (*Folsomia candida*) Reproduction Test, Czarnynoga M., 2024, Study code: G-44-24
Guideline(s):	OECD 232
Deviations:	Yes, but all mentioned deviations did not affect the results of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No
Aim of the study:	The aims of the study were to assess the impact of MEZOFLOR 103 SC on reproduction of the collembolans, *Folsomia candida* and to determine the EC10, EC20, EC50, and NOEC.
Summary:	Eleven concentrations of the test item were used. These were 2.8, 5.0, 9.1, 16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6 and 1000.0 mg of the test item/kg of dry weight of the artificial soil. Each concentration was divided into four replicates. There was also an untreated control group divided into eight replicates. The test item in form of aqueous suspension was mixed with the artificial soil. The control artificial soil was

	mixed with deionized water alone. The exposure period lasted 28 days. After that, the collembolans were extracted from the artificial soil. The numbers of adults and juveniles were determined separately.
Materials and methods:	**Test item:** MEZOFLOR 103 SC **Artificial soil:** 5% sphagnum peat, 20% kaolin clay, 74.88% air-dried industrial sand and 0.12% calcium carbonate **Test organism:** the collembolan, *Folsomia candida* obtained from a standard laboratory culture at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Laboratory of Soil Organisms Toxicology. The collembolans used in the study were between 9 and 12 days old. **Test design:** exposure period: 28 days number of replicates: 4 replicates / concentration + 8 replicates / control; number of collembolans: 10 / replicate **Concentrations of the test item:** a control, 2.8, 5.0, 9.1, 16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6 and 1000.0 mg of the test item/kg of dry weight of the artificial soil
Test conditions:	temperature: 20.1 – 22.0°C; pH at the beginning of the test: 5.31 – 5.68; pH at the end of the test: 5.52 – 5.76; soil moisture content at the beginning of the test: 17.1 – 17.5% (45.3 – 46.3% of the maximum water holding capacity); soil moisture content at the end of the test: 16.7 – 17.7% (44.2 – 46.9% of the maximum water holding capacity); lighting: 16 h light and 8h dark; light intensity at the beginning of the experiment: 769.3 – 779.3 lux; light intensity at the end of the experiment: 758.5 – 770.3 lux;
Statistical analysis:	EC10, EC20, EC50 – probit analysis using linear max. likelihood regression LC10, LC20, LC50 – probit analysis using linear max. likelihood regression NOEC (number of juveniles): Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Trend analysis by Contrasts (Monotonicity of Concentration/Response), Dunnett's Multiple t-test Procedure NOEC (survival): Qualitative Trend Analysis by Contrasts (Monotonicity of Concentration/Response) Chi2 2x2 Table Test with Bonferroni Correction
Endpoint:	EC10, EC20, EC50, NOEC LC10, LC20, LC50, NOEC
Results:	After the application of the test item at the concentrations ranging from 2.8 to 1000 mg/kg dry weight of the artificial soil, the mortality of adults ranged from 0.0 to 10.0%. As for the control group, it was equal to 7.5%. The endpoint values showing the impact of the test item on the survival of adult collembolans are presented in the Table given below.

Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg of mesotrione/kg dry weight of the artificial soil]	Value [mg of florasulam/kg dry weight of the artificial soil]
LC10	> 1000.0	> 94.25	> 2.83
LC20	> 1000.0	> 94.25	> 2.83

LC50	> 1000.0	> 94.25	> 2.83
NOEC	≥ 1000.0	≥ 94.25	≥ 2.83

After the exposure of collembolans to the test item at the concentrations ranging from 2.8 to 1000.0 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 631.8 and 772.0 per replicate. As for the control group, the number of juveniles was equal 658.3 per replicate. The endpoint values showing the impact of the test item on reproduction of *Folsomia candida* are presented in the Table given below.

Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg of mesotrione/kg dry weight of the artificial soil]	Value [mg of florasulam/kg dry weight of the artificial soil]
EC10	> 1000.0	> 94.25	> 2.83
EC20	> 1000.0	> 94.25	> 2.83
EC50	> 1000.0	> 94.25	> 2.83
NOEC	≥ 1000.0	≥ 94.25	≥ 2.83

zRMS comment:

The new data with toxicity endpoints recalculation and justification that in this case the best solution is to use endpoints based on nominal concentration in risk assessment for soil organisms was provided by Applicant. The recalculation should be treated as additional source information. In this case, when the concentration values fall below the limit of quantification of the analytical method, it is not possible to determine reliable toxicity endpoints based on geometrically measured concentrations. In addition, it can be noted that a decrease in the content of the active substance in the experimental system, caused by the decomposition of this substance (documented low DT_{50}, DT_{90} in soil, confirmed by analytical tests) usually causes a decrease in the toxicity caused by this substance. In the case of unstable substances, precise specification of reliable endpoints based on the measured geometric mean is not possible, especially when the concentration values fall below the limit of quantification of the analytical method. In order to reliably measure the decrease in such rapidly dispersing compounds, more time points of sampling at much shorter intervals (even within the first day) and a much higher number of repetitions may be necessary. Detailed technical guidance on the design of ecotoxicological studies is needed before analytical measurements are routinely included in ecotoxicological studies involving soil invertebrates. Therefore, the revision and validation of the technical guidelines (OECD 222, 232, 226; 2016 a, b, c) is necessary to achieve greater clarity. The study of soil organisms in ecotoxicology differs significantly from, for example, routine aquatic testing procedures, therefore the implementation of such appropriate analysis in the relevant tests cannot be easily adapted or transferred. The risk assessment for soil organisms, in this case, based on toxicity endpoints based on nominal concentration was accepted by zRMS. **This should be considered at the level of Member States.**

Study 1- amendment to the final report

Report	MEZOFLOR 103 SC Collembolan (*Folsomia candida*) Reproduction Test, Czarnynoga M., 2024, Study code: G-44-24- AMENDMENT NO 1. TO THE FINAL REPORT
Guideline(s):	OECD 232
GLP:	Yes
Acceptability:	Yes
Duplication	No

(if vertebrate study)

Reason for the amendment no. 1 to the final report

The Amendment No. 1 to the Final Report coded as G-44-24 was prepared on the Sponsor request (e-mail dated on November 05, 2024).
The Amendment no. 1 to the Final Report was prepared in order to provide the endpoints (EC10, EC20, EC50, LC10, LC20, LC50 and NOEC values) corrected by the recovery factor calculated based on the results of the analytical measurements during the study. The recovery factor was determined by the geometric mean of recovery of the active substances.

Changes in the final report

The Amendment No.1 introduces the supplementation into the content of the point 3.6.7. Observations and the evaluation of the experiment of the Final Report. Additionally the Amendment introduces the Tables 11 - 14.

Endpoint:

EC10, EC20, EC50, NOEC
LC10, LC20, LC50, NOEC

Results:

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Table 11. The mean recovery rates for active substances

Active substance	Nominal concentration of active substance [mg/kg d.w.]	Geometric mean of measured concentrations of active substance [mg/kg d.w.]	Recovery [%]
mesotrione	94.25	66.80	70.87
florasulam	2.83	2.50	88.36

Table 12. The determined recovery factor

Active substance	Product	
	Nominal [mg active substance/kg dry weight of the artificial soil]	Recovered [mg active substance/kg dry weight of the artificial soil]
mesotrione	94.25	66.80
florasulam	2.83	2.50
SUM:	97.08	69.30
Recovery factor*	0.714	

*** Recovery factor = (sum of recovered values)/(sum of nominal values)**

Table 14. The endpoint values increased by the recovery factor

Endpoint	Value [mg of the test item/kg dry weight of the artificial soil]	Endpoint values corrected by the recovery factor [mg of the test item/kg dry weight of the artificial soil]
LC_{10}	> 1000.0	> 1714.0
LC_{20}	> 1000.0	> 1714.0
LC_{50}	> 1000.0	> 1714.0
NOEC (survival)	≥ 1000.0	≥ 1714.0
EC_{10}	> 1000.0	> 1714.0
EC_{20}	> 1000.0	> 1714.0
EC_{50}	> 1000.0	> 1714.0
NOEC (reproduction)	≥ 1000.0	≥ 1714.0

Comments of zRMS:	The study was accepted by zRMS. Study was carried out according to OECD 232 and all validity criteria were met. Deviation from the study: **DEVIATIONS IN THE STUDY** The study was performed according to OECD Guideline No. 226 (2016), Study Plan and the SOPs mentioned in chapter 8. Deviations from the OECD Guideline No. 226 (2016): There are four deviations from the OECD Guideline No. 226 (2016), however they did not affect the results: 1. According to the OECD Guideline No. 226 (2016) in order to calculate EC_x and NOEC, eight test concentrations of the test item should be used. In the study eleven concentrations were used 2. According to the OECD Guideline No. 226 (2016) the water content of the artificial soil should be maintained throughout the test by weighing and if needed re-watering the vessels periodically. In the study to maintain proper moisture content, a small sample of soil was drying at 105°C and re-weighing at the beginning, after 7 days of the test and at the end of the test 3. Due to the use of the temperature extraction method, there was no need for euthanasia of the extracted organisms since the mites are fixed in a 70% ethanol solution 4. Due to the use of the temperature extraction method, it was not possible to record the symptoms with behavioral and morphology changes of the extracted predatory mites All above mentioned deviations did not affect the results of the study. **The validity criteria:** **VALIDITY CRITERIA** The results are considered valid because the following criteria were satisfied in the control: • mean adult mortality: 3.8% (criterion: ≤ 20%), • the mean number of juveniles per vessel at the end of the test: 127.8 (criterion: ≥ 50 juveniles at the end of the test), • the coefficient of variation for the number of juveniles: 16.3% (criterion: ≤ 30%). **Agreed toxicity endpoints:**

The results are summarized in the table given below.

Concentration [mg/kg dry weight of the artificial soil]	Adult mites			Number of juveniles (mean)
	Number of tested mites	Dead mites after 14 days of exposure		
		No.	%	
control	80	3	3.8	127.8
2.8	40	0	0.0	113.3
5.0	40	5	12.5	108.3
9.1	40	4	10.0	98.0
16.3	40	0	0.0	99.0
29.4	40	3	7.5	104.5
52.9	40	3	7.5	111.0
95.3	40	0	0.0	119.3
171.5	40	0	0.0	121.5
308.6	40	1	2.5	117.3
555.6	40	4	10.0	115.5
1000.0	40	3	7.5	96.0

Endpoint values – the impact of the test item on reproduction and on mortality of the predatory mites *(Hypoaspis aculeifer)*.

Endpoint	Value [mg of the test item/kg dry weight of the artificial soil]	Value [mg of mesotrione/kg dry weight of the artificial soil]	Value [mg of florasulam/kg dry weight of the artificial soil]
EC_{10}	> 1000.00	> 94.25	> 2.83
EC_{20}	> 1000.00	> 94.25	> 2.83
EC_{50}	> 1000.00	> 94.25	> 2.83
NOEC (reproduction)	≥ 1000.00	≥ 94.25	≥ 2.83
LC_{10}	> 1000.00	> 94.25	> 2.83
LC_{20}	> 1000.00	> 94.25	> 2.83
LC_{50}	> 1000.00	> 94.25	> 2.83
NOEC (survival)	≥ 1000.00	≥ 94.25	≥ 2.83

The studies for formulation of **MEZOFLOR 103 SC** for *Hypoaspis aculeifer* with risk assessment was accepted by zRMS only provisionally. The toxicity endpoints were based on nominal concentration. At the end on the studies concentration of substances active – mesotrione fell under 80% of nominal. The TWA or geometric mean measured concentration should be calculated over the duration of the test and used if the concentration falls under 80% of nominal. Please complete the calculation the toxicity endpoints based on geometric mean measured concentration.

The summary results of determination of active substances of test item in the test samples are shown in table below.

Nominal test item concentration [mg/kg d.w.]	Nominal concentration of florasulam [mg/kg d.w.]	Mean concentration of florasulam determined (n=3) in test samples	
		[mg/kg d.w.]	Recovery ± RSD of nominal concentration [%]
Day 0			
Control	0.0	--	---
1000	2.8	3.1577	113.0 ± 0.3
Day 7			
Control	0.0	--	---
1000	2.8	2.6650	95.2 ± 0.3
Day 14			
Control	0.0	--	---
1000	2.8	2.6570	94.9 ± 1.5

LOQ = 0.2 mg/kg; LOD = 0.025 mg/kg; -- not detected; --- not calculated

Nominal test item concentration [mg/kg d.w.]	Nominal concentration of mesotrione [mg/kg d.w.]	Mean concentration of mesotrione determined (n=3) in test samples	
		[mg/kg d.w.]	Recovery ± RSD of nominal concentration [%]
Day 0			
Control	0.0	--	---
1000	94.3	93.442	99.1 ± 0.4
Day 7			
Control	0.0	--	---
1000	94.3	69.673	73.9 ± 1.9
Day 14			
Control	0.0	--	---
1000	94.3	74.239	78.7 ± 0.9

LOQ = 0.2 mg/kg; LOD = 0.025 mg/kg; -- not detected; --- not calculated

It should be considered at MSs level.

Study 2

Report	MEZOFLOR 103 SC Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test, Górska M., 2024, Study code: G-45-24
Guideline(s):	OECD 226
Deviations:	Yes, but all mentioned deviations did not affect the results of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No
Aim of the study:	The aims of the study were to assess the impact of MEZOFLOR 103 SC on repro-

	duction of the predatory mite, *Hypoaspis* (*Geolaelaps*) *aculeifer* and to determine the EC10, EC20, EC50, and NOEC.
Summary:	Eleven concentrations of the test item were used (a deviation from OECD Guideline No. 226). These included: 2.8, 5.0, 9.1, 16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6, and 1000.0 mg/kg dry weight of the artificial soil. Each concentration was divided into four replicates. There was also an untreated control group divided into eight replicates. The test item in the form of aqueous suspension was mixed with the artificial soil. The control artificial soil was mixed with deionized water alone. The exposure period lasted 14 days. After that, the mites were extracted from the artificial soil (48-hour extraction). The numbers of adults and juveniles were determined separately.
Materials and methods:	**Test item:** MEZOFLOR 103 SC **Artificial soil:** 5% sphagnum peat, 20% kaolin clay, 74.88% air-dried industrial sand and 0.12% calcium carbonate **Test organism:** the predatory mites, *Hypoaspis (Geolaelaps) aculeifer* (adult female mites from a synchronized culture) obtained from a standard laboratory culture at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Laboratory of Soil Organisms Toxicology. The mites were introduced 7-14 days after becoming adult **Test design:** exposure period: 14 days number of replicates: 4 replicates / concentration + 8 replicates / control; number of mites: 10 / replicate **Concentrations of the test item:** a control, 2.8, 5.0, 9.1, 16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6 and 1000.0 mg of the test item/kg of dry weight of the artificial soil
Test conditions:	temperature: 20.7 – 22.0°C pH at the beginning of the test: 5.51 – 5.98 pH at the end of the test: 5.28 – 5.53 soil moisture content at the beginning of the test: 16.7 – 18.3% (44.2 – 48.4% of the maximum water holding capacity) soil moisture content in the middle of the test: 16.7 – 18.0% (44.2 – 47.6% of the maximum water holding capacity) soil moisture content at the end of the test: 16.2 – 17.3% (42.9 – 45.8% of the maximum water holding capacity) light-dark cycle: 16 h light and 8 h dark light intensity at the beginning of the test: 593.8 – 667.3 lux light intensity at end of the test: 628.3 – 693.8 lux
Statistical analysis:	EC10, EC20, EC50 – probit analysis using linear max. likelihood regression LC10, LC20, LC50 – weibull analysis using linear max. likelihood regression NOEC: offspring number – Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Trend analysis by Contrasts (Monotonicity of Concentration/Response), Williams Multiple Sequential t-test Procedure. survival – Qualitative Trend Analysis by Contrasts (Monotonicity of Concentration/Response), Tarone's Test Procedure, Step-down Rao-Scott-Cochran-Armitage Test Procedure.
Endpoint:	EC10, EC20, EC50, NOEC LC10, LC20, LC50, NOEC
Results:	Mortality of the predatory mites exposed to the test item at the concentrations ranging from 2.8 to 1000.0 mg/kg dry weight of the artificial soil was between 0.0% and 12.5%. Mortality of the control group was equal to 3.8%. After the application of the test item at the concentrations ranging from 2.8 to 1000.0 mg/kg dry weight of the artificial soil the mean number of juveniles was between 96.0 and 121.5 per replicate. The mean number of juveniles in the control group was equal to 127.8 per

replicate.

Endpoint	Value [mg of the test item/kg dry weight of the artificial soil]	Value [mg of mesotrione/kg dry weight of the artificial soil]	Value [mg of florasulam/kg dry weight of the artificial soil]
EC10	> 1000.00	> 94.25	> 2.83
EC20	> 1000.00	> 94.25	> 2.83
EC50	> 1000.00	> 94.25	> 2.83
NOEC (reproduction)	≥ 1000.00	≥ 94.25	≥ 2.83
LC10	> 1000.00	> 94.25	> 2.83
LC20	> 1000.00	> 94.25	> 2.83
LC50	> 1000.00	> 94.25	> 2.83
NOEC (survival)	≥ 1000.00	≥ 94.25	≥ 2.83

Study 2- amendment to the final report

Report	MEZOFLOR 103 SC Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test, Górska M., 2024, Study code: G-45-24, AMENDMENT NO 1. TO THE FINAL REPORT
Guideline(s):	OECD 226
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No
Reason for the amendment no. 1 to the final report	. The Amendment No. 1 to the Final Report coded as G-45-24 was prepared on the Sponsor request (e-mail dated on November 05, 2024). The Amendment No. 1 to the Final Report was prepared in order to provide the endpoints (EC10, EC20, EC50, LC10, LC20, LC50, NOEC) corrected by the recovery factor calculated based on the results of the analytical measurements during the study. The recovery factor was determined by the geometric mean of recovery of the active substances
Changes in the final report	The Amendment No. 1 introduces the supplementation into the content of the point 3.5.9. Evaluation of the Final Report. Additionally the Amendment introduces the Tables 11-14.
Endpoint:	EC10, EC20, EC50, NOEC LC10, LC20, LC50, NOEC
Results:	.

Table 11. The mean recovery rates for active substances

Active substance	Nominal concentration of active substance [mg/kg d.w.]	Geometric mean of measured concentrations of active substance [mg/kg d.w.]	Recovery [%]
mesotrione	94.25	78.48	83.27
florasulam	2.83	2.82	99.55

Table 12. The determined recovery factor

Active substance	Product	
	Nominal [mg active substance/kg dry weight of the artificial soil]	Recovered [mg active substance/kg dry weight of the artificial soil]
mesotrione	94.25	78.48
florasulam	2.83	2.82
SUM:	97.08	81.30
Recovery factor*	0.837	

*** Recovery factor = (sum of recovered values)/(sum of nominal values)**

Table 14. The endpoint values increased by the recovery factor

Endpoint	Value [mg of the test item/kg dry weight of the artificial soil]	Endpoint values corrected by the recovery factor [mg of the test item/kg dry weight of the artificial soil]
LC_{10}	> 1000.0	> 1837.40
LC_{20}	> 1000.0	> 1837.40
LC_{50}	> 1000.0	> 1837.40
NOEC (survival)	≥ 1000.0	≥ 1837.40
EC_{10}	> 1000.0	> 1837.40
EC_{20}	> 1000.0	> 1837.40
EC_{50}	> 1000.0	> 1837.40
NOEC (reproduction)	≥ 1000.0	≥ 1837.40

zRMS comment:

The new data with toxicity endpoints recalculation and justification that in this case the best solution is to use endpoints based on nominal concentration in risk assessment for soil organisms was provided by Applicant. The recalculation should be treated as additional source information. In this case, when the concentration values fall below the limit of quantification of the analytical method, it is not possible to determine reliable toxicity endpoints based on geometrically measured concentrations. In addition, it can be noted that a decrease in the content of the active substance in the experimental system, caused by the decomposition of this substance (documented low DT_{50}, DT_{90} in soil, confirmed by analytical tests) usually causes a decrease in the toxicity caused by this substance. In the case of unstable substances, precise specification of reliable endpoints based on the measured geometric mean is not possible, especially when the concentration values fall below the limit of quantification of the analytical method. In order to reliably measure the decrease in such rapidly dispersing compounds, more time points of sampling at much shorter intervals (even within the first day) and a much higher number of repetitions may be necessary. Detailed technical guidance on the design of ecotoxicological studies is needed before analytical measurements are routinely included in ecotoxicological studies involving soil invertebrates. Therefore, the revision and validation of the technical guidelines (OECD 222, 232, 226; 2016 a, b, c) is necessary to achieve greater clarity. The study of soil organisms in ecotoxicology differs significantly from, for example, routine aquatic testing procedures, therefore the implementation of such appropriate analysis in the relevant tests cannot be easily adapted or transferred. The risk assessment for soil organisms, in this case, based on

toxicity endpoints based on nominal concentration was accepted by zRMS. **This should be considered at the level of Member States.**

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 accepted by zRMS. Study was carried out according to OECD 216 and all validity criteria were met.

Deviation from the study:

Deviation from the OECD Guideline No. 216 (2000), the EU Method C.21:

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 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

All above mentioned deviations did not affect the results of the study.

The validity criteria:

VALIDITY CRITERION

The coefficients of variation (CV) in the control group were 9.6, 8.0, 8.9, 5.0 and 2.0%, after 0, 7, 14, 28 and 42 days of incubation. The validity criterion was met, because the variation between replicate control samples is less than 15%.

Agreed toxicity endpoints:

Nitrate formation rate* [mg nitrate/kg dry weight of soil/day] for selected time intervals.

Time interval [d]	Control						PEC						5 x PEC					
	Replicate			Mean	±	SD	Replicate			Mean	±	SD	Replicate			Mean	±	SD
	I	II	III				I	II	III				I	II	III			
0 – 7	-5.694	-5.714	-5.760	-5.723	±	0.03	-7.378	-7.278	-7.204	-7.286+	±	0.09	-8.911	-8.617	-8.758	-8.762+	±	0.15
0 – 14	-2.482	-2.385	-2.377	-2.415	±	0.06	-3.288	-3.265	-3.240	-3.265+	±	0.02	-4.060	-4.058	-4.053	-4.057+	±	0.004
0 – 28	2.520	2.945	2.779	2.748	±	0.21	4.230	3.691	3.511	3.811+	±	0.37	3.591	3.359	3.484	3.478+	±	0.12
0 – 42	4.514	4.302	4.383	4.399	±	0.11	5.388	5.425	5.456	5.423+	±	0.03	5.238	5.453	5.610	5.434+	±	0.19

* - Rate of nitrate ions formation per a day = [(mg nitrate / kg of soil dry weight on sampling day 'a') - (mg nitrate / kg of soil dry weight on day 0)]/ 'a' day; 'a' = 7, 14, 28 and 42 day

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

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

Time interval [d]	PEC	5 x PEC
0 – 7	-27,3	-53,1
0 – 14	-35,2	-68,0
0 – 28	-38,7	-26,6
0 – 42	-23,3	-23,5

Values obtained using ToxRat 2.10. computer software.

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

Report	MEZOFLOR 103 SC Soil Microorganisms: Nitrogen Transformation Test, Wołany M., 2021, Study code: G-64-20
Guideline(s):	OECD 216/Method C.21
Deviations:	Yes (The deviation had no impact on the results)
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Aim of the study:
The aim of this study was to detect long-term adverse effects of MEZOFLOR 103 SC on the process of nitrogen transformation in aerobic surface soils.

Summary:
The aim of the study was to detect long-term adverse effects of MEZOFLOR 103 SC on the processes of nitrogen transformation in aerobic surface soils. The freshly collected agricultural soil was used in the experiment. It was manually cleared of large objects and sieved to a particle size of 2 mm.
Two concentrations of the test item were used in the experiment:
- PEC: to 1.42 mg test item/kg dry weight of soil (i.e. 0.14 mg of mesotrione + 0.004 mg of florasulam/kg dry weight of soil).
- 5 x PEC: 7.08 mg of the test item/kg dry weight of soil (i.e. 0.68 mg of mesotrione + 0.02 mg of florasulam/kg dry weight of soil).
The treated and the control soils were divided into three replicates. On days 0, 7, 14, 28 and 42 of incubation, soil samples were collected to determine the quantities of nitrate.
The method involves a measurement of the nitrates ions concentration in a soil extract obtained by using deionised water. The pH/ION 7320 digital meter and the NO 800 nitrate electrode were used.
The nitrate formation rate in each treated group was compared with that in the control, and the percent deviation of the treated from the control was calculated.

Materials and methods:
Test material: MEZOFLOR 103 SC
batch no.: SNS-H-05-15
Active substance: mesotrione: 101.6 g/L
florasulam: 3.0 g/L

Soil: Agricultural soil collected from a place belonging to the Łukasiewicz Research Network - Institute of Industrial Organic Chemistry Branch Pszczyna.

Test design: Three portions of soil (3 x 1500 g), i.e. one control group and two treated groups. Every portion was divided into three replicates (3 x 500 g). The soil was enriched with the organic substrate, i.e. lucerne at dose of 5 g/kg dry weight of soil. Test duration: 42 days.

Concentrations of the test item:	control; PEC: to 1.42 mg test item/kg dry weight of soil (i.e. 0.14 mg of mesotrione + 0.004 mg of florasulam/kg dry weight of soil). 5 x PEC: 7.08 mg of the test item/kg dry weight of soil (i.e. 0.68 mg of mesotrione + 0.02 mg of florasulam/kg dry weight of soil).
Test conditions:	temperature: 19.6 – 21.8°C, soil moisture: 44.7 – 52.7% of the maximum water holding capacity, incubation in darkness
Endpoints:	The concentration of nitrate [mg/kg dry soil] after 0, 7, 14, 28 and 42 days of incubation. The nitrate formation rate [mg/kg dry weight of soil/day] for selected time intervals of soil incubation, i.e. 0 – 7, 0 – 14, 0 – 28, 0 - 42 days. Percent deviation from the control in nitrate formation rate calculated for selected time intervals i.e. 0 – 7, 0 – 14, 0 – 28, 0 - 42 days.
Statistical analysis:	- Shapiro-Wilk's test on Normal Distribution - Levene's Test on Variance Homogeneity (with Residuals) - Williams Multiple Sequential t-test Procedure

Results: On 28 day of analysis the percent deviation from the control calculated on the basis of the nitrate formation rate of the soil treated with the test item at the concentration corresponding to the PEC and 5 x PEC exceeded 25%, therefore, according to the OECD No. 216, EU Method C.21 and the study plan, the experiment was continued. 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: 1.42 mg test item/kg dry weight of soil (i.e. 0.14 mg of mesotrione + 0.004 mg of florasulam/kg dry weight of soil) and 5 x PEC: 7.08 mg of the test item/kg dry weight of soil (i.e. 0.68 mg of mesotrione + 0.02 mg of florasulam/kg dry weight of soil) did not exceed 25% on 42 day of analysis.
Conclusions: On the basis of the results, it was concluded that MEZOFLOR 103 SC at the concentrations corresponding to the PEC: 1.42 mg test item/kg dry weight of soil (i.e. 0.14 mg of mesotrione + 0.004 mg of florasulam/kg dry weight of soil) and 5 x PEC: 7.08 mg of the test item/kg dry weight of soil (i.e. 0.68 mg of mesotrione + 0.02 mg of florasulam/kg dry weight of soil) did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.

A 2.6 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 accepted by zRMS. Study was carried out according to OECD 227 and all validity criteria were met. Deviation from the study:

Deviation from OECD Guideline No. 227:

According to OECD Guideline No. 227 (2006), the light intensity should be 350 ± 50µE/m^2/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 100.8 and 188.8 µE/m^2/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.

All above mentioned deviations did not affect the results of the study.

The validity criteria:

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 **MEZOFLOR 103 SC** on vegetative vigour of terrestrial plants were met:

- the seedling emergence of plants (validity criterion: at least 70%) was as follows:
  - 85.7 – 92.9 – pea,
  - 83.3 – 97.6 – cabbage,
  - 80.0 – 97.5 – carrot,
  - 80.0 – 92.5 – onion,
  - 82.5 – 95.0 – perennial ryegrass,
  - 82.5 – 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.

Agreed toxicity endpoints:

The ER_{50} and NOER values 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.

	Pea *Pisum sativum*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	694.07	>1000.00	>1000.00	758.85	>1000.00	>1000.00
NOER	37.04	>1000.00	≥ 1000.00	12.35	>1000.00	>1000.00
Shoot length (plants without roots)						
ER_{50}	38.26	>1000.00	258.92	56.18	>1000.00	>1000.00
NOER	4.12	12.35	12.35	4.12	333.33	≥ 1000.00
Plant dry weight (plants without roots)						
ER_{50}	37.13	13.67	169.28	19.12	>1000.00	>1000.00
NOER	4.12	1.37	12.35	1.37	111.11	≥ 1000.00

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

	Pea *Pisum sativum*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	70.518	>101.600	>101.600	77.099	>101.600	>101.600
NOER	3.763	>101.600	≥ 101.600	1.254	>101.600	>101.600
Shoot length (plants without roots)						
ER_{50}	3.887	>101.600	26.306	5.708	>101.600	>101.600
NOER	0.419	1.254	1.254	0.418	33.866	≥ 101.600
Plant dry weight (plants without roots)						
ER_{50}	3.772	1.389	17.199	1.943	>101.600	>101.600
NOER	0.418	0.139	1.254	0.139	11.289	≥ 101.600

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

	Pea *Pisum sativum*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	2.082	>3.000	>3.000	2.277	>3.000	>3.000
NOER	0.111	>3.000	≥ 3.000	0.037	>3.000	>3.000
Shoot length (plants without roots)						
ER_{50}	0.115	>3.000	0.777	0.169	>3.000	>3.000
NOER	0.012	0.037	0.037	0.012	1.000	≥ 3.000
Plant dry weight (plants without roots)						
ER_{50}	0.111	0.041	0.508	0.057	>3.000	>3.000
NOER	0.012	0.004	0.037	0.004	0.333	≥ 3.000

	Plant damage at the end of the exposure					
	Pea *Pisum sativum*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Avena sativa*
	mL of the test item/ha					
ER_{50}	15.758 (14.312 – 17.350)	18.401 (15.677 – 21.599)	98.637 (82.474 – 117.969)	20.090 (16.667 – 24.218)	>1000.000	>1000.000
	g of mesotrione/ha					
ER_{50}	1.601 (1.454 – 1.763)	1.870 (1.593 – 2.194)	10.022 (8.379 – 11.986)	2.041 (1.693 – 2.461)	>101.600	>101.600
	g of florasulam/ha					
ER_{50}	0.047 (0.043 – 0.052)	0.055 (0.047 – 0.065)	0.296 (0.247 – 0.354)	0.060 (0.050 – 0.073)	>3.000	>3.000

ER_{50} values were calculated using ToxRatPro Version 3.3.0.

Study 1

Report	MEZOFLOR 103 SC Terrestrial Plant Test: Vegetative Vigour Test, Pieczka P., 2021, Study code: G-65-20
Guideline(s):	OECD 227
Deviations:	Yes (The deviation had no impact on the results)
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Aim of the study:
The aims of this study were to assess the impact of the test item on vegetative vigour of selected terrestrial plant species and to determine ER25, ER50 and NOER for chosen parameters of the test.

Summary:
The study, aimed at evaluating the effect of MEZOFLOR 103 SC on vegetative vigour of 6 terrestrial plants, was conducted on 3 dicotyledonous and 3 monocotyledonous species. Seeds of the test plant species were sown in plastic pots (6 seeds/pot for pea and cabbage; 10 seeds/pot for carrot, onion, perennial ryegrass and oats). 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 concentration were: - pea: 3 plants/pot – 21 plants/application rate (7 pots/application rate); - cabbage: 3 plants/pot – 21 plants/application rate (7 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). The pot is defined as a replicate. The test item was sprayed onto the plants. For each species, eight application rates were used. Untreated control group was conducted simultaneously. The treated and the control groups were divided into four replicates for carrot, onion, perennial ryegrass onion and oats; 7 replicates for pea and cabbage. The experiment was conducted in a plant growth room where suitable environmental conditions for each test species were provided. During the experiment, the plants were observed for visual phytotoxicity (7, 14 and 21 days after the test item application). The experiment finished 21 days after the spraying. At the end of the experiment, the number of surviving plants was counted. Next, the plants were cut down, and the lengths of their shoots were determined. Finally, they were dried at 60ºC to a constant weight and weighed. The results concerning the

shoot length, the dry weight, and the number of plants at the end of the experiment were statistically analyzed to determine the ER25, ER50 and NOER.

Materials and methods: Test item: MEZOFLOR 103 SC
batch number: SNS-H-05-15
active substances: mesotrione – 101.6 g/L
florasulam – 3.0 g/L

Test species: pea (Pisum sativum), cabbage (Brassica oleracea var. capitata), carrot (Daucus carota), onion (Allium cepa), perennial ryegrass (Lolium perenne), oats (Avena sativa)

Soil: Sandy loam

Study design: number of rates: 8 + control;
number of replicates/rate: 7 (pea, cabagge), 4 (carrot, onion, perennial ryegrass, oats). The total number of plants per application rate – 21 (pea, cabbage) or 20 (carrot, onion, perennial ryegrass, oats)
exposure termination: 21 days after spraying

Application rates:

- a control,
- 0.46 mL of the test item /ha (0.046 g of mesotrione + 0.001 g of florasulam/ha),
- 1.37 mL of the test item /ha (0.139 g of mesotrione + 0.004 g of florasulam/ha),
- 4.12 mL of the test item /ha (0.418 g of mesotrione + 0.012 g of florasulam/ha),
- 12.35 mL of the test item /ha (1.254 g of mesotrione + 0.037 g of florasulam/ha),
- 37.04 mL of the test item /ha (3.763 g of mesotrione + 0.111 g of florasulam/ha),
- 111.11 mL of the test item /ha (11.289 g of mesotrione + 0.333 g of florasulam/ha),
- 333.33 mL of the test item /ha (33.867 g of mesotrione + 1.000 g of florasulam/ha),
- 1000.00 mL of the test item /ha (101.600 g of mesotrione + 3.000 g of florasulam/ha).

Volume of deionized water used to prepare the highest rate corresponded to 200 L spraying liquid/ha.

Test conditions: temperature: 18.9 – 25.7°C,
humidity: 46.0 – 83.3%,
lighting: 16 h light : 8 h dark;
light intensity: 100.8 – 188.8 µE/m2 /s;
carbon dioxide concentration: 322 – 387 ppm

Statistical analysis: ER25, ER50 – probit analysis, 3-param. Normal CDF or Weibull analysis NOER:
In order to determine the NOER values, the following tests were used:
- for the emergence of plants: Fisher's Exact Binomial Test with Bonferroni Correction, Qualitative Trend Analysis by Contrasts (Monotonicity of Rate/Response), Tarone's Test Procedure, Step-down Rao-Scott-Cochran-Armitage Test Procedure
- for the 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, Multiple Sequentially-rejective Welsh t-test After Bonferroni-Holm, Dunnett's Multiple t-test Procedure,
- for the plant weight: Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Trend analysis by Contrasts (Monotonicity of Rate/Response), Non-parametric Trend analysis by Contrasts (Monotonicity of Rate/Response), Step down Jonckheere-Terpstra Test Procedure, Williams Multiple Sequential t-test Procedure, Multiple Sequentially-rejective Welsh t-test After Bonferroni-Holm, Dunnett's Multiple t-test Procedure,

Endpoints: ER25, ER50, NOER

Results and conclusions The ER50 and NOER values 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.

	Pea *Pisum sativum*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	694.07	>1000.00	>1000.00	758.85	>1000.00	>1000.00
NOER	37.04	>1000.00	≥ 1000.00	12.35	>1000.00	>1000.00
Shoot length (plants without roots)						
ER_{50}	38.26	>1000.00	258.92	56.18	>1000.00	>1000.00
NOER	4.12	12.35	12.35	4.12	333.33	≥ 1000.00
Plant dry weight (plants without roots)						
ER_{50}	37.13	13.67	169.28	19.12	>1000.00	>1000.00
NOER	4.12	1.37	12.35	1.37	111.11	≥ 1000.00

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

	Pea *Pisum sativum*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	70.518	>101.600	>101.600	77.099	>101.600	>101.600
NOER	3.763	>101.600	≥ 101.600	1.254	>101.600	>101.600
Shoot length (plants without roots)						
ER_{50}	3.887	>101.600	26.306	5.708	>101.600	>101.600
NOER	0.419	1.254	1.254	0.418	33.866	≥ 101.600
Plant dry weight (plants without roots)						
ER_{50}	3.772	1.389	17.199	1.943	>101.600	>101.600
NOER	0.418	0.139	1.254	0.139	11.289	≥ 101.600

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

	Pea *Pisum sativum*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	2.082	>3.000	>3.000	2.277	>3.000	>3.000
NOER	0.111	>3.000	≥ 3.000	0.037	>3.000	>3.000
Shoot length (plants without roots)						
ER_{50}	0.115	>3.000	0.777	0.169	>3.000	>3.000
NOER	0.012	0.037	0.037	0.012	1.000	≥ 3.000
Plant dry weight (plants without roots)						
ER_{50}	0.111	0.041	0.508	0.057	>3.000	>3.000
NOER	0.012	0.004	0.037	0.004	0.333	≥ 3.000

The test item, i.e. MEZOFLOR 103 SC applied at rates ranging from 0.46 to 1000.00 mL/ha had an impact on vegetative vigour of pea, cabbage, carrot and onion. In cultivation of perennial ryegrass, slight impact on the process of growth was observed. No effect of the test item in cultivation of oats was noticed.
The mortality of plants was noticed in cultivation of pea, carrot and onion.
On the basis of NOER, ER25 and ER50 values determined from the shoot length it was proved that the test item inhibit the process of growth of pea, cabbage, carrot and onion and slightly inhibited the process of growth of perennial ryegrass.
On the basis of NOER, ER25 and ER50 values determined from the dry shoot weight it was proved that the test item inhibited the process of growth of pea, cabbage, carrot, onion and moderately inhibited the process of growth of perennial ryegrass.
During the experiment the phytotoxic symptoms of the test item in cultivation of pea, cabbage, carrot, onion and perennial ryegrass were observed. Among plant damages, theese were: stunted growth (pea, cabbage, carrot, onion, perennial ryegrass), chlorosis (pea, cabbage, carrot, onion, perennial ryegrass), necrosis (pea, cabbage), wilting (pea, cabbage, carrot, onion) and deformations (carrot).

Comments of zRMS:	The study was accepted by zRMS. Study was carried out according to OECD 208 and all validity criteria were met. Deviation from the study: **DEVIATIONS IN THE STUDY** Deviations from OECD Guideline No. 208: According to OECD Guideline No. 208 (2006), the light intensity should be 350 ± 50µE/m^2/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 82.72 and 156.4 µE/m^2/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing. All above mentioned deviations did not affect the results of the study. **The validity criteria:**

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 **MEZOFLOR 103 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% – pea,
- 90.5% – cabbage,
- 80.0% – carrot,
- 90.0% – onion,
- 90.0% – perennial ryegrass,
- 100.0% – oats,
- the mean survival of the emerged control seedlings was 100% for cabbage, pea, carrot, onion, perennial ryegrass and oats (validity criterion: 90%);
- the control seedlings did not exhibit any visible phytotoxic effects;
- environmental conditions for all plants of the same species were identical.

Agreed toxicity endpoints:

Results and conclusions

The ER_{50} and NOER values 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.

	Pea *Pisum sativum*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	331.95	>1000.00	>100.00	>1000.00	>1000.00	>1000.00
NOER	37.04	≥1000.00	333.33	111.11	≥1000.00	≥1000.00
Shoot length (plants without roots)						
ER_{50}	211.65	94.14	366.29	520.07	>1000.00	>1000.00
NOER	12.35	12.35	111.11	111.11	≥1000.00	≥1000.00
Plant dry weight (plants without roots)						
ER_{50}	118.21	68.24	377.49	531.79	>1000.00	>1000.00
NOER	12.35	12.35	111.11	111.11	≥1000.00	≥1000.00

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

	Pea *Pisum sativum*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	33.726	>101.600	>101.600	>101.600	>101.600	>101.600
NOER	3.763	≥101.600	33.867	11.289	≥101.600	≥101.600
Shoot length (plants without roots)						
ER_{50}	21.504	9.565	37.215	52.839	>101.600	>101.600
NOER	1.254	1.254	11.289	11.289	≥101.600	≥101.600
Plant dry weight (plants without roots)						
ER_{50}	12.010	6.933	38.353	54.030	>101.600	>101.600
NOER	1.254	1.254	11.289	11.289	≥101.600	≥101.600

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

	Pea *Pisum sativum*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	0.996	>3.000	>3.000	>3.000	>3.000	>3.000
NOER	0.111	≥3.000	1.000	0.333	≥3.000	≥3.000
Shoot length (plants without roots)						
ER_{50}	0.635	0.282	1.099	1.560	>3.000	>3.000
NOER	0.037	0.037	0.333	0.333	≥3.000	≥3.000
Plant dry weight (plants without roots)						
ER_{50}	0.355	0.205	1.132	1.595	>3.000	>3.000
NOER	0.037	0.037	0.333	0.333	≥3.000	≥3.000

	Plant damage at the end of the exposure					
	Pea *Pisum sativum*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Avena sativa*
mL of the test item/ha						
ER_{50}	77.705 (69.462 – 86.927)	48.864 (43.688 – 54.652)	171.950 (161.934 – 182.585)	255.113 (225.237 – 288.953)	>1000.00	>1000.00
g of mesotrione/ha						
ER_{50}	7.895 (7.057 – 8.832)	4.965 (4.439 – 5.553)	17.470 (16.452 – 18.551)	25.919 (22.884 – 29.358)	>101.600	>101.600
g of florasulam/ha						
ER_{50}	0.233 (0.208 – 0.261)	0.147 (0.131 – 0.164)	0.516 (0.486 – 0.548)	0.765 (0.676 – 0.867)	>3.000	>3.000

Study 2

Report	MEZOFLOR 103 SC Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test, Wołany M., 2021, Study code: G-66-20
Guideline(s):	OECD 208
Deviations:	Yes (The deviation had no impact on the results)
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Aim of the study:
The aims of this study were to assess the impact of the test item on seedling emergence and seedling growth of selected terrestrial plant species and to determine the ER25, ER50, and NOER for chosen parameters of the test.

Summary:
The study, aimed at evaluating the effect of MEZOFLOR 103 SC on seedling emergence and seedling growth of 6 terrestrial plants, was conducted on 3 dicotyledonous and 3 monocotyledonous species. The test item was sprayed onto the soil surface. For each species, eight application rates were used. There was also a concurrent control group. Seeds of the test plant species were sown in plastic pots. There were 3 (pea, cabbage) or 5 (carrot, onion, perennial ryegrass and oats) seeds/pot. The experiment was conducted in a special room. Suitable environmental conditions for each test species were provided. During the experiment, the plants were observed for emergence (every day until the emergence of 50% of the control seedlings and then every 1 – 3 days) and visual phytotoxicity (after 7 and 14 days). The experiment finished 14 days after the emergence of 50% of the control seedlings. At the end of the experiment, the number of surviving plants was determined. Next, the plants were cut down, measured, dried to a constant weight at 60ºC, and weighed. The results concerning the emergence, the shoot length, and the dry weight were statistically analyzed in order to determine the ER25, ER50, and NOER.

Materials and methods:
Test item: MEZOFLOR 103 SC
batch number: SNS-H-05-15
active substances: mesotrione: 101.6 g/L

florasulam: 3.0 g/L

Test species: pea (Pisum sativum), cabbage (Brassica oleracea var. capitata), carrot (Daucus carota), onion (Allium cepa), perennial ryegrass (Lolium perenne), oats (Avena sativa)

Soil: sandy loam

Study design: number of rates: 8 + control;
number of replicates/rate: 4 (carrot, onion, perennial ryegrass, oats) or 7 (pea, cabbage). The total number of seeds per application rate – 20 (carrot, onion, perennial ryegrass, oats)) or 21 (pea, cabbage).
test termination: 14 days after the emergence of 50% of the control seedlings

Application rates:
- 0.46 mL of the test item/ha (0.046 g of mesotrione + 0.001 g of florasulam/ha),
- 1.37 mL of the test item/ha (0.139 g of mesotrione + 0.004 g of florasulam/ha),
- 4.12 mL of the test item/ha (0.418 g of mesotrione + 0.012 g of florasulam/ha),
- 12.35 mL of the test item/ha (1.254 g of mesotrione + 0.037 g of florasulam/ha),
- 37.04 mL of the test item/ha (3.763 g of mesotrione + 0.111 g of florasulam/ha),
- 111.11 mL of the test item/ha (11.289 g of mesotrione + 0.333 g of florasulam/ha),
- 333.33 mL of the test item/ha (33.867 g of mesotrione + 1.000 g of florasulam/ha),
- 1000.00 mL of the test item/ha (101.600 g of mesotrione + 3.000 g of florasulam/ha).

Test conditions: temperature: 18.0 – 26.4°C,
humidity: 46.0 – 79.0%,
lighting: 16 h light : 8 h dark;
light intensity: 82.72 – 156.4 µE/m2 /s;
carbon dioxide concentration: 336 – 374 ppm

Statistical analysis:
ER25, ER50 – probit analysis using linear max. likelihood regression, 3- param. normal CDF

NOER: In order to determine the NOER values, the following tests were used: - for the emergence of plants: Qualitative Trend Analysis by Contrasts (Monotonicity of Rate/Response), Tarone's Test Procedure, Step-down Cochran-Armitage Test Procedure, Chi2 2x2 Table Test with Bonferroni Correction, Fisher's Exact Binomial Test with Bonferroni Correction, Step-down Rao-Scott-Cochran-Armitage Test Procedure
- for the 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, Multiple Sequentially-rejective Median (2x2 Table) Test After Bonferroni-Holm, Multiple Sequentially-rejective t-test After Bonferroni-Holm.
- for the plant dry weight: Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Trend analysis by Contrasts (Monotonicity of Rate/Response),Non-parametric Trend analysis by Contrasts (Monotonicity of Rate/Response), Williams Multiple Sequential t-test Procedure, Multiple Sequentially-rejective t-test After Bonferroni-Holm, Dunnett's Multiple t-test Procedure, Step-down Jonckheere-Terpstra Test Procedure.

Endpoints: ER25, ER50, NOER

Results and conclusions:
The ER50 and NOER values 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.

	Pea *Pisum sativum*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	331.95	>1000.00	>100.00	>1000.00	>1000.00	>1000.00
NOER	37.04	≥1000.00	333.33	111.11	≥1000.00	≥1000.00
Shoot length (plants without roots)						
ER_{50}	211.65	94.14	366.29	520.07	>1000.00	>1000.00
NOER	12.35	12.35	111.11	111.11	≥1000.00	≥1000.00
Plant dry weight (plants without roots)						
ER_{50}	118.21	68.24	377.49	531.79	>1000.00	>1000.00
NOER	12.35	12.35	111.11	111.11	≥1000.00	≥1000.00

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

	Pea *Pisum sativum*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	33.726	>101.600	>101.600	>101.600	>101.600	>101.600
NOER	3.763	≥101.600	33.867	11.289	≥101.600	≥101.600
Shoot length (plants without roots)						
ER_{50}	21.504	9.565	37.215	52.839	>101.600	>101.600
NOER	1.254	1.254	11.289	11.289	≥101.600	≥101.600
Plant dry weight (plants without roots)						
ER_{50}	12.010	6.933	38.353	54.030	>101.600	>101.600
NOER	1.254	1.254	11.289	11.289	≥101.600	≥101.600

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

	Pea *Pisum sativum*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	0.996	>3.000	>3.000	>3.000	>3.000	>3.000
NOER	0.111	≥3.000	1.000	0.333	≥3.000	≥3.000
Shoot length (plants without roots)						
ER_{50}	0.635	0.282	1.099	1.560	>3.000	>3.000
NOER	0.037	0.037	0.333	0.333	≥3.000	≥3.000
Plant dry weight (plants without roots)						
ER_{50}	0.355	0.205	1.132	1.595	>3.000	>3.000
NOER	0.037	0.037	0.333	0.333	≥3.000	≥3.000

Study 3

Report	MEZOFLOR 103 SC Terrestrial Plant Test: Vegetative Vigour Test, Pieczka P., 2021, Study code: G-65-20- AMENDMENT NO. 1 TO THE FINAL REPORT
Guideline(s):	OECD 227
Deviations:	Yes (The deviation had no impact on the results)
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No
Aim of the study:	The Amendment No. 1 to the Final Report coded as G-65-20 was prepared on the Registration Authority and Sponsor request (e-mail dated December 12, 2023). The Amendment no. 1 to the Final Report was prepared in order to provide the endpoints (ER50 values) calculated on the basis of percentage of plant damages at the end of the exposure period for all tested species
Summary:	The content of the point is supplemented with following information: The ER50 values based on percentage plant damages at the end of exposure were determined using statistical tests, i.e. probit (cabbage, carrot, oats) or logit (pea, onion, perennial ryegrass) analysis using linear max. likelihood regression. The ToxRatPro Version 3.3.0 computer software was used [10], [SOP/G/80].

Results and conclusions: The Amendment No. 1 to the final report supplements the results of the experiment with a statistical analysis of phytotoxic symptoms observed on the tested plant species. Therefore, the description of the results is completed with an information on the numbering of the rate - effect curves showing the influence of the test item on the percentage of phytotoxic symptoms. The following graphs were introduced to the amendment:
- rate-effect curve showing the influence of the test item on phytotoxic symptoms of pea at the end of exposure – Figure 14;

- rate-effect curve showing the influence of the test item on phytotoxic symptoms of cabbage at the end of exposure – Figure 15;

- rate-effect curve showing the influence of the test item on phytotoxic symptoms of carrot at the end of exposure – Figure 16;

- rate-effect curve showing the influence of the test item on phytotoxic symptoms of onion at the end of exposure – Figure 17;

- rate-effect curve showing the influence of the test item on phytotoxic symptoms of perennial ryegrass at the end of exposure – Figure 18;

- rate-effect scatter plot showing the influence of the test item on phytotoxic symptoms of oats at the end of exposure – Figure 19.
The ER50 values determined on the basis of plant damages at the end of the exposure are presented in the Table 33.

Plant damage at the end of the exposure						
	Pea *Pisum sativum*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Avena sativa*
mL of the test item/ha						
ER_{50}	15.758 (14.312 – 17.350)	18.401 (15.677 – 21.599)	98.637 (82.474 – 117.969)	20.090 (16.667 – 24.218)	>1000.000	>1000.000
g of mesotrione/ha						
ER_{50}	1.601 (1.454 – 1.763)	1.870 (1.593 – 2.194)	10.022 (8.379 – 11.986)	2.041 (1.693 – 2.461)	>101.600	>101.600
g of florasulam/ha						
ER_{50}	0.047 (0.043 – 0.052)	0.055 (0.047 – 0.065)	0.296 (0.247 – 0.354)	0.060 (0.050 – 0.073)	>3.000	>3.000

ER_{50} values were calculated using ToxRatPro Version 3.3.0.

Comments of zRMS:	The calculations was accepted by zRMS.

Study 4

Report	MEZOFLOR 103 SC Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test, Wołany M., 2021, Study code: G-66-20 - AMENDMENT NO. 1 TO THE FINAL REPORT
Guideline(s):	OECD 208
Deviations:	Yes (The deviation had no impact on the results)
GLP:	Yes
Acceptability:	Yes

Duplication (if vertebrate study)	No
Aim of the study:	The Amendment No. 1 to the Final Report coded as G-66-20 was prepared on the Registration Authority and Sponsor request (e-mail dated December 12, 2023). The Amendment no. 1 to the Final Report was prepared in order to provide the endpoints (ER50 values) calculated on the basis of percentage of plant damages at the end of the exposure period for all tested species.
Summary:	The content of the point is supplemented with following information: The ER50 values based on percentage plant damages at the end of exposure were determined using statistical tests, i.e. probit (cabbage, carrot, onion, perennial ryegrass, oats) or logit (pea) analysis using linear max. likelihood regression. The ToxRatPro Version 3.3.0 computer software was used [10], [SOP/G/80].
Results and conclusions:	The Amendment No. 1 to the final report supplements the results of the experiment with a statistical analysis of phytotoxic symptoms observed on the tested plant species. Therefore, the description of the results is completed with an information on the numbering of the rate - effect curves showing the influence of the test item on the percentage of phytotoxic symptoms. The following graphs were introduced to the amendment: rate-effect curve showing the influence of the test item on phytotoxic symptoms of pea at the end of exposure - Figure 19; rate-effect curve showing the influence of the test item on phytotoxic symptoms of cabbage at the end of exposure - Figure 20; rate-effect curve showing the influence of the test item on phytotoxic symptoms of carrot at the end of exposure - Figure 21; rate-effect curve showing the influence of the test item on phytotoxic symptoms of onion at the end of exposure - Figure 22; rate-effect scatter plot showing the influence of the test item on phytotoxic symptoms of perennial ryegrass at the end of exposure – Figure 23; rate-effect scatter plot showing the influence of the test item on phytotoxic symptoms of oats at the end of exposure – Figure 24. The ER50 values determined on the basis of plant damages at the end of the exposure are presented in the Table 39.

Plant damage at the end of the exposure						
	Pea *Pisum sativum*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Avena sativa*
mL of the test item/ha						
ER_{50}	77.705 (69.462 – 86.927)	48.864 (43.688 – 54.652)	171.950 (161.934 – 182.585)	255.113 (225.237 – 288.953)	>1000.00	>1000.00
g of mesotrione/ha						
ER_{50}	7.895 (7.057 – 8.832)	4.965 (4.439 – 5.553)	17.470 (16.452 – 18.551)	25.919 (22.884 – 29.358)	>101.600	>101.600
g of florasulam/ha						
ER_{50}	0.233 (0.208 – 0.261)	0.147 (0.131 – 0.164)	0.516 (0.486 – 0.548)	0.765 (0.676 – 0.867)	>3.000	>3.000

Comments of zRMS:	The calculations was accepted by zRMS.

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