FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: M-100SC-OR2-C

Product name(s): Juzan Extra 100 SC

Chemical active substance:

mesotrione, 100 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: CIECH Sarzyna S.A.

Submission date: 05/2022

MS Finalisation date: 05/2023

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Version history

When	What
May 2022	First submission for the product authorisation
January 2023	Draft assessment performred by zRMS
May 2023	The final version of RR after commenting period

Table of Contents

9 Ecotoxicology (KCP 10)

zRMS comments:

All comments and conclusions of the zRMS are presented in grey.
Minor changes are introduced directly in the text and highlighted in grey.
Not agreed or not relevant information are struck through and shaded for transparency.

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use-No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: devel-opmental stages of the pest or pest group)	**Application**				**Application rate**			PHI (days)	Remarks: e.g. g saf-ener/ synergist per ha	**Conclusion**						
					Method / Kind	Timing / Growth stage of crop & season	Max. num-ber 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 arthro-	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	PL	Maize (ZEAMX)	F	Monotyledonous weeds (TTDMS); Dicotyledonous weeds (TTDSS)	spraying	BBCH 12 - 18	a) 1 b) 1	n.a.	a) 1,5 L/ha b) 1,5 L/ha	a) 150 g as/ha b) 150 g as/ha	200 / 400	n.a.	Dose range: 0,75 -1,5 L/ha	A	C	R	A	A	A	R
Interzonal uses (use as seed treatment, in greenhouses (or other closed places of plant production), as post-harvest treatment or for treatment of empty storage rooms)																				
Minor uses according to Article 51 (field uses)																				
2	PL	sugar maize (ZEAMS); Popcorn (ZEAME);	F	Monotyledonous weeds (TTDMS); Dicotyledonous weeds (TTDSS)	spraying	BBCH 12 - 18	a) 1 b) 1	n.a.	a) 1,5 L/ha b) 1,5 L/ha	a) 150 g as/ha b) 150 g as/ha	200 / 400	n.a.	Dose range: 0,75 -1,5 L/ha	A	C	R	A	A	A	R
Minor uses according to Article 51 (interzonal uses)																				

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

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

Explanation for column 15 – 21 “Conclusion”

A	Acceptable, Safe use
R	Further refinement and/or risk mitigation measures required

C	To be confirmed by cMS
N	No safe use

Remarks table:

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

9.1.1 Overall conclusions

9.1.1.1 zRMS comment: Metabolites of mesotrione potentially relevant for effect assessment. Metabolites of mesotrione and their required exposure assessment as summarized in Table 9.1.-3 are in agreement with EFSA Journal 2016;14(3):4419 and are considered acceptable by the zRMS.

9.1.1.2 Effects on birds (KCP 10.1.1), The acute and long-term risks of Juzan Extra 100 SC to birds were assessed from toxicity exposure ratios between toxicity endpoints estimated from studies with mesotrione and maximum residues occurring on food items following applications according to the proposed use pattern. The risk to birds from exposure via drinking water was also assessed and showed acceptable risk. Risk of secondary poisoning for mesotrione was not assessed as the log Pow is <3.0.

zRMS comment: The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438). The presented above birds risk assessment is agreed by the zRMS. All TER values exceed the relevant triggers indicating that Juzan Extra 100 SC does not pose an unacceptable risk to birds following applications according to recommended use pattern. On the basis of performed calculations, acceptable acute and long-term risk to birds may be concluded from proposed uses of Juzan Extra 100 SC.

9.1.1.3

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

The acute and long-term risks of Juzan Extra 100 SC to birds were assessed from toxicity exposure ratios between toxicity endpoints estimated from studies with mesotrione and maximum residues occurring on food items following applications according to the proposed use pattern. The risk to birds from exposure via drinking water was also assessed and showed acceptable risk. Risk of secondary poisoning for mesotrione was not assessed as the log Pow is <3.0.

The acute and long-term risks of Juzan Extra 100 SC to mammals were assessed from toxicity exposure ratios between toxicity endpoints, estimated from studies with mesotrione and maximum residues occurring on food items following applications according to the proposed use pattern. Risk of secondary poisoning has not been assessed, as mesotrione has log P_{OW} <3.0. The TER values, calculated for recommended scenarios, exceed the trigger value of 10 for acute risk. However, first-tier TER value calculated for a few scenarios did not exceed the relevant trigger values of 5 for reproductive risk and acceptable risk to mammals was confirmed based on higher tier assessment. The risk to mammals is acceptable following use of Juzan Extra 100 SC according to the proposed use pattern.

Regarding effects on other terrestrial vertebrate wildlife (reptiles and amphibians) no data/information available.

9.1.1.5 Effects on aquatic organisms (KCP 10.2)

Based on PEC/RAC calculations, no unacceptable risk is indicated for aquatic organisms considering all envisaged GAP uses for Juzan Extra 100 SC, assuming that following risk mitigation measures are taken into account:

- a vegetative buffer strip of 20m to surface water bodies is required when conventional spraying techniques are applied.

9.1.1.6 Effects on bees (KCP 10.3.1)

The evaluation of the risk for bees has been performed in line with SANCO/10329/2002 rev 2 final.

Based on results obtained in oral and contact studies on honeybees all calculated hazard quotients are considerably less than trigger values, indicating that the formulation poses a low risk to bees. Therefore, a low risk to bees is expected from the application Juzan Extra 100 SC according to the proposed GAP and no mitigation measures are required.
According to Commission regulation (EU) No 284/2013, point 10.3.1. (Effects on bees): the Applicant provided also the chronic test on bees and chronic test for larvae for formulated product.

9.1.1.7 Effects on arthropods other than bees (KCP 10.3.2)

The risk assessment was conducted according to the ESCORT 2 Guidance Document (2000) and the Guidance Document on Terrestrial Ecotoxicology (2002).

The in-field and off-field risk from exposure to mesotrione applied as Juzan Extra 100 SC for the intended uses in major and minor crops is indicated to be acceptable for non-target arthropods other than bees

based on Tier 2 data without the need for risk mitigation measures.

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

The risk from exposure to mesotrione and relevant soil degradation products applied as Juzan Extra 100 SC for all intended uses is indicated to be acceptable for the soil meso- and macrofauna.

The risk to soil microorganisms is acceptable since negligible effects on the nitrogen transformations are foreseen at higher levels than the calculated PEC soil values for the active when the intended use of pattern for the Juzan Extra 100 SC is considered.

9.1.1.9 Effects on non-target terrestrial plants (KCP 10.6).

For the proposed use of Juzan Extra 100 SC, based on the highest application rate the risk for non-target plants in the off-crop area is indicated to be acceptable when either 1 m buffer strip with 90% drift reduction or a 5 m buffer strip with 50% drift reduction, or 10 m buffer strip with no drift reduction is applied as risk mitigation measure.

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

No further relevant data available and considered necessary.

9.1.2 Grouping of intended uses for risk assessment

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

Table 9.1-2: Critical use pattern of Juzan Extra 100 SC grouped according to crop

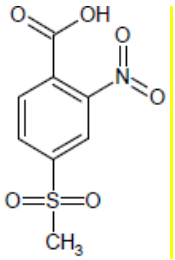
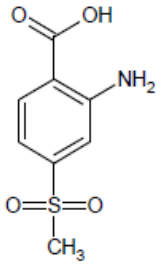
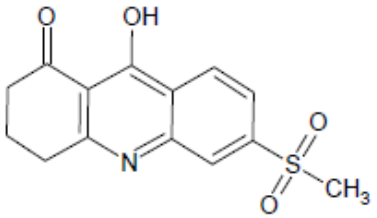
Grouping according to crop			
Group	**Intended uses**	**relevant use parameters for grouping**	**Risk assessment**
1.	Maize, sugar maize and popcorn	-the highest exposure scenario -highest PEC soil for mesotrione and relevant metabolites -highest PECsw for mesotrione and its metabolites -highest SV value	- risk assessment for bees, arthropods other than bees, non-target plants - risk assessment for soil organisms - risk assessment for aquatic organisms - risk assessmnet for birds and mammals

9.1.3 Consideration of metabolites

A list of metabolites found in environmental compartments is provided below. The need for conducting a metabolite-specific risk assessment in the context of the evaluation of Juzan Extra 100 SC is indicated in

the table.

Table 9.1-3 Metabolites of mesotrione

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
MNBA NOA437130 4-(methylsulfonyl)-2-nitrobenzoic acid		245	Soil: 57.2 % of a.s (aerobic laboratory degradation and soil photolysis studies)	Yes, for aquatic and soil organisms
AMBA NOA422848 2-amino-4-(methylsulfonyl) benzoic acid		215	Soil: 9.7 % of a.s. (aerobic laboratory degradation studies and soil photolysis studies)	Yes, for aquatic and soil organisms
SYN546974		291	Soil: max 1 E-10 % AR. Maximum occurence observed in sediment/water studies: 33 %	Yes, for aquatic organisms

zRMS comment: Metabolites of mesotrione potentially relevant for effect assessment. Metabolites of mesotrione and their required exposure assessment as summarized in Table 9.1.-3 are in agreement with EFSA Journal 2016;14(3):4419 and are considered acceptable by the zRMS.

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with mesotrione. Full details of these studies are provided in the respective EU DAR and related documents.
Effects on birds of Juzan Extra 100 SC were not evaluated as part of the EU assessment of mesotrione.

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

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

Species	Substance	Exposure System	Results	Reference
Bobwhite quail (Colinus virginianus)	Mesotrione	Oral 1 d Acute	LD50>2000 mg a.s./kg bw (corrected to 3776 mg a.s./kg bw)	EFSA Journal 2016;14(3):4419
Mallard duck (Anas platyrhynchos)	Mesotrione	Dietary 8 d Short-term	LC50>5200 mg/kg diet	EFSA Journal 2016;14(3):4419
Mallard duck (Anas platyrhynchos)	Mesotrione	Dietary Reproductive toxicity	NOEL= 20.6 mg a.s./kg bw/d	EFSA Journal 2016;14(3):4419

zRMS comment: zRMS confirms that the reported toxicity data in table 9.2-1 are in accordance with the EU agreed end-points and will be used for risk assessment

9.2.2 Risk assessment for spray applications

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

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

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

Table 9.2-2: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of Juzan Extra 100 SC in maize

Intended use	**Maize (***also minor uses i.e.: sugar maize, popcorn***);**				
Active substance/product	mesotrione				
Application rate (g/ha)	150				
Acute toxicity (mg/kg bw)	≥ 2000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening step	Small omivorous bird	158.8	1.0	23.8	84.0
Reprod. toxicity (mg/kg bw/d)	20.6				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening step	Small omivorous bird	64.8	1.0 x 0.53	5.15	**4.0**

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

Table 9.2-3: Tier 1 assessment of the long-term/reproductive risk for birds due to the use of Juzan Extra 100 SC in maize and minor uses.

Intended use	**Maize (***also minor uses i e: sugar maize, popcorn***);**				
Active substance/product	mesotrione				
Application rate (g/ha)	150				
Reprod. toxicity (mg/kg bw/d)	20.6				
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 BBCH 10 - 19	Small insectivorous bird “wagtail”	11.3	1.0 x 0.53	0.90	22.9
Maize BBCH 10 - 29	Medium granivorous bird “gamebird”	3.0	1.0 x 0.53	0.24	86.4
Maize BBCH 10 - 29	Medium	22.7	1.0 x 0.53	1.80	11.4

	herbivorous/granivorous bird "pigeon"				
Maize BBCH 10 - 29	Small omnivorous bird "lark"	10.9	1.0 x 0.53	0.87	23.8

9.2.2.2 Higher-tier risk assessment

Not required

9.2.2.3 Drinking water exposure

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

Leaf scenario

Since Juzan Extra 100 SC is not 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 14 ml/g (worst case), mesotrione belongs to the group of less sorptive substances.

Effective application rate (g/ha)*	=	150		
Acute toxicity (mg/kg bw)	=	2000	quotient =	0.075
Reprod. toxicity (mg/kg bw/d)	=	20.6	quotient =	7.3

The resulting ratios fall below the trigger of 50 indicating that the further assessment of the acute and long term risk to birds from drinking water from puddles is not required.

zRMS comment: Agreed.

9.2.2.4 Effects of secondary poisoning

The log P_{ow} of mesotrione and its main metabolites MNBA, AMBA and SYN546974 amounts to 0.11, -1.3. 0.32 and 1.62 respectively and thus do not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

Risk assessment for earthworm-eating birds via secondary poisoning

Not required.

Risk assessment for fish-eating birds via secondary poisoning

Not required.

zRMS comment: Agreed.

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.2.4 Overall conclusions

The acute and long-term risks of Juzan Extra 100 SC to birds were assessed from toxicity exposure ratios between toxicity endpoints estimated from studies with mesotrione and maximum residues occurring on food items following applications according to the proposed use pattern. The risk to birds from exposure via drinking water was also assessed and showed acceptable risk. Risk of secondary poisoning for mesotrione was not assessed as the log Pow is <3.0.

zRMS comment: The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438). The presented above birds risk assessment is agreed by the zRMS. All TER values exceed the relevant triggers indicating that Juzan Extra 100 SC does not pose an unacceptable risk to birds following applications according to recommended use pattern. On the basis of performed calculations, acceptable acute and long-term risk to birds may be concluded from proposed uses of Juzan Extra 100 SC.

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

9.3.1 Toxicity data

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

Effects on mammals of Juzan Extra 100SC were not evaluated as part of the EU assessment of mesotrione. The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

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

Species	Substance	Exposure System	Results	Reference
Rat	mesotrione	Acute	LD50>5000 mg a.s./kg bw	EFSA Journal 2016;14(3):4419
Rat	mesotrione	Long term	NOEL=0.3 mg a.s./kg bw/d NOEL=1.2 mg a.s./kg bw/d*	EFSA Journal 2016;14(3):4419

*For details, please see the consideration below.

zRMS comment: zRMS confirms that the reported toxicity data in table 9.3-1 are in accordance with the EU agreed end-points and will be used for risk assessment.

9.3.2 Risk assessment for spray applications

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

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

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

Table 9.3-2: Screening step assessment of the acute and long-term/reproductive risk for mammals due to the use of Juzan Extra 100SC in maize and minor corps: maize

Intended use	Maize (*also minor uses i. e.: sugar maize, popcorn*);				
Active substance/product	mesotrione				
Application rate (g/ha)	150				
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**
maize	Small herbivorous mammals	136.4	1.0	20.46	244.4
Reprod. toxicity (mg/kg bw/d)	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}**
maize	Small herbivorous mammals	72.3	1.0 x 0.53	5.75	**0.05**

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 comment: 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.

Table 9.3-3: Tier-1 step assessment of the long-term risk for mammals due to the use of Juzan Extra 100SC in maize

Intended use	Maize (***also minor uses i e: sugar maize, popcorn***);				
Active substance/product	mesotrione				
Application rate (g/ha)	150				
Reprod. toxicity (mg/kg bw/d)	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}**
Maize BBCH 10-19	Small insectivorous mammal – "shrew"	4.2	1.0 x 0.53	0.33	**0.90**
Maize BBCH 10-29	Small herbivorous mammal – "vole"	72.3	1.0 x 0.53	5.75	**0.05**
Maize BBCH 10-29	Small omnivorous mammal – "mouse"	7.8	1.0 x 0.53	0.62	**0.48**

zRMS comment: For mesotrione the TER_{LT} values from the tier 1 reproductive risk assessment are below the trigger for all scenarios. Refinement risk assessment was not accepted by zRMS. Further refinement for long-term risk assessment is required.

Due to unacceptable reproductive risk to mammals in post-emergence use as the risk refinement new decline residues studies were performed to determine DT50 of mesotrione in maize (Peda T., 2021, SGS study code: 21SGS76).

The objective of the study was the determination of the residues of mesotrione and its degradation time (DT50) in maize (RAW Agricultural Commodity) after one application of M-100SC-OR2-C under field conditions. Application was made at BBBCH 12 of the crop with dose rate of 1.5 L/ha (150g a.s./ha) of formulated product. Field trials were set up in Poland, Germany[1], Hungary and Northern France.

The study consisted of field phase and analytical phase (both are full GLP).

Sampling was performed just after application at 1 h, 2 h, 4 h, 6h, 20 h, 24 h after application (HAA), and at 2 days, 3 days, 4 days and 5 days after application (DAA).
Mesotrione residues were analysed in samples harvested during the field phase.
DT50 was determined using a statistical tool.

SUMMARIZED RESIDUE RESULTS

Residues in control samples were non-detectable or below the limt of quantification. The residues for mesotrione in the treated specimens are summarized below:

	Mesotrione residues (mg/kg)			
Timing	**21SGS76-01 Poland**	**21SGS76-03 Hungary**	**21SGS76-05 Poland**	**21SGS76-06 France**
1 HAA	8.53	14.16	15.06	16.55
2 HAA	7.73	14.08	14.50	15.22
4 HAA	8.34	12.29	14.45	13.34
6 HAA	6.76	12.16	13.95	13.35
20 HAA	6.07	10.06	10.11	11.45
24 HAA	6.24	10.03	10.51	10.13
2 DAA	2.63	3.91	5.27	5.14
3 DAA	2.50	3.38	3.73	2.76
4 DAA	0.64	2.15	2.02	0.41
5 DAA	0.34	0.10	0.79	0.19

	% Mesotrione			
Timing	**21SGS76-01 Poland**	**21SGS76-03 Hungary**	**21SGS76-05 Poland**	**21SGS76-06 France**
1 HAA	100	100	100	100
2 HAA	7.73	14.08	14.50	15.22
4 HAA	8.34	12.29	14.45	13.34
6 HAA	6.76	12.16	13.95	13.35
20 HAA	6.07	10.06	10.11	11.45
24 HAA	6.24	10.03	10.51	10.13
2 DAA	2.63	3.91	5.27	5.14
3 DAA	2.50	3.38	3.73	2.76
4 DAA	0.64	2.15	2.02	0.41
5 DAA	0.34	0.10	0.79	0.19

Based on the new DT50 in crop, new fTWA was calculated using DT_{50} from SFO kinetic evaluation.

[1] Due to the deviation occurred in Germany, it was decided to repeat the trial in Polan 14.45d (trial no.: 21SGS76-05)

Trial	DT_{50} [h]	DT_{50} [days]	Error [%]
21SGS76-01	34.4	1.43	9.34
21SGS76-03	32.6	1.36	7.8
21SGS76-05	33.6	1.40	3.93
21SGS76-06	29.5	1.23	8.09

Calculation of fTWA 21-days, based on the equation:
$fTWA = (1-e^{-kt}) / kt$
where $k = \ln(2) / DT_{50}$
t = averaging time in days
DT50 = 1.355 days (geometric mean)
fTWA = 0.0931

Estimated new fTWA based on residue decline study will be used as a risk refinement for reproductive risk to mammals in post-emergence use in maize.

zRMS comment: A higher tier risk assessment based on the refinement parameters such as foliage residue dissipation (DT_{50}) was not accepted by RMS.

The presented by the Applicant refinement risk assessment for the vertebrates was evaluated by the RMS, but found not acceptable due to the uncertainties related to the kinetic analysis of the data of the residue trials, what in turn put a question mark over the reliability of RUD value. However that analysis may be found acceptable in case the Applicant satisfactorily clarifies all identified problems.

A detailed explanation is provided by zRMS on page 60 of the above report.

Refinement of DT_{50} should be considered at MSs level.

Table 9.3-4: Higher-tier assessment of the long-term risk for mammals due to the use of Juzan Extra 100SC in maize

Intended use	maize				
Active substance/product	mesotrione				
Application rate (g/ha)	1 x 150				
Reprod. toxicity (mg/kg bw/d)	0.3				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**$MAF_m \times TWA$**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize BBCH 10-19	Small insectivorous mammal – "shrew"	4.2	0.0931	0.059	5.08
Maize BBCH 10-29	Small herbivorous mammal – "vole"	72.3	0.0931	1.01	**0.30**
Maize BBCH 10-29	Small omnivorous mammal – "mouse"	7.8	0.0931	0.109	**2.75**

Based on the new fTWA, application for vole and mouse is still unacceptable. However, based on EFSA Conclusion 2016 voles are not representative species in maize. The lowest first tier TER is for small herbivorous mammals "voles". Although voles are listed as relevant focal species, it is widely acknowledged that voles are not relevant for arable crops.

- Gurney, *et al.* (1998)[2] reports the feeding habit of field voles (*Microusagrestis*) to be mainly rough, ungrazed grassland, including thick grass ground cover. In a two year study of small mammals on Scottish arable land and set-aside (Rodgers 19931) 159 field voles were caught, which were reported to have an almost exclusive preference for rough grassland and were completely absent from the wood and also infrequent in set-aside and crops.
- In a three year study of small mammals on an arable farm in Oxfordshire Tew (19941) failed to capture any field voles away from hedgerows around cereal fields. In the Boxworth project, field voles were occasionally caught in the fields but this was restricted to areas with dense ground cover, such as patches infested with blackgrass (Johnson *et al*., 19921).
- No data are available from radio tracking studies for the bank vole or the field vole. Radio tracking has been tried unsuccessfully in both species (Plesner-Jensen 1993). Trapping studies have shown that although both species do not use arable fields as main habitat, they are common in hedgerows and woods adjacent to arable fields (Pollard &Relton 1970; Jefferies et al 1973; Green 1979; Loman 1991; Johnson et al. 1992).The preference of the common vole for non-cropped areas are discussed in Jacob *et al* (2014), in which it states: "The common vole is primarily a grassland species that is well adapted to steppe habitats. Primary habitats are meadows, set-aside land, flower strips, grassy field verges and alfalfa and clover fields. It prefers to inhabit undisturbed short vegetation and can be found in grass leys in forests after clear cuts and other grassy habitats."

Furthermore, information from DEFRA's research project on "Estimating wildlife exposure to pesticides in crops: additional scenarios and data" (2009) supports the non-relevance of the vole. The aim of this work was to provide further information on use of crops by wildlife by extensive surveying and by review of public literature. The following table taken from this report shows the number of captures of small mammals in the various habitat types.

Table 9.3-5: Captures of small mammals during 11,000 trap-events in different agricultural habitats (Table 3 from Report PS2328)

Captures per 100 trap events

[2] Gurney at al., 1998; Update CONTRACT PN0910/PN0919 MILESTONE REPORT Mammals and farming: information for risk assessmentCSL Project No. M37

	Potatoes	Arable hedge	Cereal	Sugar beet	Other non-crop	Orchard hedge	Orchard crop
Field vole	0	0.15	0.08	0	0	1.52	1.31
Pygmy shrew	0.02	0.53	0.23	0	0.34	1.82	0.51
Common shrew	0.38	6.43	1.36	1.00	6.38	3.33	1.85
Bank vole	0.02	6.43	1.44	0	1.55	4.24	0.27
Woodmouse	0.82	8.06	7.04	0.50	2.76	7.88	2.49
Total	1.24	21.6	10.15	1.5	11.03	18.79	6.43

Trap events in this habitat	5020	2630	2570	200	580	330	2970

Although the study did not specifically include maize fields, the results clearly show that wood mice are much more prevalent in arable crops (including potatoes, cereal and sugar beet) than voles. A follow on research project by DEFRA, on "Small mammal activity in soft fruit, cane fruit and top fruit orchards" (2012), states that wood mice are omnivorous and forage above ground, while shews and voles tend to forage beneath thatch and litter layers.

Taking all of the above into consideration and the fact that maize fields do not contain thatch and litter layers, voles are not considered to be a relevant focal species. Instead the wood mouse is considered a relevant focal species. The wood mouse is widespread and common in the agricultural landscape and occurs in a number of farmland habitats . The wood mouse was also considered to be the most relevant small mammal focal species in the RAR (2015), based on observations from field monitoring studies submitted by the Notifier.
However, as voles are not considered to be appropriate focal species, consideration should be given to the relevance of another herbivorous focal species. EFSA's Bird and Mammal Guidance Document (2009) identifies the European rabbit (Oryctolagus cuniculus) as the representative species for large herbivorous mammals. This species is abundant across Europe and may be associated with arable crops. Gurney et al. (1998) reports the feeding habitat of the rabbit to be areas of short grass; naturally occurring, dry heaths or closely grazed agricultural pastures with secure refuge nearby. The brown hare (Lepus europaeus) is also widespread and abundant across Europe. It is found in all sorts of open agricultural landscape such as intensively farmed areas, areas with mixed farming and pastoral landscapes (Northern Zone Guidance). Gurney et al. (1998) reports the preferred feeding habitat of the brown hare to be arable land where cereals predominate with available grass fields for summer feeding. Based on this, the brown hare is considered the most relevant focal species to represent herbivorous mammals in the following risk assessment.
The brown hare was also considered to be the most relevant herbivorous mammal focal species in the RAR (2015), based on observations from field monitoring studies submitted by the Notifier.
Overall the following higher tier risk assessment focuses on the wood mouse (omnivorous) and the brown hare (herbivorous) as relevant focal species for the proposed use of mesotrione on maize. This selection of focal species is in line with the approach taken in the RAR (2015), which considered use of mesotrione on maize at BBCH 12-18.

During renewal of the active substance –mesotrione a detailed refined long-term risk assessment for mammals and a range of studies for identification of focal species and PT values. The results of 3 monitoring studies indicate that the omnivorous wood mouse (Apodemus sylvaticus) and the herbivorous European brown hare (Lepus europaeus) are appropriate focal species for maize at the early stages after germination. From the results of 2 monitoring studies a maximum PT of 0.139 has been taken for the omnivorous wood mouse in early maize for use in risk assessment.

Table 9.3-6: Higher-tier assessment of the long-term risk for mammals due to the use of Juzan Extra 100SC in maize

Intended use	Maize (***also minor uses i e: sugar maize, popcorn***);mesotrione
Active substance/product	

Application rate (g/ha)	mesotrione 1 × 150
Reprod. toxicity (mg/kg bw/d)	0.3
TER criterion	5

Focal species	**Food category, % in diet**	**Sv**	**FIR/bw**	**RUD_m**	**PT**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize, pos-emergence	Brown hare *(Lepus europaeus)*	-	0.334*	54.2	1	0.0931	0.252	**1.2**
	Wood mouse *(Apodemus sylvaticus)*	7.8	-	-	0.139	0.0931	0.015	20

The long-term risk assessment is still not acceptable for brown hare. Therefore, further refinement is required for this species as the TERLT is below the trigger of 5.

Refinement of focal species

zRMS comment: The focal species for maize at early BBCH growth stages such as wood mouse and brown hare were accepted by zRMS.

Refinement of PT value

zRMS comment: The PT value of 0.139 for wood mouse was accepted at the EU level during mesotrione evaluation.

Toxicity endpoint

In terms of the long-term endpoint for use 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 discussed further under section B.6 (Toxicology and metabolism). ***The NOAEL for reproductive performance 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 appropriate 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.*

Therefore the NOAEL of 1.2 mg/kg bw/d has been used in the risk assessment.

~~**Table 9.3-7: Higher-tier assessment of the long-term risk for mammals due to the use of Juzan Extra 100SC in maize**~~

~~**Intended use**~~	~~Maize (***also minor uses i e: sugar maize, popcorn***);~~
~~**Active substance/product**~~	~~mesotrione~~
~~**Application rate (g/ha)**~~	~~1 × 150~~
~~**Reprod. toxicity (mg/kg bw/d)**~~	~~1.2~~

~~**TER criterion**~~	~~**5**~~

~~**Focal species**~~	~~**Food category, % in diet**~~	~~**FIR/bw**~~	~~**RUD_m**~~	~~**SVm**~~	~~**$MAF_m \times TWA$**~~	~~**DDD_m (mg/kg bw/d)**~~	~~**TER_{lt}**~~
~~*Maize, pos-emergence*~~	~~*Brown hare (100 % plant material)*~~	~~0.334~~	~~54.2~~	~~-~~	~~0.0931~~	~~0.25~~	~~**4.8**~~
	~~*Apodemus sylvaticus*~~	~~-~~	~~-~~	~~7.8~~	~~0.0931~~	~~0.109~~	~~11.00~~

Refinement of toxicity endpoint

zRMS comment: The applicant's proposal to change the mammalian endpoint was not accepted. 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. Therefore the meeting agreed that the NOAEL of 0.3 mg/kg bw/day should be used in the risk assessment.

(justification: 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). However, endpoint to be used in the mammalian risk assessment has been discussed during the Pesticides Peer Review experts Meeting 136 in December 2015. The experts decided that NOAEL of 0.3 mg a.s./kg bw/d should be used).

Intended use	Maize
Active substance/product	Mesotrione
Application rate (g a.s./ha)	1 × 150
Reprod. toxicity (mg/kg bw/d)	0.3
TER criterion	5

Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**PT**	**$MAF_m \times TWA$**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize BBCH 10-29	Brown hare *Lepus europaeus* (100% grass)	17.3[1)]	1	1 x 0.53	1.38	**0.22**

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 × 150
Reprod. toxicity (mg/kg bw/d)	0.3
TER criterion	5

Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**PT**	**$MAF_m \times TWA$**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize BBCH 10-29	*Apodemus sylvaticus*	7.8[1)]	0.139	1 x 0.53	0.086	**3.48**

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)

The trigger value for both species are still below the trigger of 5.
Therefore, further refinement is required for this species as the TER_{LT} is below the trigger of 5.
Refinement of long-term risk assessment with refinement toxicity endpoints for mammals should be considered at MSs level.

PT refinement for brown hare

Prosser (2010) reports radio-tracking data for brown hare, although, data specifically on maize are not available. The 90th percentile PT values ranged from 0.88 to 1.0 for "all crops" during spring/summer (data for consumers only). Without specific data on maize crops, as a worst-case approach a PT value of 1.0 has been applied to the risk assessment for hare. However, as brown hare have large home ranges (29 - 138 ha reported in the Northern Guidance Document and 20 – 40 ha reported by Gur-ney *et al.* 1998) this implies that hare would not feed only in the treated field over a long-term period.
It should also be noted that, it would be more appropriate to use mean rather then 90th percentile PT values in a long-term risk assessment, along with the general approach taken in EFSA (2009). However, mean values have not been presented in the report by Prosser (2010).

It seems unrealistic that brown hares obtain 100% of their diet from early maize fields treated with terbuthylazine and a PT of 1 seems not appropriate.
This assumption is supported by a comprehensive study on the ecology of brown hares in arable land in south-western Germany. Späth (1989)[3] investigated the habitat use and home range sizes of brown hares. A total of nine individual brown hares were radio-tracked between March and November. The study area consisted of winter and spring cereals, meadows, set-asides, sugar beet, maize, rape, clover and strawberry field, where on average cereals and maize were grown on 60% of the study area. The time individual brown hares spent in different habitats was recorded and quantified several times for each individual. For the PT estimation, only the period of fresh germinated seedlings of maize have to be considered. The 90th percentile was determined for the mean of the single daily values of the individual. The 90th percentile PT in maize (May) was 26% (Table 9.3-8).

Table 9.3-8: PT values [in %] for radio-tracked brown hares in maize fields during 15 days in May in South-Western Germany (Späth 1989)

Month	Individual hares								
	Annie	Dollie	Egon	Hoho	Iwan	Petra	Reißer	Seppl	Willi
	PT (%) in maize								
May 01				20				0	
May 02	3	21	29	43	9	51	22	0	0
May 03	42	30	39	43	23	57	38	0	32
mean	22.5	25.5	34	35.3	16	54	30	0	16
90%tile	**26**								

In conclusion, considering the large home ranges size in intensive managed agricultural area from the data above, it is unlikely that brown hares feed exclusively on one single field. Therefore, it seems unrealistic that brown hares obtain 100% of their diet from fields treated with terbuthylazine and a PT of 1 seems not appropriate. The PT value of 0.26 as shown above is assumed for maize. Taking the double of this value (PT=0.52) for the long-term risk is considered highly conservative.

PT refinement for brown hare

zRMS comment: PT refinement for brown hare = 0.52 was not accepted by zRMS. There is not enough

[3] Späth, V., 1989. Untersuchungen zur Populationsökologie des Feldhasen (*Lepus europaeus* PALLAS) in der Oberrheinebene. Freiburger Waldschutz-Abhandlungen, 8: 101

information in the summary to reliably assess the PT value taken from this study. In the case of a study for 4 - 10 individuals tracked in the field - it should be used for assessment maximum PT value; not the 90_{th} percentile PT value.

Refinement of DT_{50} should be considered at MSs level.

~~**Table 9.3-9: Higher-tier assessment of the long-term risk for mammals due to the use of Juzan Extra 100SC in maize**~~

~~**Intended use**~~ ~~**Active substance/product**~~ ~~**Application rate (g/ha)**~~	~~Maize (*also minor uses i. e.: sugar maize, popcorn*);~~ ~~mesotrione~~ ~~1 × 150~~							
~~**Reprod. toxicity (mg/kg bw/d)**~~ ~~**TER criterion**~~	~~1.2~~ ~~5~~							
~~**Focal species**~~	~~**Food category, % in diet**~~	~~**FIR/bw**~~	~~**RUD$_m$**~~	~~**SVm**~~	~~**MAF$_m$ × TWA**~~	~~**PT**~~	~~**DDD$_m$** (mg/kg bw/d)~~	~~**TER$_{lt}$**~~
~~*Maize, pos-emergence*~~	~~*Brown hare (100 % plant material)*~~	~~0.334~~	~~54.2~~	~~-~~	~~0.0931~~	~~0.52~~	~~0.13~~	~~9.23~~

New data (new refinement risk assessment for mammals) was provided by Applicant:

Mesotrione selection of endpoint for wild mammals assessment

EFSA long-term mammal endpoint for mesotrione

The long-term endpoint for mammals for mesotrione was discussed at the Pesticides Peer Review Experts Meeting 136 in December 2015 (EFSA, 2016). The experts agreed on a NOAEL of 0.3 mg/kg bw per day based on the effects on litter size of the F2 generation in the 3-generation study (1997) submitted with the original DAR in 1999. The original study is not accessible, data and information reported here were taken from the 2015 Renewal Assessment Report (RAR). The multigeneration study was conducted between 1995-1996 and it is considered to be compliant with OECD 416 (2001) by RMS. Rats (26/sex/dose level) were fed diet containing 0, 2.5, 10, 100 or 2500 ppm mesotrione. Animals were mated after 10 weeks and allowed to rear litters (F1) to weaning. Selected F1 animals were similarly bred to produce F2 litters after a 10-week pre-mating period. F2 animals were fed experimental diets until Week 14, after which approximately half of the animals continued with the same treatment (F2CT) while the remainder were assigned to a recovery sub-group (F2R) and fed control diet. At Week 18, F2 sub-groups were mated to produce F3 litters (F3CT and F3R). Test diets were fed continuously throughout the study with the exception of the recovery sub-groups (RAR, 2015). The mammal reproductive endpoint is based from the litter size dose-response results reported in **Błąd! Nie można odnaleźć źródła odwołania.**. Results indicated that F1 litter sizes were decreased at concentration ≥10 ppm, although values attained statistical significance only at 2500 ppm. F2 litter size was decreased in all treated groups, however no clear dose-response was seen and values attained statistical significance only at 2500 ppm. The magnitude of the reduction in F2 pup number at 2500 ppm was greater than that seen in F1 litters. Reductions in litter size were seen at ≥10 ppm in F3CT and F3R litters. F3R litter size at 2.5 ppm was reduced compared to the concurrent control value but was similar to F3CT controls.

Mesotrione effects on the litter size from the multigeneration study in rats (RAR (2015))

Parameter	**Generation**	**Dose Level (ppm)**				
		0	**2.5**	**10**	**100**	**2500**
Litter size (no. pups)	F1	11.7	12.4	10.9	10.3	9.2**
	F2	11.8	9.8	9.5	10	7.8**

	F3CT	10.6	10.8	8.4	8.5	5.5**
	F3R	11.7	10.5	9.6	9.2*	8.2*

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

The NOAEL for reproductive performance was determined to be 2.5 ppm (equivalent to 0.3 mg/kg bw/day) based on reduced litter sizes in the F2 generation at ≥10 ppm. Values did not attain statistical significance; however, effects were consistent in all generations and are therefore considered to be of significance. The concentrations in diet (ppm) were converted into mg/kg bw/d dose levels using the standard factor of 0.12 for two-generation study in rats from the birds and mammals guidance document (EFSA 2009).

Selection of Endpoint for Wild Mammals Assessment

The long-term endpoint for mammals for mesotrione is based on the effects on litter size of the F2 generation from the multigeneration rat study (EFSA, 2016). Here is presented a discussion to propose the use of a NOAEL specific for the F1 generation, as more relevant the ecotoxicology risk assessment in contrast with the mammalian toxicology assessment.

In mammalian toxicology, the aim of protection is the individual, whereas in ecotoxicology the goal is to protect animal populations. This means that, although the same tests are considered in both mammalian toxicology and ecotoxicology, each effect and endpoint must be considered from a different perspective.

Developmental data for the F1 generation from the RAR for mesotrione (2015) are presented in the **Błąd! Nie można odnaleźć źródła odwołania.** below. 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.

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)

As it can be observed in **Błąd! Nie można odnaleźć źródła odwołania.**, the reduction in pup survival observed at 10ppm can not be incidental because the trend in pup survival reduction is carried on in the higher doses. Pup survival in tested animals shows a 2.7, 7.8, 2.9 and 16% of reduction from untested animals at 2.5, 10, 100 and 2500 ppm, respectively. A similar trend is observed in all the other tested endpoints:

- Gestation length: increase of gestation duration of 0.4, 2.2 and 2.7 % compared to control for 10, 100 and 2500 ppm respectively.
- Litter size: reduction in litter size of 6.8, 12.0 and 21.4 % compared to the control for 10, 100 and 2500 ppm respectively.
- Litter weight: reduction in litter weight of 6.4, 9.9 and 18.9 % compared to the control for 10, 100 and 2500 ppm respectively.

This demonstrates that the effects observed at the 10 ppm treatment are clearly not incidental as all tested

endpoints at 100ppm show a damaging effect compared to control (although not significant).

The use of the NOAEL of 10 ppm (1.2 mg/kg bw/d) was proposed by the applicant in the mesotrione RAR (2015), and the RMS commented that "the ecotoxicity assessment will need to consider whether exposure of to F2 generation needs to be considered and if not whether the reduction in litter size of 6.8% which is seen in the F1 generation is acceptable for wild populations."

The effects in the second generation of multigeneration reproductive tests could potentially be the result of exposure during a critical developmental phase and, this being the case, it should be considered relevant in deriving a risk assessment endpoint. The NOAEL value currently considered in the risk assessment (2.5 ppm) results in a reduction in litter size of the F2 generation of 16.9%; however, at the same concentration pup survival is not affected and actually shows a survival increase of 5.7%. Also, the gestation length is similar across generations (

An additional reason for why data from F2 generation are not relevant in the risk assessment concerns the use pattern of mesotrione. Mesotrione is a selective herbicide applied to maize BBCH 12-18 once per season, which means the F2 generation are unlikely to be exposed in the wild situation and therefore effects seen in this generation are not applicable to the ecotoxicology risk assessment.

The litter size effects on which the EFSA NOAEL is based on are from a repeated exposure for several weeks to rats from F0 through mature F1 animals, corresponding to more than 20 weeks of exposure to the test substance. According to OECD 416 test protocol, the test substance should be administered via diet or drinking water preferably on a 7-days-a-week basis, dosing shall be continued for at least 10 weeks before the mating period and also during the 2-week mating period. In the laboratory experiment, F2 litter size is thus an effect of 12 weeks of exposure of the parent animals and 12 weeks of exposure of F1 adult animals. The total exposure in the multigenerational laboratory experiment is thus much higher than the possible real exposure in a treated field, as mesotrione should be applied just once per season. Consequently, developmental data from the F1 generation are considered to be more appropriate to calculate the long-term mammals endpoint for environmental risk assessment and a NOAEL of 10 ppm (1.2 mg/kg bw/d) is proposed.

), and adults from the F2 generation that continued with the same dosing treatment (F2CT) did not show any effect at the two higher doses, similarly to adults from F2 generation that were assigned to a recovery sub-group (F2R) and fed the control diet. These results indicate that mesotrione effects were not the result of exposure during a critical developmental phase and data from F2 generation might not be relevant in the risk assessment.

An additional reason for why data from F2 generation are not relevant in the risk assessment concerns the use pattern of mesotrione. Mesotrione is a selective herbicide applied to maize BBCH 12-18 once per season, which means the F2 generation are unlikely to be exposed in the wild situation and therefore effects seen in this generation are not applicable to the ecotoxicology risk assessment.
The litter size effects on which the EFSA NOAEL is based on are from a repeated exposure for several weeks to rats from F0 through mature F1 animals, corresponding to more than 20 weeks of exposure to the test substance. According to OECD 416 test protocol, the test substance should be administered via diet or drinking water preferably on a 7-days-a-week basis, dosing shall be continued for at least 10 weeks before the mating period and also during the 2-week mating period. In the laboratory experiment, F2 litter size is thus an effect of 12 weeks of exposure of the parent animals and 12 weeks of exposure of F1 adult animals. The total exposure in the multigenerational laboratory experiment is thus much higher than the possible real exposure in a treated field, as mesotrione should be applied just once per season. Consequently, developmental data from the F1 generation are considered to be more appropriate to calculate the long-term mammals endpoint for environmental risk assessment and a NOAEL of 10 ppm (1.2 mg/kg bw/d) is proposed.

Mesotrione effects on gestation length from the multigeneration study in rats in the RAR (2015)

Parameter	Generation	Dose Level (ppm)

		0	2.5	10	100	2500
Gestation length (d)	F0	22.7	22.4*	22.6	22.7	22.9
	F1	22.3	22.3	22.4	22.8**	22.9**
	F2CT	23	22.9	22.5*	22.9	23.1
	F2R	22.4	22.4	22.7	22.8	22.7

Conclusions of the toxicity endpoint

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.

Higher-Tier risk assessment for mammals exposed to mesotrione following application on maize

According to EFSA (2009), the omnivorous wood mouse (*Apodemus sylvaticus*) and the herbivorous European brown hare (*Lepus europaeus*) are considered as appropriate focal species for maize at the early stages after germination (BBCH 10-16).

Refinement of mammals reproductive risk assessment was performed using the relevant focal species in early maize (BBCH 12-18), with consideration of the refined PT (proportion of time spent on foraging in the crop) and PD (the assumed proportion of food items in the diet) values, Food Intake Rate and residue decline data. The NOAEL for reproductive performance of 1.2 mg/kg bw/d is also used as additional refinement.

Refined risk assessment for the wood mouse (*Apodemus sylvaticus*)

Determination of PT for focal species
The agreed PT value of 0.139 from mesotrione EFSA conclusion (2016) is used for the wood mouse.

PD values
For purposes of the risk refinement assumption, the standard diet of the wood mouse consisting of 25% weeds, 50% weed seeds and 25% of ground arthropods as indicated in EFSA (2009) is used in the risk assessment.

Food Intake Rate
FIR/bw of 0.27 is used for the wood mouse based on calculation performed in line with indications of Appendix G of EFSA (2009) with consideration of the bodyweight of 21.7 g and the mixed diet indicated in EFSA guidance (Table 3-1).

Food intake rate calculations for the wood mouse

Maize	April-May	Plant material[f]	Ground arthropods	Weed seeds
Fraction of food item in mixed diet [a]	PD*i*,fresh (%)	25%	25%	50%
Food energy of food item [*i*] in mixed diet [b]	FE (kJ/dry g)	17.6	22.7	21.7
Moisture content of food item [*i*] in mixed diet [b]	MC (%)	76.4	68.8	9.9
Assimilation efficiency of food item [*i*] in mixed diet [%] [c]	AE (%)	47	87	84

Food energy of food item in diet [d]	FE*item,fresh* (kJ/g fresh weight)	0.488	1.54	8.21
Food energy of total mixed diet [d]	FE*total,fresh* (kJ/g fresh weight)	10.2		
Daily energy expenditure [d]	DEE (kJ/day)	59		
Food intake rate of total mixed diet [d]	FIR*total,fresh* (kJ/g fresh weight)	5.76		
bw [e]	(g)	21.7		
FIR/bw	(g fresh weight/bw/day)	0.27		

[a] PD for wood mouse Tier I EFSA mixed diet
[b] from table 3 of Appendix G in EFSA (2009)
[c] from table 4 of Appendix G in EFSA (2009)
[d] calculated according to EFSA (2009) Appendix G
[e] Body weight of wood mouse from EFSA (2009)
[f] Plant material is assumed to be equal in maize shoot (using the default value for grasses and cereal shoots)

Refined RUD values

Default RUD values for maize indicated in the EFSA birds and mammals' guidance document (2009) originate from the residue trials performed on grass + cereals with no maize trials included in derivation of the RUD. The lack of residue decline data for maize was addressed also by Lahr et al. (2018) in the EFSA external scientific report "Data collection for the estimation of ecological data (specific focal species, time spent in treated areas collecting food, composition of diet), residue level and residue decline on food items to be used in the risk assessment for birds and mammals", which reported a large number of data available for maize and calculated a RUD value for maize of 29.7 mg/kg. This refined value has been also included in the new Birds and Mammals guidance document (2023) as the default RUD value for maize. Therefore, for the refinements presented here, the RUD value of **29.7** mg/kg from Lahr et al. (2018) was used.

Residue decline and f_{TWA}

In order to determine the DT_{50} value in maize, a residue decline study by Peda (2021, Final report for study 21SGS76) was submitted. The residue decline field trails on maize were conducted in Poland (at two sites), Hungary and France. Mesotrione was applied at a nominal rate of 150 g a.i./ha to maize BBCH 12. These data were fitted using the single first order model to derive the DT_{50} values of 1.44 and 1.41 for Poland, 1.37 for Hungary and 1.23 for France. Here the geomean DT_{50} value of **1.36** for all four locations was used for fTWA refinement.

Higher-tier assessment including the refinements presented above demonstrates an unacceptable risk to the wood mouse following an application of mesotrione at 150 g a.i./ha (Table 3-2) when using the EFSA agreed long-term endpoint for mesotrione of 0.3 mg/kg bw/d.

However, when these calculations were performed with the more relevant long-term mammal endpoint of 1.2 mg/kg bw/d, an acceptable risk is concluded (Table 3-3).

Higher-tier assessment using the long-term/reproductive risk of 0.3 mg/kg/bw/d

Intended use	Maize
Active substance/product	Mesotrione
Application rate (g/ha)	1 x 150
Reprod. toxicity (mg/kg bw/d)	0.3
TER criterion	5

Focal species	**Food category, % in diet**	**FIR/bw**	**RUD_{90} × DF (mg/kg food)**	**MAF_m x TWA**	**PT**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Wood mouse	Maize, 0.25	0.27	29.7	0.093	0.139	0.0039	
	Seeds, 0.5	0.27	40.2	0.53	0.139	0.0600	

	Arthropods, 0.25	0.27	3.5	0.53	0.139	0.0026	
	Whole diet					0.0665	**4.51**

FIR/bw: Food intake rate per body weight; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Higher-tier assessment using the refined long-term/reproductive endpoint of 1.2 mg/kg/bw/d

Intended use	Maize
Active substance/product	Mesotrione
Application rate (g/ha)	1 x 150
Reprod. toxicity (mg/kg bw/d)	1.2
TER criterion	5

Focal species	**Food category, % in diet**	**FIR/bw**	**RUD_{90} × DF (mg/kg food)**	**MAF_m x TWA**	**PT**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Wood mouse	Maize, 0.25	0.27	29.7	0.093	0.139	0.0039	
	Seeds, 0.5	0.27	40.2	0.53	0.139	0.0600	
	Arthropods, 0.25	0.27	3.5	0.53	0.139	0.0026	
	Whole diet					0.0665	18.05

FIR/bw: Food intake rate per body weight; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Refined risk assessment for the European brown hare (*Lepus europaeus*)

Determination of PT for focal species

As the only agreed PT value from the mesotrione EFSA conclusion (2016) is for brown hare, the PT value used was **1** as a worst-case scenario.

PD values

Brown hare diet relevant for maize in spring indicated in the Northern Zone Guidance Document is used here (i.e., 84% maize and 16% dicotyledons weeds). The derived PD values were based on published data from studies carried out in Sweden (Frylestam 1980), England (Tapper and Barnes 1986), France (Chapuis 1990) and Denmark (Olesen & Asferg 2006; Hansen 1990).

Food Intake Rate

FIR/bw of **0.328** is used for the brown hare based on calculation performed in line with indications of Appendix G of EFSA (2009) with consideration of the bodyweight of 3800 g and the mixed diet indicated in EFSA guidance (Table 3-4).

Food intake rate calculations for the brown hare

Maize	**April-May**	**Maize shoots** [f]	**Non-grass herbs**
Fraction of food item in mixed diet [a]	PD*i*,fresh (%)	84%	16%
Food energy of food item [*i*] in mixed diet [b]	FE (kJ/dry g)	17.6	17.8
Moisture content of food item [*i*] in mixed diet [b]	MC (%)	76.4	88.1
Assimilation efficiency of food item [*i*] in mixed diet [%] [c]	AE (%)	47	76
Food energy of food item in diet [d]	FE*item,fresh* (kJ/g fresh weight)	1.640	0.258
Food energy of total mixed diet [d]	FE*total,fresh* (kJ/g fresh weight)	1.897	

Daily energy expenditure [d]	DEE (kJ/day)	2363.40
Food intake rate of total mixed diet [d]	FIR*total,fresh* (kJ/g fresh weight)	1245.61
bw [e]	(g)	3800
FIR/bw	(g fresh weight/bw/day)	0.328

[a] PD for hare according to the Northern Zone Guidance Document
[b] from table 3 of Appendix G in EFSA (2009)
[c] from table 4 of Appendix G in EFSA (2009)
[d] calculated according to EFSA (2009) Appendix G
[e] Body weight of brown hare from EFSA (2009)
[f] Plant material is assumed to be equal in maize shoot (using the default value for grasses and cereal shoots)

Refined RUD values
Same RUD refinements presented above for the wood mouse.

Residue decline and fTWA
The same residue decline study and calculated DT_{50} and fTWA values presented above for the wood mouse are used for the brown hare.

The risk assessment based on refined parameters, including the refined toxicity endpoint, demonstrated acceptable risk to the brown hare from exposure to mesotrione applied at 150 g a.i./ha.

Higher-tier assessment using the long-term/reproductive endpoint of 0.3 mg/kg/bw/d

Intended use	Maize						
Active substance/product	Mesotrione						
Application rate (g/ha)	1 x 150						
Reprod. toxicity (mg/kg bw/d)	0.3						
TER criterion	5						
Focal species	**Food category, % in diet**	**FIR/bw**	**RUD_{90} × DF (mg/kg food)**	**MAF_m x TWA**	**PT**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Brown hare	Maize, 0.84	0.328	29.7	0.093	1	0.114	
	Dicots weeds, 0.16	0.328	28.7	0.53	1	0.120	
	Whole diet					0.234	**1.28**

FIR/bw: Food intake rate per body weight; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Higher-tier assessment using the long-term/reproductive endpoint of 1.2 mg/kg/bw/d

Intended use	Maize						
Active substance/product	Mesotrione						
Application rate (g/ha)	1 x 150						
Reprod. toxicity (mg/kg bw/d)	1.2						
TER criterion	5						
Focal species	**Food category, % in diet**	**FIR/bw**	**RUD_{90} × DF (mg/kg food)**	**MAF_m x TWA**	**PT**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Brown hare	Maize, 0.84	0.328	29.7	0.093	1	0.114	
	Dicots weeds, 0.16	0.328	28.7	0.53	1	0.120	5.13

	Whole diet	0.234	

FIR/bw: Food intake rate per body weight; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

RMS comment: The new refinement risk assessment for mammals based on new refined RUD values and refinement f_{TWA} has not been evaluated by RMS in Core Dossier. The evaluation of new data should be considered at Ms level.

9.3.2.2 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 156, mesotrione belongs to the group of less sorptive substances.

Effective application rate (g/ha)*	=	150			
Acute toxicity (mg/kg bw)	=	5000	quotient	=	0.03
Reprod. toxicity (mg/kg bw/d)	=	0.3	quotient	=	**500**

With a K(f)oc of 14 (as a worst case), mesotrione belongs to the group of less sorptive substances. Since the ratio of effective application rate (150 g/ha) to relevant endpoint (0.3 mg/kg bw/d) exceeds the critical value of 50 for at least one use scenario, a quantitative risk assessment (calculation of TER values) is necessary and presented in Table 9.3-9

The predicted environmental concentration in puddles is calculated as follows in accordance with the EFSA Guidance Document:

$$PEC_{puddle} = \frac{AR/10}{1000\ (w + Koc \times s)}$$

where:
AR = application rate (g/ha); divisor of 10 to achieve rate in mg/m²
w = 0.02 (pore water term; volume)
s = 0.0015 (soil term: volume, density, organic carbon content)

Table 9.3-9: Assessment of the risk for mammals due to exposure to mesotrione via contaminated drinking water in puddles

Intended use	miaze
Active substance	mesotrione
Application rate (g/ha)	1 x 150
Reprod. toxicity (mg/kg bw/d)	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_a / TER_{lt}
150	50 (geomean)	0.158	0.24	0.0379	7.92
150	14 (worst-cse in Efsa conslusion)	0.366	0.24	0.0878	**3.42**

For the proposed use the resulting TER values are below the trigger of 5 (for KOC wrost case value of 14) indicating unacceptable chronic risk to mammals from drinking water from puddles.
Taking into account the refined NOAEL value 1.2 mg a.s./kg bw the TER reaches the acceptable value as presented in the following table:

Table 9.3-10: Assessment of the risk for mammals due to exposure to mesotrione via contaminated drinking water in puddles

Intended use	miaze				
Active substance	mesotrione				
Application rate (g/ha)	1 x 150				
Reprod. toxicity (mg/kg bw/d)	1.2				
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_a / TER_{lt}**
150	14 (worst-cse in Efsa conslusion)	0.366	0.24	0.0878	13.66

zRMS comment: The refinement assessment of the risk for mammals due to exposure to mesotrione via contaminated drinking water in puddles provided by the Applicant has not been accepted by zRMS. The applicant's proposal to change the mammalian endpoint was not accepted. 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. Therefore the meeting agreed that the NOAEL of 0.3 mg/kg bw/day should be used in the risk assessment.

However, NOEAL of 1.2 mg a.s./kg bw should be considered at MSs level.

9.3.2.3 Effects of secondary poisoning

The log P_{ow} of mesotrione and its main metabolities MNBA, AMBA and SYN546974 amount to 0.11, -1.3, 0.32 and 1.62 respectively and thus do not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

Risk assessment for earthworm-eating mammals via secondary poisoning

Not required.

zRMS comment: The evaluation of the risk of secondary poisoning for earthworm-eating mammals for mesotrione is not triggered due to log Pow being <3.

Risk assessment for fish-eating mammals via secondary poisoning

Not required.

zRMS comment: The evaluation of the risk of secondary poisoning for fish-eating-mammals for mesotrione is not triggered due to log Pow being <3.

9.3.2.4 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.3.4 Overall conclusions

The acute and long-term risks of Juzan Extra 100 SC to mammals were assessed from toxicity exposure ratios between toxicity endpoints, estimated from studies with mesotrione and maximum residues occurring on food items following applications according to the proposed use pattern. Risk of secondary poisoning has not been assessed, as mesotrione has log P_{OW} <3.0. The TER values, calculated for recommended scenarios, exceed the trigger value of 10 for acute risk. However, first-tier TER value calculated for a few scenarios did not exceed the relevant trigger values of 5 for reproductive risk and acceptable risk to mammals was confirmed based on higher tier assessment. The risk to mammals is acceptable following use of Juzan Extra 100 SC according to the proposed use pattern.

zRMS comment: In the screening step the TER_A values for mesotrione exceeds the trigger value (10), indicating that Juzan Extra 100 SC presents an acceptable acute risk to mammals.

The TER_{LT} values from the tier 1 reproductive risk assessment are below the trigger of 5 for the use on maize, indicating that Juzan Extra 100 EC 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 was not accepted by zRMS.

No safe use was concluded following application of Juzan Extra 100 EC at 1.5 L/ha (corresponding to 150 g a.s./ha) and further refinement is required.

The high long-term drinking water risk was identified for dose rate 150 g a.s./ha using the Koc value for pH 7.8 (14 L/kg). Applicant should submit additional refinement option which should be considered at MS level.

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

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

No relevant data available.

9.5 Effects on aquatic organisms (KCP 10.2)

9.5.1 Toxicity data

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

Effects on aquatic organisms of Juzan Extra 100 SC were not evaluated as part of the EU assessment of mesotrione. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
Rainbow trout (Oncorhynchus mykiss)	Mesotrione	96 h, s	LC_{50} >120 mg a.s./L ~~nm~~ (nom)	EFSA Journal 2016;14(3):4419
Bluegill sunfish (Lepomis macrochirus)	Mesotrione	96 h, s	LC_{50} >120 mg a.s./L ~~nm~~ (nom)	EFSA Journal 2016;14(3):4419
Fathead minnow (Pimephales promelas)	Mesotrione	36-d (flow-through)	NOEC=12.5 mg a.s./L ~~nm~~ (nom)	EFSA Journal 2016;14(3):4419
Rainbow trout (Oncorhynchus mykiss)	MNBA	96 h, s	LC_{50} >120 mg a.s./L ~~nm~~ (nom)	EFSA Journal 2016;14(3):4419
Rainbow trout (Oncorhynchus mykiss)	AMBA	96 h, s	LC_{50} =150 mg a.s./L ~~nm~~ (nom)	EFSA Journal 2016;14(3):4419
Daphnia magna	Mesotrione	48 h, s	EC_{50}> 622 mg a.s./L mm	EFSA Journal 2016;14(3):4419
Daphnia magna	Mesotrione	21 d, ss	NOEC (reproduction & length)=180 mg a.s./L ~~nm~~ (nom)	EFSA Journal 2016;14(3):4419
Daphnia magna	MNBA	48 h, s	EC_{50}=130 mg a.s./L ~~nm~~ (nom)	EFSA Journal 2016;14(3):4419
Daphnia magna	AMBA	48 h, s	EC_{50} = 160 mg a.s./L ~~nm~~	EFSA Journal 2016;14(3):4419

Species	Substance	Exposure System	Results	Reference
			(nom)	
Pseudokirchneriella subcapitata	Mesotrione	120 h, s	E_bC_{50}= 3.5 mg a.s./L ~~nm~~ (nom) E_rC_{50}= 13 mg a.s./L mm (nom)	EFSA Journal 2016;14(3):4419
Pseudokirchneriella subcapitata	MNBA	72-h, s	E_bC_{50}= 38 mg a.s./L ~~nm~~ (nom) E_rC_{50}= 42 mg a.s./L (nom)	EFSA Journal 2016;14(3):4419
Pseudokirchneriella subcapitata	AMBA	72-h, s	E_bC_{50}= 9.4 mg a.s./L ~~nm~~ (nom) E_rC_{50}= 14 mg a.s./L ~~nm~~ (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
Lemna gibba	MNBA	7 d, ss	E_rC_{50}/ E_yC_{50} (for both)>97 mg a.s./L mm	EFSA Journal 2016;14(3):4419
Lemna gibba	AMBA	7 d, ss	E_rC_{50}/ E_yC_{50} (for both)>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.)~~>35 mg a.s./L~~ =93 mg a.s./L mm	EFSA Journal 2016;14(3):4419
Higher-tier studies (micro- or mesocosm studies)				
No further tests submitted				

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

Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – Juzan Extra 100SC

Species	Substance	Exposure System	Results	Reference
Daphnia magna	M-100 SC-OR2-C	48 h,	LC_{50} = 1872.63 mg/L $_{nom}$ LC_{50} = 186.90 mg as/L $_{nom}$	Szlauer S., 2022, EMI/4/5/2021
Navicula pelliculosa	M-100 SC-OR2-C	72 h, s	E_rC_{50} = 179.448 mg formulation/L	Szlauer S., 2022,

Species	Substance	Exposure System	Results	Reference
			E_rC_{50} = 17.012 mg as/L E_yC_{50} = 11.563 mg formulation/L E_yC_{50} = 1.154 mg as/L	EMI/4/2/2021
Pseudokirchneriella subcapitata	M-100 SC-OR2-C	72 h, s	E_rC_{50} = 74.71 mg formulation/L E_rC_{50} = 7.46 mg as/L E_yC_{50} = 10.65 mg formulation/L E_yC_{50} = 1.06 mg as/L	Szlauer S., 2022, EMI/4/3/2021
Lemna sp.	M-100 SC-OR2-C	7d, ss	*Results based on geometric mean concentratins:* *Frond number* E_rC_{50} = 2.224 mg formulation/L E_rC_{50} = 0.222 mg as/L E_yC_{50} = 0.225 mg ormulation/L E_yC_{50} = 0.022 mg as/L *Dry weight* E_rC_{50} = 1.44 mg formulation/L E_rC_{50} = 0.144 mg as/L E_bC_{50} = 0.239 mg formulation/L E_bC_{50} = 0.024 mg as/L	Szlauer S., 2022, EMI/4/6/2021
Higher-tier studies (micro- or mesocosm studies)				
n.r.				

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

9.5.1.1 Justification for new endpoints

No new data for active substances is presented with this application.

9.5.2 Risk assessment

The evaluation of the risk for aquatic and sediment-dwelling organisms was performed in accordance with the recommendations of the "Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters in the context of Regulation (EC) No 1107/2009", as provided by the Commission Services (SANTE-2015-00080, 15 January 2015).
The relevant global maximum FOCUS Step 1, 2 and 3 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the table below.

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

Table 9.5-3: 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 Juzan Extra 100 SC in maize

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic plamts**
Test spe-cies		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna Gib-ba***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_bC_{50}	E_bC_{50}
(µg/L)		120000	12500	622000	180000	3500	7.7
AF		100	10	100	10	10	10
RAC (µg/L)		1200	1250	6220	18000	350	0.77
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
pH 5.1	42.738	0.04	0.03	0.01	0.00	0.122	**55.504**
pH 6.5	48.1259	0.04	0.039	0.01	0.00	0.138	**62.501**
pH 7.9	50.246	0.04	0.04	0.01	0.00	0.144	**62.255**
Step 2							
pH 5.1	6.349	-	-	-	-	-	**8.245**
pH 6.5	6.565	-	-	-	-	-	**8.526**
pH 7.9	1.380	-	-	-	-	-	**1.792**
Step 3 pH 5.1							

Group		Fish acute	Fish pro-longed	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic plamts
D3/ditch	**0.787**	-	-	-	-	-	**1.02**
D4/pond	0.085	-	-	-	-	-	0.11
D4/stream	0.678	-	-	-	-	-	0.88
R1/pond	0.116	-	-	-	-	-	0.15
R1/stream	**2.428**	-	-	-	-	-	**3.15**
Step 3 pH 6.5							
D3/ditch	**0.788**	-	-	-	-	-	**1.02**
D4/pond	0.033	-	-	-	-	-	0.04
D4/stream	0.677	-	-	-	-	-	0.88
R1/pond	0.073	-	-	-	-	-	0.09
R1/stream	**1.673**	-	-	-	-	-	**2.17**
Step 3 pH 7.9							
D3/ditch	**0.787**	-	-	-	-	-	**1.02**
D4/pond	0.032	-	-	-	-	-	0.04
D4/stream	0.674	-	-	-	-	-	0.88
R1/pond	0.032	-	-	-	-	-	0.04
R1/stream	**0.739**	-	-	-	-	-	0.96

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 uses calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms for mesotrioe (risk for *Lemna Gibba* as characterised by an EC_{50} for species of 7.7 µg/L in connection with an assessment factor of 10) FOCUS Steps 1-3 scenarios.. Therefore, further PEC/RAC ratios were calculated based on risk mitigation in FOCUS Step 4 PECsw considering reduced exposure of surface water bodies.

Table 9.5-4: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for mesotrione based on FOCUS Step 4 calculations and toxicity data for most sensitive species *Lemna Gibba* with mitigation of spray drift and run-off for the use of Juzan Extra 100 SC in crop (maize).

Intended use **Active substance** **Application rate (g/ha)**		maize mesotrione 1 × 150 **pH 5.1**		maize mesotrione 1 × 150 **pH 6.5**		maize mesotrione 1 × 150 **pH 7.9**	
Nozzle reduction	**No-spray buffer (m)**	10	20	10	20	10	20
	Vegetated filter strip (m)	10	20	10	20	10	20
None	D3/ditch	0.1368	0.0711	0.1372	0.0714	0.1368	0.07108
None	D4/pond	0.08542	0.08542	0.0214	0.0153	0.02041	0.01363
None	D4/stream	0.1545	0.1383	0.1533	0.0810	0.1505	0.07819
None	R1/pond	0.05021	0.0269	0.0337	0.0186	0.02040	0.01362
None	R1/stream	1.099	0.5746	0.6864	0.3462	0.3032	0.1529
RAC (µg/L) 0.77		**PEC/RAC ratio**		**PEC/RAC ratio**		**PEC/RAC ratio**	
None	D3/ditch	0.18	-	0.18	-	0.18	-
None	D4/pond	0.11	-	0.03	-	0.03	-
None	D4/stream	0.20	-	0.20	-	0.20	-
None	R1/pond	0.07	-	0.04	-	0.03	-
None	R1/stream	**1.43**	0.75	0.89	-	0.39	-

Table 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite (MNBA) of mesotrione for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of Juzan Extra 100 SC in maize.

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Aquatic plamts**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna Gibba***
Endpoint		LC_{50}	EC_{50}	EbC50	EbC50
(µg/L)		120000	130000	38000	97000
AF		100	100	10	10
RAC (µg/L)		1200	1300	3800	9700
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**				
Step 1					
PEC/RAC	23.4824	0.02	0.02	0.01	0.00

Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite (AMBA) of mesotrione for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of Juzan Extra 100 SC in maize.

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Aquatic plamts**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna Gibba***
Endpoint		LC_{50}	EC_{50}	EbC50	EbC50
(µg/L)		150000	160000	9400	90000
AF		100	100	10	10
RAC (µg/L)		1500	1600	940	9000

Group		Fish acute	Inverteb. acute	Algae	Aquatic plamts
FOCUS Scenario	PEC gl-max (µg/L)				
Step 1					
PEC/RAC	10.8276	0.01	0.01	0.02	0.00

Table 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite (SYN 546974) of mesotrione for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of Juzan Extra 100 SC in maize.

Group		Aquatic plamts
Test species		*Lemna Gibba*
Endpoint (µg/L)		EbC50 93000
AF		10
RAC (µg/L)		9300
FOCUS Scenario	PEC gl-max (µg/L)	
Step 1		
PEC/RAC	0.7725	0.00

Risk assessment for formulation to aquatic organisms

Table 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for formulation based on Drift Calculator in SWASH MODEL v. 5.3 calculations for the use of Juzan Extra 100 SC in maize.

Intended use	maize

Formulation **Application rate (g/ha)**	Juzan Extra 100 SC 150
Buffer zone [m]	**PECsw forumaltion [µg/L]**
1	10.1252
5	2.7445
10	1.4556
RAC (µg/L) Lemna Gibba =ErC_{50} **1444 µg/L** RAC=~~28.5~~ 144.4 (AF=10)	**PEC/RAC**
1	~~0.01~~ 0.07
5	0.019
10	0.01

9.5.3 Overall conclusions

Based on PEC/RAC calculations, no unacceptable risk is indicated for aquatic organisms considering all envisaged GAP uses for Juzan Extra 100 SC, assuming that following risk mitigation measures are taken into account:

- a vegetative buffer strip of 20m to surface water bodies is required when conventional spraying techniques are applied.

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).

The PEC_{sw} calculations for mesotrione have been approved for applications proposed in GAP. PEC_{sw} and PEC_{sed} calculations were carried out according to the FOCUS recommendations. The Applicant has been used FOCUS models: STEPS 1-2 and Step 3. $PEC_{sw/sed}$ were also carried out at Step 4 according to FOCUS L&M Guidance for 10m and 20m buffer zone.

The relevant predicted environmental concentrations in water (PEC_{sw}) for risk assessments covering the proposed use pattern are taken from Part B Section 8 (Environmental Fate). Details on PEC_{sw} calculations for mesotrione and formulation Juzan Extra 100 SC are included in Section B8.

In the risk assessment for mesotrione the dependence on pH was taken to consideration (pH 5.1, pH 6.5 and pH 7.9). In step 3 and 4, only the following scenarios were considered: D3/ditch; D4/pond; D4/stream; R1/pond; R1/stream. For all soils the buffer zones are required. Moreover, based on standard assessment an unacceptable risk was identified for following scenarios:

- R1 at pH 5.1 – 20m vegetative buffer zone
- R1 and D3 at pH 6.5 – 10m vegetative buffer zone
- D3 at pH 7.9 – 10m vegetative buffer zone

The acceptability of proposed risk mitigation measures should be taken at MSs level.

Conclusion from the risk assessment based on the active substance mesotrione:
The ratios between predicted environmental concentrations in surface water bodies (PEC_{SW}, PEC_{SED}) and regulatory acceptable concentrations (RAC) for a.s.- mesotrione based on the worst case for aquatic organisms were <1 indicating acceptable risk to aquatic organism with applying 20 m vegetative buffer zone (the worst case scenario – R1 stream – pH 5.1).

Conclusion from the risk assessment based on the formulated product:
The risk assessment for the formulated product is resolved without requiring mitigation options.

Final risk mitigation measures should be considered at MSs level.

9.6 Effects on bees (KCP 10.3.1)

9.6.1 Toxicity data

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

Effects on bees of Juzan Extra 100 SC were not evaluated as part of the EU assessment of active substance mesotrione. New data submitted with this application are summarised in Appendix 2.

Table 9.6-1: 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 a.s./bee	EFSA Journal 2016;14(3):4419
Apis mellifera	mesotrione	Contact	LD_{50} = 100 µg a.s./bee	EFSA Journal 2016;14(3):4419
Apis mellifera	Callisto 100 SC (A12739A)	Oral	LD_{50} = 79.7 µg a.s./bee	EFSA Journal 2016;14(3):4419
Apis mellifera	Callisto 100 SC (A12739A)	Contact	LD_{50} = 52.5 µg a.s./bee	EFSA Journal 2016;14(3):4419
Apis mellifera	Callisto 100 SC (A12739A)	Chronic 10 day-LD_{50}	LD_{50} = 19.2 µg a.s./bee/day	EFSA Journal 2016;14(3):4419
Apis mellifera	Callisto 100 SC (A12739A)	Bee brood development (7day exposure study) NOEDlarvae	$NOED_{larvae}$ = 57.8 µg a.s./larva	EFSA Journal 2016;14(3):4419
Apis mellifera	Formulation M-100SC-OR2-C (Juzan Extra 100 SC)	Oral	LD50 ≥ 200 µg/bee (>19.35 µg a.s./bee)	Knapik M., 2020, B-86-20
Apis mellifera	Formulation M-100SC-OR2-C (Juzan Extra 100 SC)	Contact	LD_{50} ≥ 200 µg/bee (>19.35 µg a.s./bee)	Knapik M., 2020, B-87-20
Apis mellifera	Formulation M-100SC-OR2-C (Juzan Extra 100 SC)	Chronic 10days	LDD_{50} > 58.248 µg of the test item/bee/day NOEDD > 58.248 µg of the test item/bee/day	Woźniak A., 2020, 0016/0093/E
Apis mellifera	Formulation M-100SC-OR2-C (Juzan Extra 100 SC)	Larval toxicity test, repeated exposure, 22 days	22-day LC_{50} > 650 mg product/kg of food 22-day NOEC ≥ 650 mg product/kg of food 22-day NOEDD ≥ 100 µg product/larvae	Woźniak A., 2020, 0016/0091/E
Higher-tier studies (tunnel test, field studies)				
Not relevant				

9.6.1.1 Justification for new endpoints

In addition to EU agreed endpoints for the active substances, new formulation studies have been submitted for acute oral and contact exposure, chronic exposure to adult bees, and larval development.

9.6.2 Risk assessment

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

9.6.2.1 Hazard quotients for bees

Table 9.6-2: First-tier assessment of the risk for bees due to the use of Juzan Extra 100 SC in maize and also in minor uses mentioned in the GAP table

Intended use **Active substance** **Application rate (g a.s./ha)**	maize mesotrione 1 × 150		
Test design	**LD_{50} (lab.) (µg a.s./bee)**	**Single application rate (g a.s./ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	11	150	13.63
Contact toxicity	100		1.5
Product **Application rate (g/ha)**	Juzan Extra 100 SC 1 × 1567*		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	≥200	1567	< 7.835
Contact toxicity	≥200		< 7.835

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.
* Application rate of = 1.5 L product ha-1 x (relative density 1.045) = 1567.5 g product/ha

The risk assessment for bees presented in this draft registration report includes also exposure resulting from the use of Juzan Extra 100 SC in protection of the requested minor crops (the maximum single application rate for all of requested crops is the same as in group of maize presented in the GAP).

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

Not relevant.

9.6.3 Effects on bumble bees

No data/information available.

9.6.4 Effects on solitary bees

No data/information available.

9.6.5 Overall conclusions

The acute risk assessments for the active substance as well as for the formulated product Juzan Extra 100 SC with Hazard Quotients well below the trigger for acceptability of effects indicate an acceptable risk

for bees exposed in accordance with the intended uses in maize and in proposed minor crops. Therefore, a low risk to bees is expected from the application of Juzan Extra 100 SC 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 **Juzan Extra 100 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.

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

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

Effects on non-target arthropods of Juzan Extra 100 SC were not evaluated as part of the EU assessment of mesotrione. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

Species	Substance	Exposure System	Results	Reference
Mesotrione and related formulated products from EU review				
Typhlodromus pyri (protonymphs)	Formulation A12739A	Laboratory test glass plates (2D)	LR_{50} = 93.11 g a.s./ha ER_{50} >81 g a.s./ha	EFSA Conclusion 2016
Aphidius rhopalosiphi (adults)	Formulation A12739A	Laboratory test glass plates (2D)	LR_{50} = 43.56 g a.s./ha ER_{50} >25.6 g a.s./ha	EFSA Conclusion 2016
Juzan Extra 100 SC				
Typhlodromus pyri	Formulation M-100SC-OR2-C (Juzan Extra 100 SC)	Extended labolatory study, corn leaves (2-D)	LR_{50} > 150 g a.i/ha ER_{50}> 150 g a.i/ha	Šklíba J, 2020, 20/201
Aphidius rhopalosiphi	Formulation M-100SC-OR2-C (Juzan Extra 100 SC)	Extended labolatory study, barley plants (3-D)	LR_{50} > 3.0 L/ha (>303.6 g a.i./ha) ER_{50} > 3.0 L/ha (>303.6 g a.i./ha)	Kulec-Płoszczyca, 2021, B-52-21
Chrysoperla carnea	Formulation M-100SC-OR2-C (Juzan Extra 100 SC)	Extended labolatory study, corn leaves disc (2-D)	NOER ≥ 1500 g a.i/ha ER50 = 559.4 g a.i/ha	Šklíba J., 2020, 20/200
Coccinella septempunctata	Formulation M-100SC-OR2-C (Juzan Extra 100 SC)	Extended study, bean leaves (2-D)	LR_{50} = 1107 g a.i./ha ER_{50} = 600 g a.i.ha NOER = 96 g a.i/ha	Nácarová J, 2020, 20/199

Species	Substance	Exposure System	Results	Reference
Field or semi-field tests				
Not relevant				

zRMS comment:

zRMS agrees with the toxicity endpoints proposal by the Applicant.

9.7.1.1 Justification for new endpoints

No deviation from EU agreed endpoints.

9.7.2 Risk assessment

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

9.7.2.1 Risk assessment for in-field exposure

The risk envelope approach is not applied here, since application rate is the same for all intended uses in Maize and on minor uses – 1.5 L product/ ha (i.e. 150 g a.s/ha), covering the main application with also proposed minor uses.

Table 9.7-2: Higher-tier assessment of the in-field risk for non-target arthropods due to the use of Juzan Extra 100 SC in maize and in minor crops acc.to GAP table

Intended use	Maize (field crop scenario)
Active substance/product	Mesotrione/ Juzan Extra 100 SC
Application rate (g a.s/ha)	1 x 150
MAF	1

Test species Higher-tier	**L/ER$_{50}$ [g a.s/ha]**	**PER$_{in\text{-}field}$ (g/ha)**	**PER$_{in\text{-}field}$ below rate with ≤ 50 % effect?**
Typhlodromus pyri	> 150	150	Yes; 1; ≤ 1
Aphidius rhopalosiphi	> 303.6	150	Yes; 0.49 ; ≤ 1
Chrysoperla carnea	559.4	150	Yes; 0.27 ; ≤ 1
Coccinella septempunctata	~~1107~~ 600#	150	Yes; ~~0.13~~ 0.25; ≤ 1

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.

#RMS pointed that in the study report the application of Juzan Extra 100 EC did not affect female fecundity nor egg fertility in range of the test item (38.4 – 600 g a.i/ha). Therefore, ER$_{50}$ > 600 g a.i./ha should be used for risk assessment.

zRMS comment:

zRMS pointed that in the study report for *Coccinella septempunctata* the application of Juzan Extra 100 EC did not affect female fecundity nor egg fertility in range of the test item (38.4 – 600 g a.i/ha). Therefore, ER_{50} > 600 g a.i./ha should be used for risk assessment. The in-field risk assessment was corrected by zRMS. A low risk is demonstrated for non-target arthropods - such as - *Typhlodromus pyri, Aphidius rhopalosiphi, Chrysoperla carnea* and *Coccinella septempunctata* (extended laboratory studies).

9.7.2.2 Risk assessment for off-field exposure

The risk envelope approach is not applied here, since application rate and the highest drift rate is the same for all of the intended uses in major and on minor uses (respectively 1.5L product/ha and 2.77%).
The worst PERoff field value is equal 2.77 g formulation/ha for all of the intended uses.

Table 9.7-3: Higher-tier assessment of the off-field risk for non-target arthropods due to the use of maize and in minor crops acc.to GAP table

Intended use	Maize (field crop scenario)
Active substance/product	Mesotrione/ Juzan Extra 100 SC
Application rate (g a.s/ha)	1 × 150
MAF	1
vdf	10

Test species Higher-tier	**L/ER_{50} [g a.s/ha]**	**Drift rate**	**$PER_{off-field}$ (g/ha)**	**CF**	**corr. $PER_{off-field}$ below rate with ≤ 50 % effect? HQ ≤ 1**
Typhlodromus pyri	> 150	0.0277	0.4155	5	Yes; 0.013; ≤ 1
Aphidius rhopalosiphi	> 303.6				Yes; 0.006 0.068; ≤ 1
Chrysoperla carnea	559.4				Yes; 0.004 ; ≤ 1
Coccinella septempunctata	~~1107~~ 600#				Yes; 0.0035 ; ≤ 1

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.

#RMS pointed that in the study report the application of **Juzan Extra 100 EC** did not affect female fecundity nor egg fertility in range of the test item (38.4 – 600 g a.i/ha). Therefore, ER_{50} > 600 g a.i./ha should be used for risk assessment.

The risk assessment for minor crops presented in the GAP is covered by a risk assessment performed for the product Juzan Extra 100 SC. The growth stage of presented minor uses, number of applications and doses are the same as the main intended uses in maize (filed crop scenario).

zRMS comment:

zRMS agrees with the Applicant's assessment with the of-field risk to non-target arthropods from the proposed use of **Juzan Extra 100 SC** above, however the VDF is set to 5 in the Central zone instead of 10 for only 2-D studies (In this case in 3-D studies VDF correction is 1). The calculations are given in the table below. A low risk is demonstrated to the 2 standard first tier and additional test species.

zRMS pointed that in the study report for *Coccinella septempunctata* the application of **Juzan Extra 100**

EC did not affect female fecundity nor egg fertility in range of the test item (38.4 – 600 g a.i/ha). Therefore, ER_{50} > 600 g a.i./ha should be used for risk assessment. The off-field risk assessment was corrected by zRMS. A low risk is demonstrated for non-target arthropods - such as - *Typhlodromus pyri, Aphidius rhopalosiphi, Chrysoperla carnea* and *Coccinella septempunctata* (extended laboratory studies).

Intended use	**Maize (field crop scenario)**
Active substance/product	**Mesotrione/Juzan Extra 100 SC**
Application rate (g/ha)	**1 x 150**
MAF	**1**
VDF	**5 for 2-D study and 1 for 3-D**

Test species Tier II	**LR_{50} (lab.) (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 1**
Typhlodromus pyri (2-D)	> 150	2.77%	0.831	5	0.0277
Aphidius rhopalosiphi (3-D)	> 303.6		4.155		0.068
Coccinella septempunctata (2-D)	559.4		0.831		0.007
Chrysoperla carnea (2-D)	~~1107~~ 600		0.831		0.0069

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.

The calculations are given in the table below. A low risk is demonstrated to the 2 standard first tier and additional test species.

A low risk is demonstrated to the 2 standard first tier and additional test species.
All hazard quotients (HQ) are less than 1, indicating that **Juzan Extra 100 SC** applied at the maximum use rate in maize poses no risk to non-target arthropods. No risk mitigation needed.

9.7.2.3 Additional higher-tier risk assessment

Not relevant.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

The in-field and off-field risk from exposure to mesotrione applied as Juzan Extra 100 SC for the intended uses in major and minor crops is indicated to be acceptable for non-target arthropods other than bees based on Tier 2 data without the need for risk mitigation measures.

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

9.8.1 Toxicity data

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

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of Juzan Extra 100 SC were not evaluated as part of the EU assessment of mesotrione. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

Species	Substance	Exposure System	Results	Reference
Earthworms				
Juzan Extra 100 SC				
Eisenia andrei	Juzan Extra 100 SC (M-100SC-OR2-C)	Mixed into substrate 56 d, chronic 10 % peat content	$NOEC_{reproduction}$= 41.00 mg product/kg dry soil (4.09 mg a.s./kg dry soil) **EC_{10} = 38.25 mg product/kg dry soil (3.82 mg a.s./kg dry soil)** EC_{20} = 127.42 mg product/kg dry soil (12.72 mg a.s./kg dry soil)	Swoboda T., 2021, Study Code: EMI/4/7/2021
Mesotrione, relevant degradation products and related formulated products from EU review				
Eisenia fetida	mesotrione	Mixed into substrate 28 d, acute 10 % peat content	LC_{50} > 2000 mg a.s./kg d.w. soil	EFSA Journal 2016;14(3):4419
Eisenia fetida	MNBA	-	LC_{50} > 1000 mg a.s./kg d.w. soil	EFSA Journal 2016;14(3):4419
Eisenia fetida	Callisto 100 SC (A12739A)	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 125 mg A12739A /kg d.w. soil (equivalent to 10.85 mg a.s./kg d.w. soil) EC_{10}, number of juveniles = 68.1 mg A12739A /kg d.w. soil (equivalent to 5.91 mg a.s./kg d.w. soil) EC_{20}, number of	EFSA Journal 2016;14(3):4419

Species	Substance	Exposure System	Results	Reference
			juveniles = 174.9 mg A12739A /kg d.w. soil (equivalent to 15.18 mg a.s./kg d.w. soil)	
Eisenia fetida	AMBA	Mixed into substrate 56 d, chronic 5 % peat content	NOEC = 1050 mg /kg d.w. soil EC_{10} = 1050 mg /kg d.w. soil EC_{20} = 1050 mg /kg d.w. soil	EFSA Journal 2016;14(3):4419
Eisenia fetida	MNBA	Mixed into substrate 56 d, chronic 5 % peat content	NOEC = 1050 mg /kg d.w. soil EC_{10} > 1050 mg /kg d.w. soil EC_{20} > 1050 mg /kg d.w. soil	EFSA Journal 2016;14(3):4419
Soil mesofauna				
Juzan Extra 100 SC				
Folsomia candida	Juzan Extra 100 SC (M-100SC-OR2-C)	Mixed into substrate 21 d, chronic 5 % peat content	NOEC $_{reproduction}$ = 510 mg product/kg dry soil (50.90 mg a.s./kg dry soil) **EC_{10} = 507.94 mg product/kg dry soil (50.70 mg a.s./kg dry soil)** EC_{20} = 651.30 mg product/kg dry soil (65.00 mg a.s./kg dry soil)	Swoboda T., 2021 Study Code: EMI/4/1/2021
Hypoaspis aculeifer	Juzan Extra 100 SC (M-100SC-OR2-C)	Mixed into substrate 14 d, chronic 5 % peat content	**$NOEC_{reproduction}$ > 714.29 mg product/kg dry soil (71.29 mg a.s./kg dry soil)** EC_{10} = 907.22 mg product/kg dry soil (90.55 mg a.s./kg dry soil) EC_{20} = 970.66 mg product/kg dry soil (96.88 mg a.s./kg dry soil)	Dec W., 2021 Study Code: EMI/4/8/2021
Mesotrione, relevant degradation products and related formulated products from EU review				

Species	Substance	Exposure System	Results	Reference
Folsomia candida	Callisto 100 SC (A12739A)	Mixed into substrate 21 d, chronic 5 % peat content	NOEC = 556 mg A12739A /kg d.w. soil (equivalent to 50.54 mg a.s. /kg d.w. soil) EC_{10} = 413 mg A12739A /kg d.w. soil (equivalent to 37.54 mg a.s /kg d.w. soil) EC_{20}= 620 mg A12739A /kg d.w. soil	EFSA Journal 2016;14(3):4419
Hypoaspis aculeifer	Callisto 100 SC (A12739A)	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 90.9 mg a.s. /kg d.w. soil (equivalent to 1000 mg A12739A /kg d.w. soil) EC_{10} > 1000 mg A12739A /kg d.w. soil EC_{20} > 1000 mg A12739A /kg d.w. soil	EFSA Journal 2016;14(3):4419
Field studies				
-				
Litter bag test				
-				

Please notice that as the log Pow values for mesotrione and its metabolites are below 2 no correction of the study endpoints is required to account for differences in organic content of the test soil compared to agricultural soils.

9.8.1.1 Justification for new endpoints

No deviation from EU agreed endpoints.

9.8.2 Risk assessment

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

9.8.2.1 First-tier risk assessment

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3. According to the assessment of environmental-fate data, multi-annual accumulation in soil does not need to be considered for mesotrione.
To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use maize also covers the risk for earthworms and other non-target soil organisms (meso- and macrofauna) from all other intended uses. (see 9.1.2).

Table 9.8-2: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of Juzan Extra 100 SC

Intended use	Maize, sugar maize, popcorn		
Acute effects on earthworms			
Product/active substance	**LC_{50} (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_a (criterion TER ≥ 10)**
~~mesotrione~~	~~>2000~~	~~0.15~~	~~>13333~~
~~MNBA~~	~~>1000~~	~~0.0620~~	~~>16129~~
Chronic effects on earthworms			
Product/active substance	**NOEC/EC_{10} (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Mesotrione (from Callisto 100 SC)	5.91	0.15	39.40
AMBA	1050	0.0092	114130
MNBA	1050	0.0620	16935
Mesotrione (from Juzan Extra 100 SC)	3.82	0.15	25.50
Chronic effects on other soil macro- and mesofauna			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Folsomia candida			
Mesotrione (from Callisto 100 SC)	37.54	0.15	250
Mesotrione (from Juzan Extra 100 SC)	50.70	0.15	338
Hypoaspis aculeifer			
Mesotrione (from Callisto 100 SC)	90.90	0.15	606
Mesotrione (from Juzan Extra 100 SC)	71.29	0.15	475

TER values shown in bold fall below the relevant trigger.

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

The risk from exposure to mesotrione and relevant soil degradation products applied as Juzan Extra 100 SC for all intended uses is indicated to be acceptable for the soil meso- and macrofauna.

zRMS comment: The long-term risks of **Juzan Extra 100 SC** to soil meso- and macro-organisms were assessed from toxicity exposure ratios between toxicity endpoints and maximum PEC_{soil}. The relevant predicted environmental concentrations in soil (PEC_{soil}) for risk assessments covering the proposed use pattern are taken from Part B Section 8 (Environmental Fate).
Safe use of **Juzan Extra 100 SC** in maize was confirmed based on TERLT calculations for metabolites and for formulation.

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

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

Studies on effects soil microorganisms have been carried out with mesotrione and its relevant metabolites. Full details of these studies are provided in the respective EU RAR and related documents.

Effects on soil microorganisms of Juzan Extra 100 SC were not evaluated as part of the EU assessment of mesotrione. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

Endpoint	Substance	Exposure System	Results	Reference
Juzan Extra 100 SC				
N-mineralisation	Juzan Extra 100 SC (M-100SC-OR2-C)	70 d, aerobic	**No negative effect > 25% at 70 d at 10.40 mg product/kg dry weight of soil (1.04 mg a.s./ dry weight of soil**	Swoboda T. 2021 Study Code: EMI/4/11/2021
Mesotrione, relevant degradation products and related formulated products from EU review				
N-mineralisation	Callisto 100 SC (A12739A)	28 d, aerobic	7.8% effect at day 28 at 0.53 mg a.s./kg d.w. soil (equivalent to 5.84 mg A12739A/kg d.w. soil)	EFSA Journal 2016;14(3):4419
N-mineralisation	AMBA	28 d, aerobic	-7.6%effect at day 28 at 1.13 mg /kg d.w. soil	EFSA Journal 2016;14(3):4419
N-mineralisation	MNBA	28 d, aerobic	-4.8%effect at day 28	EFSA Journal

Endpoint	Substance	Exposure System	Results	Reference
			at 1.13 mg /kg d.w. soil	2016;14(3):4419

9.9.1.1 Justification for new endpoints

No deviation from EU agreed endpoints.

9.9.2 Risk assessment

The evaluation of the risk for soil microorganisms was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).
The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.8).
To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use maize also covers the risk for the soil microorganisms from all other intended uses in groups.

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of Juzan Extra 100 SC

Intended use	Maize/sugar maize/popcorn		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
Mesotrione (from Callisto 100 SC)	0.53	0.15	yes
Mesotrione (from Juzan Extra 100 SC)	1.04	0.15	yes
AMBA	1.13	0.0092	yes
MNBA	1.13	0.0620	yes

9.9.3 Overall conclusions

The risk to soil microorganisms is acceptable since negligible effects on the nitrogen transformations are foreseen at higher levels than the calculated PEC soil values for the active when the intended use of pattern for the Juzan Extra 100 SC is considered.

zRMS comments:

The risk assessment for soil micro-organism after exposure of **Juzan Extra 100 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 **Juzan Extra 100 SC**. The results indicate no adverse effect on nitrogen transformation even at soil concentrations well higher than the ones expected following application of **Juzan Extra 100 SC.**

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

9.10.1 Toxicity data

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

Effects on non-target terrestrial plants of Juzan Extra 100 SC were not evaluated as part of the EU assessment of mesotrione. New data submitted with this application are listed in Appendix 1 summarised in Appendix 2.

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

Species	Substance	Exposure System	Results	Reference
Juzan Extra 100 SC				
Brassica oleracea var. capitata$_d$ *Solanum lycopersicon*$_d$ *Glycine max*$_d$ *Lactuca sativa*$_d$ *Allium cepa*$_m$ *Avena sativa*$_m$	Juzan Extra 100 SC (M-100SC-OR2-C)	14 d Seedling emergence	ER_{50} emergence = 745.30 ml product/ha (*Allium cepa*) ER_{50} plant number = 1384.17 ml product/ha (*Allium cepa*) **ER_{50} plant dry weight = 215.57 ml product/ha (*Solanum lycopersicon*)** ER_{50} plant height = 241.32 ml product/ha (*Solanum lycopersicon*) ER_{50} phytotoxicity = 446.53 ml product/ha (*Solanum lycopersicon*)	Dec W, 2021 Study Code: EMI/4/10/2021
Brassica oleracea var. capitata$_d$ *Solanum lycopersicon*$_d$ *Glycine max*$_d$ *Lactuca sativa*$_d$ *Allium cepa*$_m$ *Avena sativa*$_m$	Juzan Extra 100 SC (M-100SC-OR2-C)	21 d Vegetative vigour	LR_{50} plant number = 204.78 ml product/ha (*Solanum lycopersicon*) **ER_{50} plant dry weight = 38.44 ml product/ha (*Lactuca sativa*)** ER_{50} plant height = 63.37 ml product/ha (*Lactuca sativa*)	Dec W, 2021 Study Code: EMI/4/10/2021

Species	Substance	Exposure System	Results	Reference
			ER_{50} phytotoxicity = 103.07 ml product/ha (*Solanum lycopersicon*)	
Mesotrione, relevant degradation products and related formulated products from EU review				
Lactuca sativa *Allium cepa* *Avena sativa* *Lolium perenne* *Brassica oleracea* *Brassica rapa* *Cucmis sativa* *Glycine max* *Linum usitatissimum* *Lycopersicon esculentum*	Callisto 100 SC (A12739A)	21 d Seedling emergence	Lowest ER_{50} = 13.8 g a.s./ha *(Lactuca sativa)*	EFSA Journal 2016;14(3):4419
Lactuca sativa *Allium cepa* *Avena sativa* *Lolium perenne* *Brassica oleracea* *Brassica rapa* *Cucmis sativa* *Glycine max* *Linum usitatissimum* *Lycopersicon esculentum*	Callisto 100 SC (A12739A)	21 d Vegetative vigour	Lowest ER_{50} = 0.883 g a.s./ha *(Lactuca sativa)*	EFSA Journal 2016;14(3):4419

m: monocotyledonous; d: dicotyledonous

9.10.1.1 Justification for new endpoints

No deviation from EU agreed endpoints.

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Not relevant.

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

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002). It is restricted to off-field situations, as non-target plants are non-crop plants located outside the treated area.
Since maximum recommended application rate of Juzan Extra 100 SC is the same for all of the intended uses (1.5 L of product/ha), risk envelope approach was not applied here. The worst drift rate=2.77% was used.

Table 9.10-2: Assessment of the risk for non-target plants due to the use of Juzan Extra 100 SC

Intended use	Maize, sugar mazie, popcorn			
Active substance/product	Mesotrione/ Juzan Extra 100 SC			
Application rate (g/ha)	1 × 150 g/ha / 1 × 1.5 L/ha			
MAF	1			
Test species	**ER_{50} (L/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (L/ha)**	**TER criterion: TER ≥ 5**
Lactuca sativa	0.03844	2.77%	0.04155	0.93

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

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

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

Table 9.10-3: Risk assessment for non-target terrestrial plants due to the use of Juzan Extra 100 SC considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Maize, sugar maize, popcorn			
Active substance/product		Mesotrione/ Juzan Extra 100 SC			
Application rate (g/ha)		1 × 150 g/ha / 1 × 1.5 L/ha			
MAF		1			
Buffer strip (m)	**Drift rate (%)**	**$PER_{off\text{-}field}$ (L/ha)**	**$PER_{off\text{-}field}$ 50 % drift red. (L/ha)**	**$PER_{off\text{-}field}$ 75 % drift red. (L/ha)**	**$PER_{off\text{-}field}$ 90 % drift red. (L/ha)**
1	2.77	0.04155	0.02078	0.01039	0.00416
5	0.57	0.00855	0.00428	-	-
10	0.29	0.00435	-	-	-
Toxicity value ER_{50} = 0.03844 L/ha		**TER criterion: TER ≥ 5**			
1		**0.9251**	**1.8499**	**3.6997**	9.2404
5		**4.4959**	8.9813	-	-
10		8.837	-	-	-

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

9.10.3 Overall conclusions

For the proposed use of Juzan Extra 100 SC, based on the highest application rate the risk for non-target plants in the off-crop area is indicated to be acceptable when either 1 m buffer strip with 90% drift reduction or a 5 m buffer strip with 50% drift reduction, or 10 m buffer strip with no drift reduction is applied as risk mitigation measure.

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} = 38.44 mL product/ha value *(Lactuca sativa)* from vegetative vigour test and $PER_{off\text{-}field}$, indicated needs for further refinement.
The risk following mitigation measures are proposed: **Juzan Extra 100 SC** achieve the acceptability criteria TER ≥ 5 with applying:

- 10 m buffer zone without drift-reducing nozzles
- 5 m and use of 50% drift reducing nozzles
- 1 m and use of 90% drift reducing nozzles

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

Additional tests on other non-target species are not required.

9.12 Monitoring data (KCP 10.8)

Not required.

9.13 Classification and Labelling

Formulation Juzan Extra 100 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.

CLASSIFICATION	
Hazard class(es), categories:	Aquatic Acute 1 Aquatic chronic 1
LABELLING	
Hazard pictograms:	GHS09 
Signal word:	Warning
Hazard statement(s):	H410 - Very toxic to aquatic life with long lasting effects H 400 - Very toxic to aquatic life

Precautionary statement(s):	P391 - Collect spillage. P501 - Dispose of contents/container in accordance to national regulations.

zRMS	**comment**:	Agreed.

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

MS to blacken authors of vertebrate studies in the version made available to third parties/public.

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
KCP 10.1.2/01 (KCP 5.1.2/01)	Peda T.	2021	Magnitude of residues of Mesotrione in maize (Raw Agricultureal Commodity) after one appcliation of M-100SC-OR2-C – four decline curve studies in Poland, Germany, Hungary and Northern France - 2021 No. 21SGS76 SGS Poland, Warszwa GLP, Unpublished	N	CIECH Sarzyna S.A.
KCP 10.2.1/01 (KCP 5.1.2/08)	Szlauer S.	2022	*Daphnia sp*., Acute Immobilisation Test according to OECD Guidline No. 202 (2004) Study code: EMI/4/5/2021 Ecomelius Institute Sp. z o. o. GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.2.1/02 (KCP 5.1.2/09)	Szlauer S.	2022	Fresh *Alga and Cyanobacetria*, Growth Inhibition Test according to OECD Guidline No. 201 (2011) Study code: EMI/4/2/2021 Ecomelius Institute Sp. z o. o. GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.2.1/03 (KCP 5.1.2/06)	Szlauer S.	2022	Fresh *Alga and Cyanobacetria*, Growth Inhibition Test according to OECD Guidline No. 201 (2011) Study code: EMI/4/3/2021	N	CIECH Sarzyna S.A.

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**
			Ecomelius Institute Sp. z o. o. GLP Unpublished		
KCP 10.2.1/04 (KCP 5.1.2/07)	Szlauer S.	2022	*Lemna sp*., Growth Inhibition Test according to OECD Guidline No. 221 (2006) Study code: EMI/4/6/2021 Ecomelius Institute Sp. z o. o. GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.3.1.1/01	Knapik M.	2020	M-100SC-OR2-C Honeybees (*Apis mellifera* L.), Acute Oral Toxicity Test STUDY CODE: B-86-20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.3.1.1/02	Knapik M.	2020	M-100SC-OR2-C Honeybees (*Apis mellifera* L.), Acute Contact Toxicity Test STUDY CODE: B-87-20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.3.1.2/01	Woźniak A.	2020	Honey bee chronic oral toxicity test according to OECD 245 guideline Study code: 0016/0093/E Test item: M-100SC-OR2-C SORBOLAB Research Laboratory LLC GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.3.1.2/02	Woźniak A.	2020	Chronic toxicity test for honey bee larvae according to OECD GD 239 Study code: 0016/0091/E Test item: M-100SC-OR2-C SORBOLAB Research Laboratory LLC GLP	N	CIECH Sarzyna S.A.

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**
			Unpublished		
KCP 10.3.2.2/01	Kulec-Płoszczyca E.	2021	An extended laboratory test for evaluating the effects of M-100SC-OR2-C on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez) STUDY CODE: B-52-21 Łukasiewicz Research Network –Institute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.3.2.2/02	Šklíba J.	2020	Extended GLP laboratory test for evaluating the effects of a test item on the predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae) Study code: 20/201 i2L Research Europe s.r.o. GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.3.2.2/03	Nácarová J.	2020	Extended GLP laboratory test for evaluating the effects of a test item on the plant dwelling insect *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) Study code: 20/199 i2L Research Europe s.r.o. GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.3.2.2/04	Šklíba J.	2020	Extended GLP laboratory test for evaluating the effects of a test item on *Chrysoperla carnea* (Neuroptera: Chrysopidae) Study code: 20/200 i2L Research Europe s.r.o. GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.4.1/01	Swoboda T.	2021	Earthworm Reproduction Test (*Eisenia andrei*) according to the OECD Guideline for the Testing of Chemicals No. 222 (July 29, 2016) Study code: EMI/4/7/2021 Ecomelius Institute Sp. z o. o. GLP	N	CIECH Sarzyna S.A.

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**
			Unpublished		
KCP 10.4.2./01	Swoboda T.	2021	Collembolan (*Folsomia candida*) Reproduction Test according to OECD Guideline No. 232 (2016) Study code: EMI/4/1/2021 Ecomelius Institute Sp. z o. o. GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.4.2/02	Dec W.	2021	Predatory mite (*Hypoaspis* (*Geolaelaps*) *aculeifer*) Reproduction Test according to the OECD Guideline No. 226 (2016) Study code: EMI/4/8/2021 Ecomelius Institute Sp. z o. o. GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.5	Swoboda T.	2021	Soil Microorganisms: Nitrogen Transformation Test according to the OECD Guideline No. 216 (2000) Study code: EMI/4/11/2021 Ecomelius Institute Sp. z o. o. GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.6.2/01 (KCP 5.1.2/04)	Dec W	2021	Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test according to OECD Guideline No. 208 (2006) Study code: EMI/4/10/2021 Ecomelius Institute Sp. z o. o. GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.6.2/02 (KCP 5.1.2/05)	Dec W	2021	Terrestrial Plant Test: Vegetative Vigour Test according to OECD Guideline No. 227 (2006) Study code: EMI/4/9/2021 Ecomelius Institute Sp. z o. o. GLP Unpublished	N	CIECH Sarzyna S.A.

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 XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

The following tables are to be completed by MS

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**
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	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**
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	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

No additional studies were performed.

A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

No additional studies were performed

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

No additional studies were performed.

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

No additional studies were performed.

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

No additional studies were performed.

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

<table>
<tr><td>Comments of zRMS:</td><td>The method is acceptable in section B5 in dRR of Juzan Extra 100 SC.
The analytical method therefore meets the requirements of guideline SANTE/2020/12830, Rev.1.

Characteristics for the analytical method used for validation of mesotrione residues in water medium:
<table>
<tr><th></th><th>Mesotrione</th></tr>
<tr><td>Specificity</td><td>LC-MS/MS method was used during the study.Two mass transitions were evaluated and used for quantification. The specificity of the method was evaluated on the basis of the analysis of chromatograms recordered for matrix blank samples. No interferences at above 30% of the LOQ were detected at the retention time of the active substance in matrix blank sample.</td></tr>
<tr><td>Calibration</td><td>n= 6
The linearity of the detector resonse was demonstrated by single determination of matrix-matched calibration standards at six concentration levels ranging from 1 ppb to 500 ppb of mesotrione.
m/z 338.00 > 290.85 R^2= 0.9999491; R= 0.9999746; y=0.616994x-0.000567045
m/z 338.00>211.95 R^2=0.99995683; R= 0.9997841; y=0.221031x-0.000113843</td></tr>
<tr><td>Calibration range</td><td>1 ppb to 500 ppb of mesotrione</td></tr>
</table></td></tr>
</table>

Assessment of matrix effects is presented	In order to compensate for matrix effects, there were used matrix-matched standards (matrix-matched calibration) and IL-IS for quantification.
Stability	As required in SANTE/2020/12830, Rev.1., if the extracts contain an IL-IS for quantification, testing on final extract stability is not required since the IL-IS will compensate for loses during extract storage. However was extract stability was proven by the corresponding procedural recovery samples, which were stored under the same conditions together with the extracts of the specimens for residue analysis.
Limit of determination/quantification	LOQ: 0.010 mg/kg LOD: 0.002 mg/kg

For mesotrione the TER_{LT} values from the tier 1 reproductive risk assessment are below the trigger for all scenarios. A higher tier risk assessment based on the foliage residue dissipation (DT_{50}) among others not accepted by RMS. No safe use was concluded following application of Juzan Extra 100 SC at 1.5 L/ha (corresponding to 150 g a.s./ha) and further refinement is required.

Justification:

1. a full kinetic report on the determination of DT_{50} for mesotrione in accordance with current guidelines has not been submitted (including kinetic parameters, e.g. the Chi-2 error ($\chi 2$ error) and related 95% uncertainty limits.

2. the Applicant declared to perform the analysis using only one kinetic model – SFO, Applicant should perform the analysis using three kinetic models – SFO, FOMC and DFOP and will obtain the best-fit results based on available visual fit and the numerical results of each fit, such as statistical parameters of the kinetic parameters.

3. goodness of fit should be assessed using four indicators, all of which should be clearly reported. It should be noted that these indicators should be evaluated together and not in a hierarchical manner:

- ✓ Visual fit→ plot of time vs concentration should be provided. Ideally, the fitted line should pass through (or in the vicinity of) the measurement points.
- ✓ Residual plot →Plot of time vs residuals against the y=0 line should be provided. Points should ideally be scattered around the zero line. Regular patterns are generally indicative that the kinetic model used is not appropriate. Underestimation of the last time points is indicative of an under-conservative kinetic.
- ✓ Chi-square ($\chi 2$) % should be reported and should ideally be <15%. Note that Chi-square should be calculated using the mean of true replicates.
- ✓ t-test and/or confidence intervals of individual model parameters should be reported. t-test for rate constant resulting in p-values >0.05 (or confidence intervals including zero) indicate large uncertainty in the estimation of the model parameters and such results should not be accepted.

4. There is a lack of information whether the designated DT_{50} values are based on maximum values or geometric average.
5. Due to the deviation occurred in Germany, it was decided to repeat the trial in Poland 14.45 d (trial no.: 21SGS76-05). RMS pointed out that, the information about deviation is very fragmentary ("Trial number 21SG76-02 and 21SGS76-04 were cancelled. Two new trials were set up: 21SGS76-05 in Poland and 21SGS76-06 in Northern France." The Applicant should explain the detailed reason for rejecting the rehearsals made in Germany. According to the guidelines, it is recommended to use a minimum of 4 locations. After rejecting the location of Germany and repetition of samples in Poland, we only have 3 locations available at the time: Poland, Hungry and France.

The presented by the Applicant refinement risk assessment for the vertebrates was

	evaluated by the RMS, but found not acceptable due to the uncertainties related to the kinetic analysis of the data of the residue trials, what in turn put a question mark over the reliability of RUD value. However that analysis may be found acceptable in case the Applicant satisfactorily clarifies all identified problems. **Refinement of DT_{50} should be considered at MSs level.**

Reference: KCP 10.1.2 /01

Report: Magnitude of the residues of Meostrione in maize (Raw Agricultural Commodity) after application of M-100SC-OR2-C – four decline curve studies in Poland, Germany, Hungary and Northern France - 2021

Guideline(s):
- Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009 of the European Parliament and of the market and repealing Counci Direcyives 79/117/EEC and 91/414/EEC
- Commission Working Document 7029/VI/95 Rev. 5, General Recommendatios for the Design, Preparation and Realization of Residue Trials, July 22, 1997
- OECD Guidline for the testing of chemicals on Crop Field Trial (TG 509 published in September 2009)
- SANTE/2020/12830 rev. 1, 24/02/2021

Deviations: Tral number 21SGS56-02 and s1SGS76-04 were cancelled. Two new trias were set up: 21SGS56-05 in Poland and 21SGS56-06 in Northern France.

GLP: Yes

Acceptability: Yes

Summary:

The objective of the study was to determine the residues of mesotrione and its degradation time (DT50) in maize after one application of M-100SC-OR2-C under field conditions. Application was made at BBCH 12 of the crop woth dose of 1.5 L/ha (150 a.s./ha) of formulated product. Spray volume was 200-400 L/ha according to Good Agricultural Practice. Field trials were set up in Poland, Germany, Hungary and Northern France (one trial in each country).
Specimens had to be deep-frozen 15 minutes after sampling was complete. In case time exceeds 15 minutes dry ice was used.

RAC specimens were collected following the target schedule below:

Sampling Event	Plot	Timing*	Commodity	**Minimum** sample size
S1	U/T	**0-1 HAA**	Whole plant without roots	100g; 12 Units **(please see special requirements)**
S2	U/T	**2 HAA**	Whole plant without roots	100g; 12 Units
S3	U/T	**4 HAA**	Whole plant without roots	100g; 12 Units
S4	U/T	**6 HAA**	Whole plant without roots	100g; 12 Units
S5	U/T	**20 HAA**	Whole plant without roots	100g; 12 Units
S6	U/T	**24 HAA**	Whole plant without roots	100g; 12 Units
S7	U/T	**2 DAA**	Whole plant without roots	100g; 12 Units
S8	U/T	**3 DAA**	Whole plant without	100g;

			roots	12 Units
S9	U/T	**4 DAA**	Whole plant without roots	100g; 12 Units
S10	U/T	**5 DAA**	Whole plant without roots	100g; 12 Units

*HAA – Hours After Application; DAA – Days After Application

Identiciation of the field trials:

Trial number	Study type	EU Zone	Country (region)	Trial site	Zip code
21SGS76-01	DCS	Central	Poland (Kujawsko-pomorskie)	Wenecja	88-400
21SGS76-03	DCS	Central	Hungary (Szabolcs-Szatmár-Bereg)	Nyírtel	H-4461
21SGS76-05	DCS	Central	Poland (Kujawsko-pomorskie)	Zamarte	89-430
21SGS76-06	DCS	North	France (Grand Est)	Auménancourt	51110

Results and conclusions:

Residue concentration detected in analysed field samples:

	Mesotrione residues (mg/kg)			
Timing	**21SGS76-01 Poland**	**21SGS76-03 Hungary**	**21SGS76-05 Poland**	**21SGS76-06 France**
1 HAA	8.53	14.16	15.06	16.55
2 HAA	7.73	14.08	14.50	15.22
4 HAA	8.34	12.29	14.45	13.34
6 HAA	6.76	12.16	13.95	13.35
20 HAA	6.07	10.06	10.11	11.45
24 HAA	6.24	10.03	10.51	10.13
2 DAA	2.63	3.91	5.27	5.14
3 DAA	2.50	3.38	3.73	2.76
4 DAA	0.64	2.15	2.02	0.41
5 DAA	0.34	0.10	0.79	0.19

*HAA – Hours After Application; DAA – Days After Application

For each trial DT50 values were determined. For this purpose CAKE program, following single first-order kinetics (SFO) was used. Residues decay is described by:

$$c = c_0 e^{-kt}$$

c – *concentration at time t*

c_0 – *initial concentration*

k – *rate constant*

t – *time*

Trial	DT_{50} [h]	DT_{50} [days]	Error [%]
21SGS76-01	34.4	1.43	9.34
21SGS76-03	32.6	1.36	7.8
21SGS76-05	33.6	1.40	3.93
21SGS76-06	29.5	1.23	8.09

For Analytical details see section B5 of dRR

Conclusuion:

This study was fully performed as anticipated, in accordance with the study plan and the amendment issued. The collected specimens were suitable for the purpose of the study and the residue values can therefore be considered as representative of the crop and of the application timing(s) and rate(s).

The method was validated according to SANTE/2020/12830 Rev.1 guideline. (For details see Section B5 of dRR).

The limit of detection and quantification of the method was established at 0.002 and 0.010 mg/kg for maize plant, respectively

There were no interfering signals at retention time of analyzed compound in examined control matrix.
The analytical method for determining the residues of mesotrione in maize (plants) meets the criteria of SANTE/2020/12830 Rev.1 guideline in terms of precision, accuracy and uncertainty.

New data

Kinetic analysis to derive the rate of residue decline (DT_{50}) for mesotrione applied on maize plants

Executive summary

A kinetic analysis was performed for the derivation of the rate of residue decline (DT_{50}) for mesotrione applied on maize plants. Crop residue data are available from field studies performed at four locations in Europe: Poland (two sites), Hungary and France (Peda T., 2021; Final report for study 21SGS76).

The study was conducted as crop field trials for the determination of the magnitude of the pesticide residue in or on raw agricultural commodities according to OECD 509. Mesotrione was applied on maize at BBCH 12 (leaf development) at a nominal rate of 150 g a.i./ha. During the 0 to 5 day period after application, samples of plant material (excluding roots) were removed for analysis according to a 10-point time-course series.

Approximately 95% of applied mesotrione was dissipated during the study period and the measured quantity of mesotrione in all samples in the study were greater than the limit of quantification (0.01 mg/kg). The kinetic analysis for mesotrione was performed according to the general principles described in “FOCUS Generic guidelines for estimating persistence and degradation kinetics from environmental fate studies” (FOCUS, 2014).

Kinetic modelling was performed using CAKE version 3.5 (Hybrid Engineering and Syngenta, 2021), using the IRLS (iteratively reweighted least squares) optimisation algorithm. Three kinetic models: single first order (SFO), first order multi-compartment (FOMC) and double first order in parallel (DFOP) were fitted to the datasets. The best model was selected based on visual and statistical goodness of fit.

The single first order (SFO) model is the preferred model for deriving endpoints for modelling calculations and was found appropriate for describing degradation of mesotrione on maize at the field sites in Poland, Hungary and France. The derived endpoints are listed in **Błąd! Nie można odnaleźć źródła odwołania.**. The derived model parameters were considered acceptable in all cases, based on visual and statistical goodness of fit and passing of the t-test ($p<0.05$).

Summary residue decline endpoints, mesotrione

Location	Model	Model parameter, *k*	χ^2-error	DT_{50} (d)	t-test p-value
Poland 01	SFO	0.4813	9.34	1.44	$p < 0.05$
Hungary	SFO	0.5078	7.74	1.37	$p < 0.05$
Poland 05	SFO	0.4921	4.04	1.41	$p < 0.05$
France	SFO	0.5625	7.89	1.23	$p < 0.05$

Introduction

Crop field trials for the determination of the magnitude of the pesticide residue in or on raw agricultural commodities were conducted with the herbicide mesotrione (Table 9-4), which is used to control broadleaf weeds.

Mesotrione was applied by spray application onto maize plants at BBCH 12 (leaf development) at field sites in Poland, Hungary and France. Samples of whole plants excluding roots were collected over time and analysed for mesotrione. The field study was performed according to OECD 509.

The following consideration relates to the kinetic analysis of the reported residue data for the determination of the rate of decline of mesotrione residue on maize shoot material for the purpose of refining the ecotoxicology risk assessment for mammals.

Table 9-4: Mesotrione

Substance	Chemical name	Molecular mass (g/mol)
Mesotrione	2-(4-mesyl-2-nitrobenzoyl)cyclohexane-1,3-dione	339.32

Crop field trial study data

Crop field trial study with mesotrione

Mesotrione was applied by spray application onto maize plants at BBCH 12 (leaf development) at field sites in Poland, Hungary and France (Peda, 2021). Soil properties and other site characteristics at the four locations are shown in **Błąd! Nie można odnaleźć źródła odwołania.**. A range of soil textures are represented, and the test sites are typical of areas used for maize production under central and southern EU conditions.

Two other trials were not able to provide reliable data: the trial in Germany (21SGS76-02) was cancelled because in the period between sample collection and analysis the freezer in which the samples were stored malfunctioned and sample integrity was compromised. Trial 21SGS76-04 (France) was also cancelled because owing to extensive bird damage it was not possible to collect at least 100 g of plant material for analysis. The second trial in Poland (21SGS76-05), ~100 km north and under different edaphic conditions from 21SGS76-01, was established in place of the German trial; and a replacement trial was established at the site in France (21SGS76-06).

The test substance was applied as a single application in May and June 2021 (**Błąd! Nie można odnaleźć źródła odwołania.**). Spray equipment was used to apply the chemical to young maize plants at a nominal rate of 150 g a.s./ha.

Soil properties and site characteristics at the field sites

Trial ID	Location	Soil texture (USDA)	Soil organic matter (%)	pH-H_2O	Slope (%)	Air temp. (°C) Min. – max.	Rainfall (mm)
21SGS76-01	Wenecja, Poland	Sand	<0.5	7.0*	0	13.5 – 27.9	20.8 (June)
21SGS76-03	Nyírtel, Hungary	Clay loam	2.44	6.0	1	8.4 – 20.6	84.1 (May)
21SGS76-05	Zamarte, Poland	Sandy clay loam	1.5	8.7*	0	10.6 – 26.3	32.4 (June)
21SGS76-06	Auménancourt, France	Loam	2.5	8.5	0	13.0 – 24.0	114.2 (June)

*converted from reported pH-$CaCl_2$ using the German input decision tool 3.3

Application dates at the field sites

Trial ID	Location	Plot area (m^2)	Nominal crop density (Plants/ha)	Sowing date	Application date
21SGS76-01	Wenecja, Poland	1008	80,000	19 May 2021	07 June 2021
21SGS76-03	Nyírtel, Hungary	1125	77,000	09 May 2021	25 May 2021
21SGS76-05	Zamarte, Poland	1008	95,000	04 June 2021	15 June 2021
21SGS76-06	Auménancourt, France	552	75,000	08 June 2021	24 June 2021

Residue data

Single composite samples of 12 plants (without roots), of about 100 g mass, were collected on designated sampling times and dates (Table 1-5) and analysed for mesotrione. There were no residue detections < LOD (0.002 mg/kg) or < LOQ (0.01 mg/kg) in any sample, at any time.

Table 9-5: Sampling times and dates

Sampling time point No.	Hours after application	Days after application	Time (DAA)
S1	0 - 1	-	0.02
S2	2	-	0.08
S3	4	-	0.17
S4	6	-	0.25
S5	20	-	0.83
S6	24	-	1
S7	-	2	2
S8	-	3	3
S9	-	4	4
S10	-	5	5

Kinetic analysis

The kinetic analysis for mesotrione was performed according to the general principles described in "FOCUS Generic guidelines for estimating persistence and degradation kinetics from environmental fate studies" (FOCUS, 2014).

Software and kinetic models

Kinetic modelling for the mesotrione → sink transformation was performed using CAKE version 3.5 (Hybrid Engineering and Syngenta, 2021), which is a tool for Computer Assisted Kinetic Evaluation. The software fulfils the requirements by FOCUS (2014), as it provides the kinetic models recommended by the FOCUS group and provides the standard statistical information needed to assess the quality of the curve fitting (χ^2-error and t-test).

All optimisation settings were kept at the defaults set in the software. The iteratively reweighted least squares (IRLS) optimisation algorithm was used in agreement with FOCUS (2014, p.74). It has been argued that this approach yields more realistic estimates of confidence intervals for model parameters than nonlinear least squares (NLLS, OLS) as it is not limited by the assumption of equal error variances, nor does it rely upon assigning arbitrary weighting to the data (Gao *et al*. 2011).

Four primary models, simple first-order (SFO), first order-multi-compartment (FOMC), double-first order in parallel (DFOP) and hockey-stick (HS), are accepted to derived degradation endpoint values for environmental risk assessments within the EU (FOCUS, 2014). The differential equations associated with the first three models are listed below (the hockey stick model is not considered in this report).

[1] SFO: $\frac{dM}{dt} = -k\,M$

[2] FOMC: $\frac{dM}{dt} = -\frac{\propto}{\beta} M(\frac{t}{\beta} + 1)^{-1}$

[3] DFOP: $\frac{dM}{dt} = -\frac{k_1 g e^{-k_1 t} + k_2 (1-g) e^{-k_2 t}}{g e^{-k_1 t} + (1-g) e^{-k_2 t}} M$

Where:
M = total amount of chemical present at time t
t = time since the beginning of the experiment (days)
k = rate constant (d^{-1})
k_1 = rate constant in compartment 1 of DFOP model (d^{-1})
k_2 = rate constant in compartment 2 of DFOP model (d^{-1})
α = shape parameter
β = location parameter
g = fraction of parent compound applied into compartment 1

In the integrated form of the above equations, an additional variable is needed: M_0 is the amount of chemical present at time 0.

Simple first order kinetics is described by an exponential equation with only two parameters (M_0 and k). The rate of change in pesticide concentration is at any time proportional to the actual concentration remaining in the system. The SFO equation is the default degradation model for all EU kinetic assessments and is the starting point for the derivation of all degradation end-points. However, there are a number of reasons why the degradation rate of a chemical might change over time resulting in 'biphasic' degradation, as described by the FOMC and DFOP models.

In the FOMC model it is assumed that the rate of residue decline changes with time and can be described by a gamma distribution and expressed by a simple analytical equation with only 3 parameters (M_0, α and β). Dissipation is faster for larger values of α and for smaller values for β. The DFOP model assumes that the chemical residue is split into 2 unconnected compartments, each with its own rate constant.

The parameter g is required to describe the proportion of residue in the 'fast' and 'slow' compartments, and each compartment requires a rate constant (k_1 and k_2) along with the initial concentration (M_0).

Modelling strategy

The aim of the kinetic analysis was to determine the rate of decline of mesotrione residue on the raw agricultural commodity of interest (maize shoot material) for the purpose of refining the ecotoxicology risk assessment for mammals.

Single first-order (SFO) kinetics is the preferred option for deriving degradation endpoints, as the first-order DT_{50} can be used directly as input in the environmental fate modelling tools (FOCUS, 2014). Alternative models are considered if no satisfactory fit can be obtained by the SFO model. When the data shows biphasic pattern then the FOMC, or DFOP models are tested. According to the FOCUS flow diagram (Figure 7-2 in FOCUS, 2014) the FOMC model should be used when 10% of the initially measured concentration is reached within the experimental period.

Statistical assessment

For each model fit, the goodness of fit is assessed, both visually and statistically. The overall pattern was assessed visually by plotting measured data points against the fitted line of predicted concentrations and by plotting the residuals (observed minus calculated values).

The statistical goodness of fit is expressed as the χ^2 error which is calculated in CAKE according to FOCUS (2014):

$$err = 100 * \sqrt{\frac{1}{\chi^2_{tabulated}}} * \sum \frac{(C-O)^2}{\bar{O}^2}$$

Where:

C = calculated value
O = observed value
$\bar{O}$ = mean of all observed values
err = measurement error percentage (χ^2 error)
χ^2 tabulated = tabulated χ^2 value (depending on degrees of freedom)

A significance test, known as the 't-test' (FOCUS, 2014), is applied to test the probability that the optimised degradation rate parameter is different from zero. CAKE presents the probability (p value), whereby a value smaller than 0.05 indicates that the t-test is passed, *i.e.,* the degradation rate is significantly different from zero.

$$t = \frac{\hat{a}_i}{\sigma_i}$$

Where:
$\hat{a}_i$= estimate of degradation rate
σ_i= standard error of degradation rate

Note that the t-test cannot be applied to the FOMC model, as the parameters alpha (α) and beta (β) are shape parameters rather than representing kinetic rates. An alpha or beta value close to zero does not mean that degradation is slow. On the contrary, smaller values of beta indicate more rapid degradation, and alpha only indicates the shape of the curve and has nothing to do with the rate of degradation (FOCUS, 2014; p.97).

The 90% probability interval is reported because in kinetic analysis the t-test is single-sided.

Residue data

Plant residues at each sampling time were reported as concentrations expressed as mg/kg plant material and are summarized in table below.

Total residues on maize plants (mg/kg)

Sampling time point	DAA [a]	Mesotrione (mg/kg)			
		Poland 01	Hungary	Poland 05	France
S1	0.02	8.53	14.16	15.06	16.55
S2	0.08	7.73	14.08	14.50	15.22
S3	0.17	8.34	12.29	14.45	13.34
S4	0.25	6.76	12.16	13.95	13.35
S5	0.83	6.07	10.06	10.11	11.45
S6	1	6.24	10.03	10.51	10.13
S7	2	2.63	3.91	5.27	5.14
S8	3	2.5	3.38	3.73	2.76
S9	4	0.64	2.15	2.02	0.41
S10	5	0.34	0.10	0.79	0.19
Residue remaining [b] (%)		4.00	0.71	5.25	1.15

a) DAA = days after application
b) Calculated as % remaining on day 5 relative to the concentrations measured at the first sampling timepoint (0-1 h)

Modelling results mesotrione

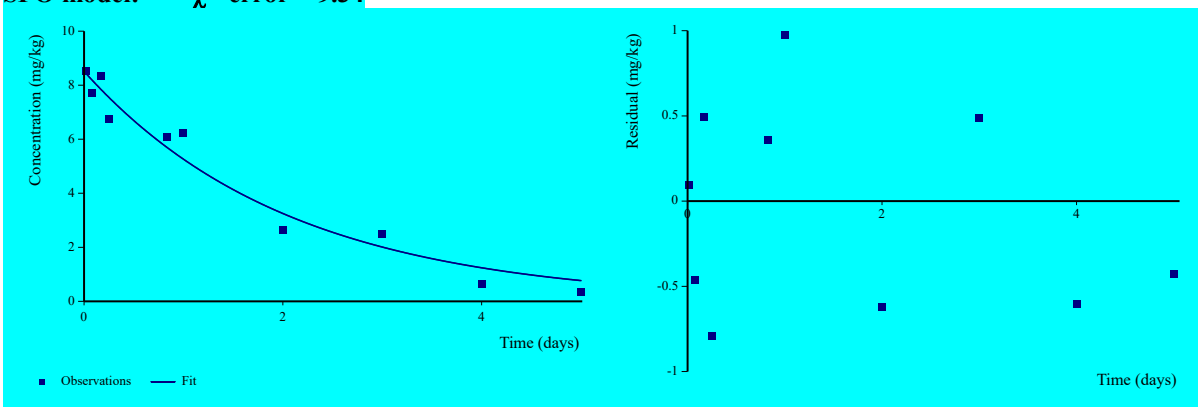
Despite mesotrione residue concentrations reaching 10 % of the initially measured concentration in all four trials, in order to achieve the best model description for the degradation of mesotrione all three kinetic models were fitted and evaluated. The results are presented in the graphs and tables below.

Poland 01 (21SGS76-01)

Degradation of mesotrione at the first site in Poland was described well by all models, which gave good statistical goodness of fit (χ^2-error <15.0) and acceptable visual fit with no systematic error. The calculated DT_{50} values for all models was 1.44 days. Overall, the statistical goodness of fit (χ^2-error = 9.34) for the SFO model was marginally better than for the other models (FOMC χ^2-error = 9.81; DFOP χ^2-error = 10.4). The SFO model was selected for deriving the DT_{50}.

Poland 01 (21SGS76-01)

SFO model: χ^2-error = 9.34

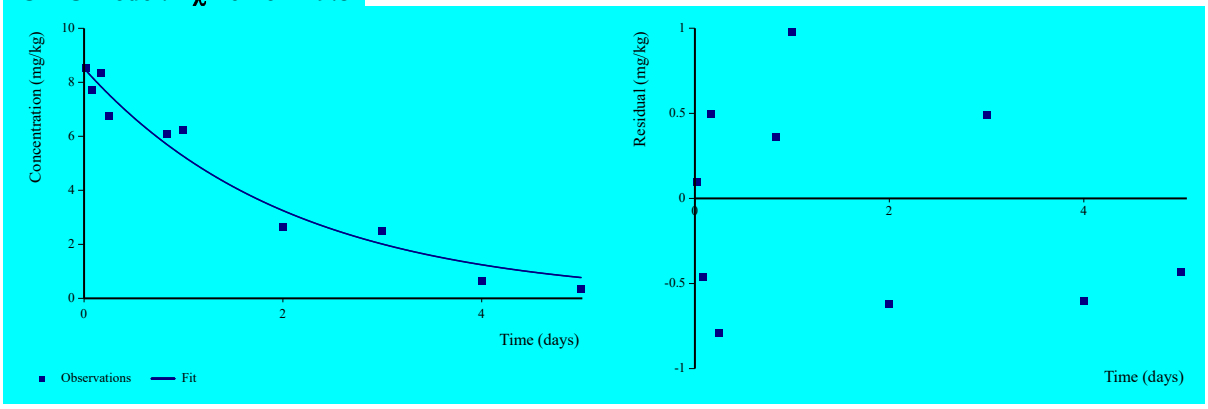


Poland 01 (21SGS76-01) - SFO model

21SGS76-01	**Model**	**χ^2-error**	**DT_{50} (d)**	**DT_{90} (d)**
Mesotrione	SFO	9.34	1.44	4.78

Parameter	**value**	**st.dev**	**t-test p-value**	**90% probability interval**	
M_0	8.515	0.3566	-	7.852	9.178
k_Parent	0.4813	0.058	1.68E-05	0.3734	0.5891

FOMC model: χ^2-error = 9.81

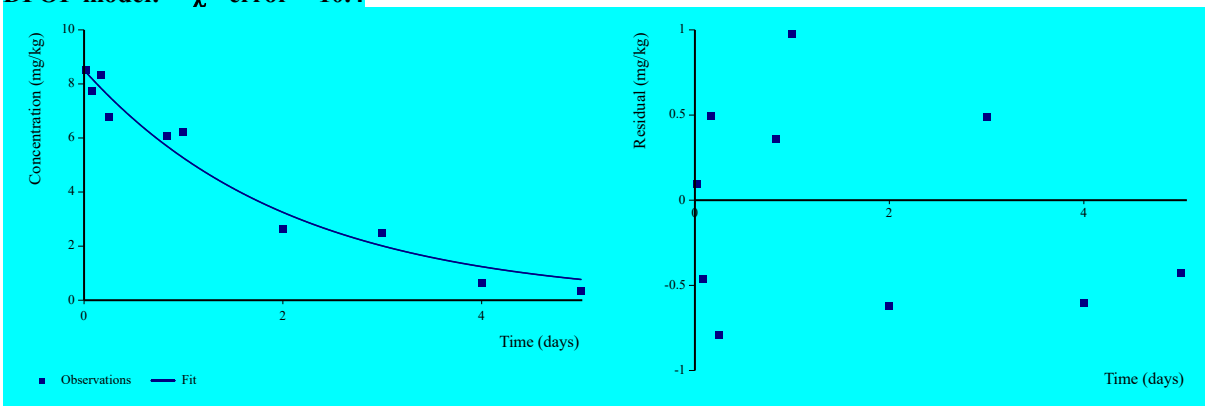


Poland 01 (21SGS76-01) - FOMC model

21SGS76-01	Model	χ^2-error	DT_{50} (d)	DT_{90} (d)
Mesotrione	FOMC	9.81	1.44	4.79

Parameter	value	st.dev	t-test p-value	90% probability interval	
M_0	8.517	0.3764	-	7.803	9.23
alpha	415.5	695.8	-	-	-
beta	861.9	1440	-	-	-

DFOP model: χ^2-error = 10.4



Poland 01 (21SGS76-01) - DFOP model

21SGS76-01	Model	χ^2-error	DT_{50} (d)	DT_{90} (d)
Mesotrione	DFOP	10.4	1.44	4.79

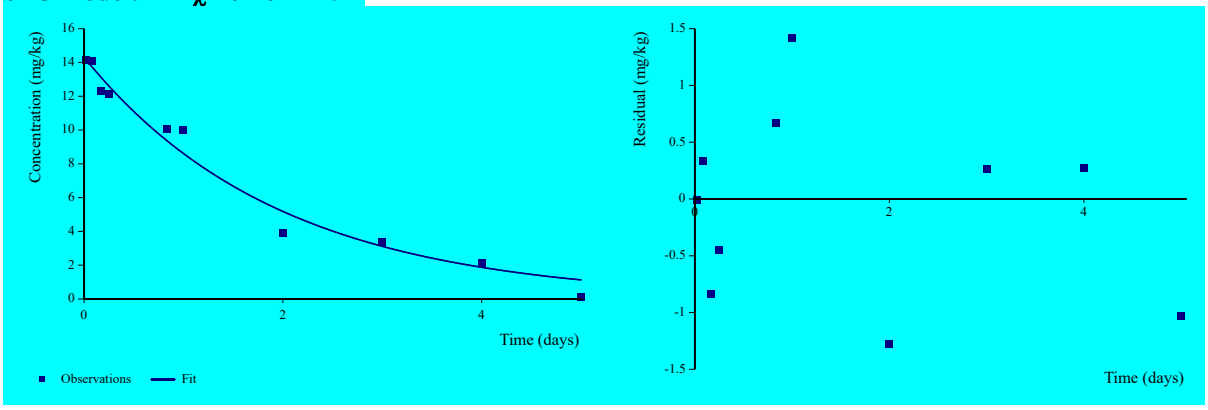
Parameter	value	st.dev	t-test p-value	90% probability interval	
M_0	8.515	0.4061	-	7.726	9.304
k1_Parent	0.4813	0.06369	0.00014	0.3575	0.605
k2_Parent	0.01252	nd	nd	nd	nd
g	1	nd	-	nd	nd

Hungary (21SGS76-03)

Degradation of mesotrione at the site in Hungary was described well by all models, which gave good statistical goodness of fit (χ^2-error <15.0) and acceptable visual fit with no systematic error. The calculated DT_{50} values ranged between 1.36 and 1.37 days. Overall, the statistical goodness of fit (χ^2-error = 7.74) for the SFO model was marginally better than for the other models (FOMC χ^2-error = 8.13; DFOP χ^2-error = 8.59). The SFO model was selected for deriving the DT_{50}.

Hungary (21SGS76-03)

SFO model: χ^2-error = 7.74

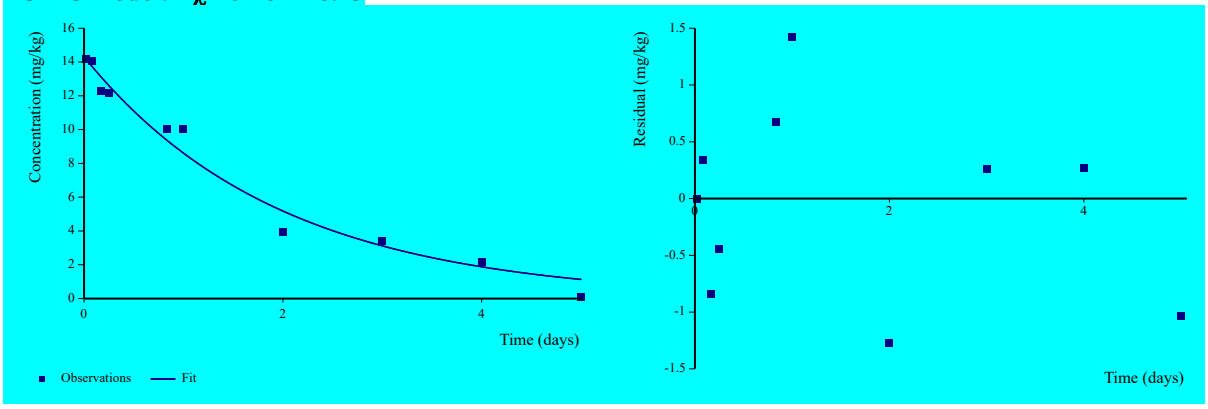


Hungary (21SGS76-03) - SFO model

21SGS76-03	**Model**	**χ^2-error**	**DT_{50} (d)**	**DT_{90} (d)**
Mesotrione	SFO	7.74	1.37	4.53

Parameter	**value**	**st.dev**	**t-test p-value**	**90% probability interval**	
M_0	14.31	0.4942	-	13.39	15.23
k_Parent	0.5078	0.05037	3.99E-06	0.4141	0.6015

FOMC model: χ^2-error = 8.13

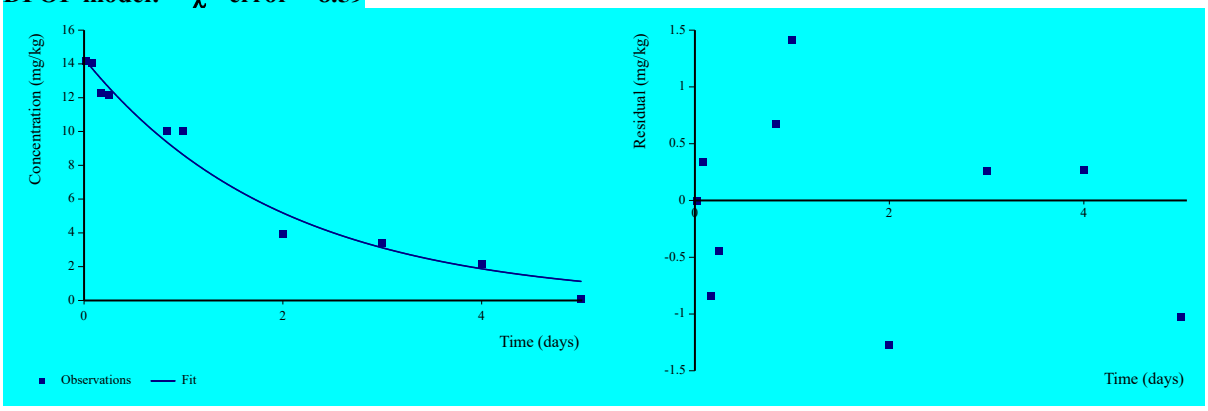


Hungary (21SGS76-03) - FOMC model

21SGS76-03	Model	χ^2-error	DT_{50} (d)	DT_{90} (d)
Mesotrione	FOMC	8.13	1.36	4.54

Parameter	value	st.dev	t-test p-value	90% probability interval	
M_0	14.31	0.5233	-	13.32	15.3
alpha	651.7	1.32E+03	-	-	-
beta	1.28E+03	2.59E+03	-	-	-

DFOP model: χ^2-error = 8.59



Hungary (21SGS76-03) - DFOP model

21SGS76-03	Model	χ^2-error	DT_{50} (d)	DT_{90} (d)
Mesotrione	DFOP	8.59	1.37	4.53

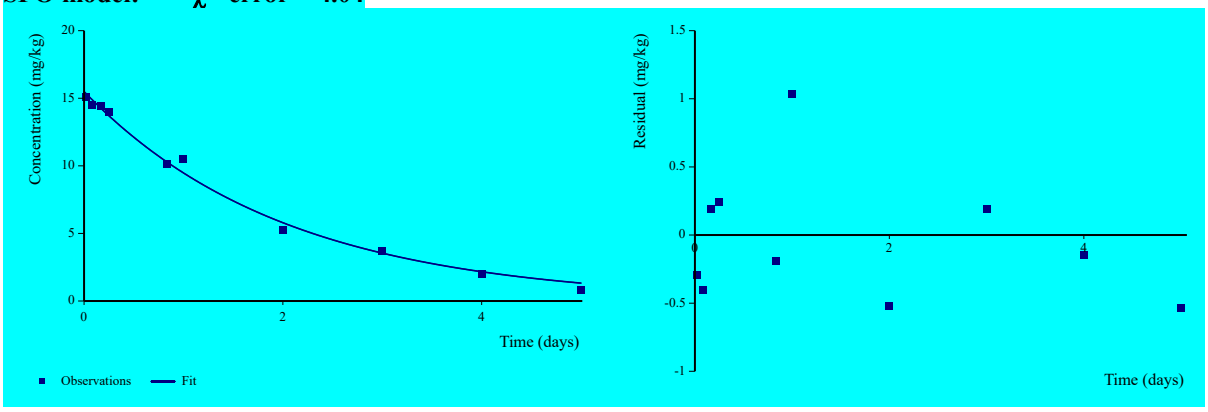
Parameter	value	st.dev	t-test p-value	90% probability interval	
M_0	14.31	0.5647	-	13.21	15.41
k1_Parent	0.5078	0.05603	5.06E-05	0.3989	0.6167
k2_Parent	0.01453	nd	nd	nd	nd
g	1	nd	-	nd	nd

Poland 05 (21SGS76-05)

Degradation of mesotrione at the second site in Poland was described very well by all models, which gave good statistical goodness of fit (χ^2-error <15.0) and acceptable visual fit with no systematic error. The calculated DT_{50} values for all models was 1.41 days. Overall, the statistical goodness of fit (χ^2-error = 4.04) for the SFO model was marginally better than for the other models (FOMC χ^2-error = 4.24; DFOP χ^2-error = 4.48). The SFO model was selected for deriving the DT_{50}.

Poland 05 (21SGS76-05)

SFO model: χ^2-error = 4.04

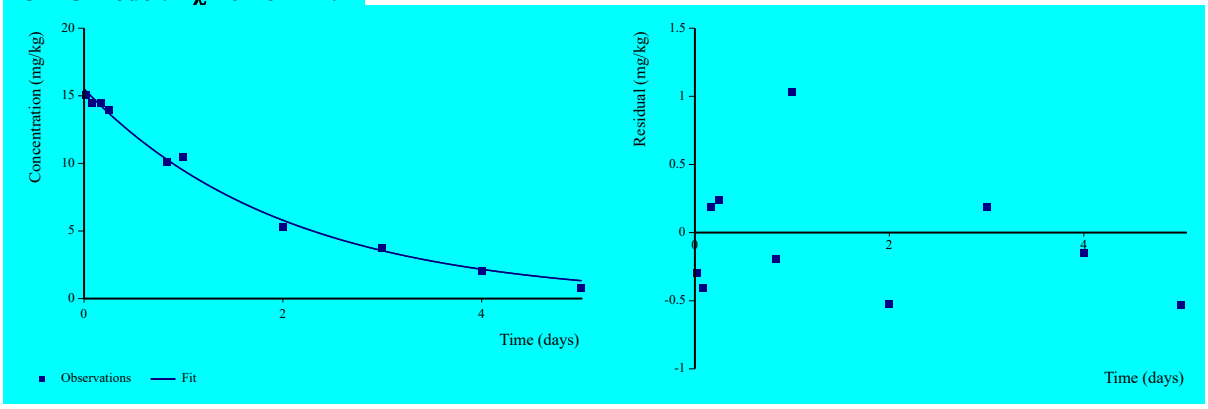


Poland 05 (21SGS76-05) - SFO model

21SGS76-05	**Model**	**χ^2-error**	**DT_{50} (d)**	**DT_{90} (d)**
Mesotrione	SFO	4.04	1.41	4.68

Parameter	**value**	**st.dev**	**t-test p-value**	**90% probability interval**	
M_0	15.5	0.2811	-	14.98	16.02
k_Parent	0.4921	0.02566	2.83E-08	0.4444	0.5398

FOMC model: χ^2-error = 4.24

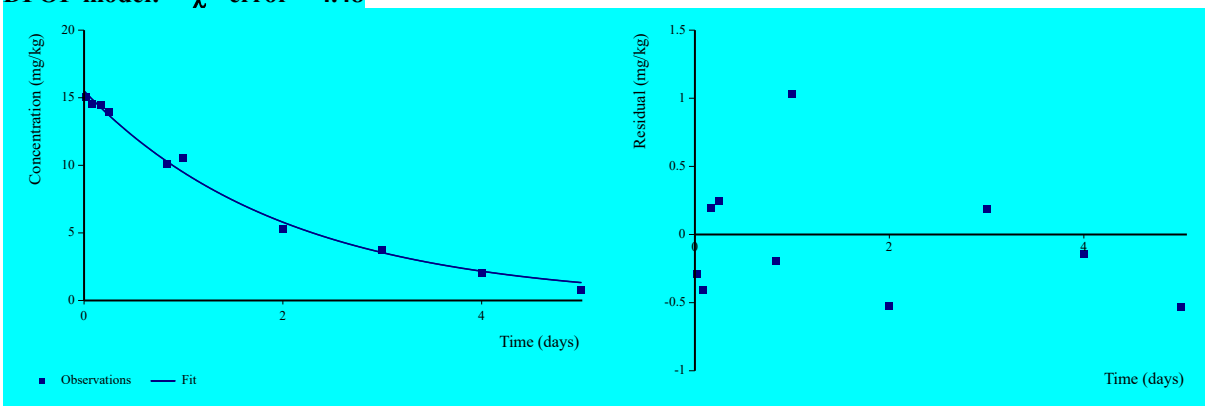


Poland 05 (21SGS76-05) - FOMC model

21SGS76-05	Model	χ^2-error	DT_{50} (d)	DT_{90} (d)
Mesotrione	FOMC	4.24	1.41	4.68

Parameter	value	st.dev	t-test p-value	90% probability interval	
M_0	15.5	0.337	-	14.87	16.14
alpha	5.71E+07	8.01E+06	-	-	-
beta	1.16E+08	1.31E+07	-	-	-

DFOP model: χ^2-error = 4.48



Poland 05 (21SGS76-05) - DFOP model

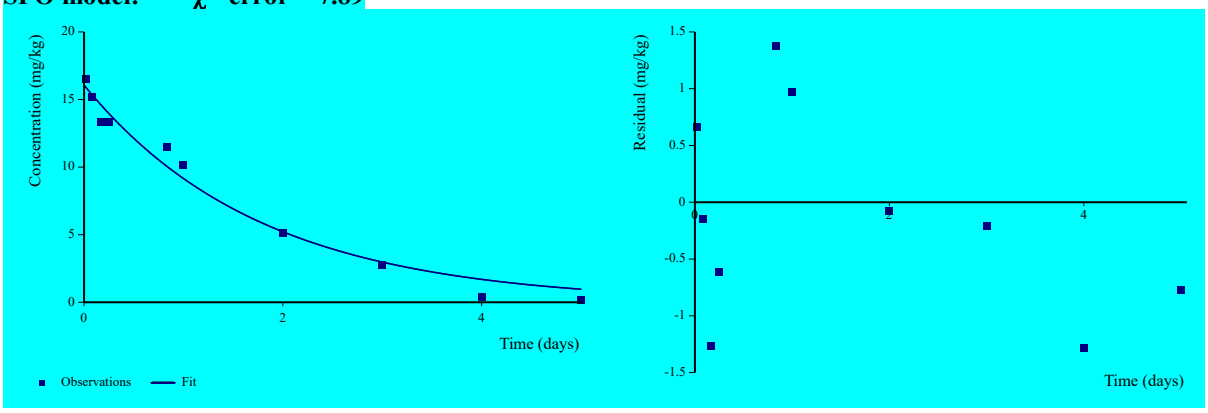
21SGS76-05	Model	χ^2-error	DT_{50} (d)	DT_{90} (d)
Mesotrione	DFOP	4.48	1.41	4.68

Parameter	value	st.dev	t-test p-value	90% probability interval	
M_0	15.5	0.3223	-	14.88	16.13
k1_Parent	0.4921	0.02889	1.31E-06	0.436	0.5482
k2_Parent	0.01078	nd	nd	nd	nd
g	1	nd	-	nd	nd

France (21SGS76-06)

Degradation of mesotrione at the site in France was described reasonably well by all models, which gave good statistical goodness of fit (χ^2-error <15.0). The visual fit was acceptable, but there was systematic over-estimation by all models between day 2 and day 5. The calculated DT_{50} values for all models was 1.23 days. Overall, the statistical goodness of fit (χ^2-error = 7.89) for the SFO model was marginally better than for the other models (FOMC χ^2-error = 8.28; DFOP χ^2-error = 8.76). The SFO model was selected for deriving the DT_{50}.

SFO model: χ^2-error = 7.89

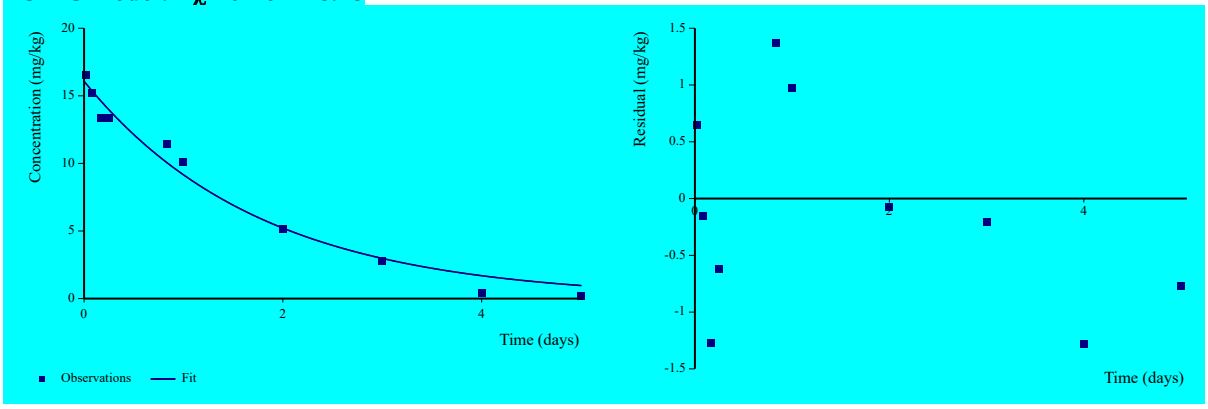


France (21SGS76-06) - SFO model

21SGS76-06	**Model**	**χ^2-error**	**DT_{50} (d)**	**DT_{90} (d)**
Mesotrione	SFO	7.89	1.23	4.09

Parameter	**value**	**st.dev**	**t-test p-value**	**90% probability interval**	
M_0	16.07	0.5534	-	15.04	17.1
k_Parent	0.5625	0.05554	3.86E-06	0.4592	0.6658

FOMC model: χ^2-error = 8.28

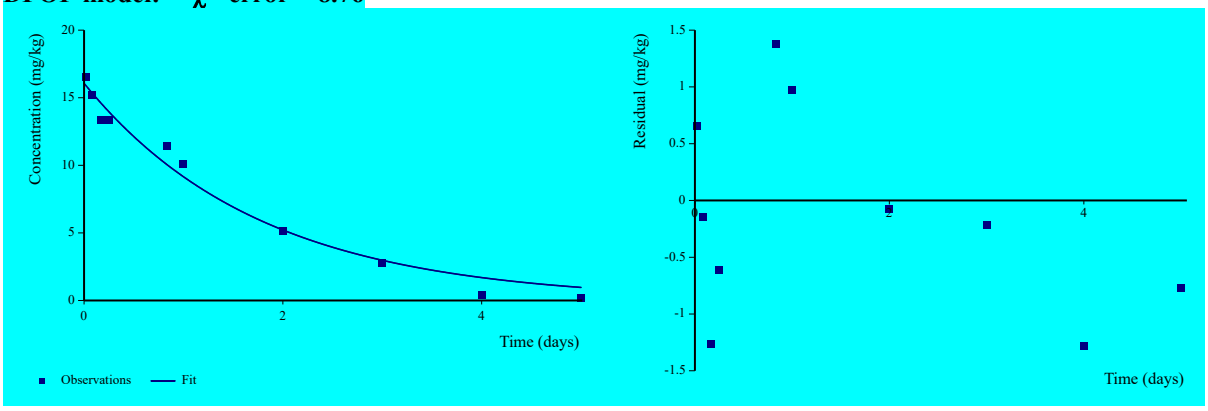


France (21SGS76-06) - FOMC model

21SGS76-06	Model	χ^2-error	DT_{50} (d)	DT_{90} (d)
Mesotrione	FOMC	8.28	1.23	4.09

Parameter	value	st.dev	t-test p-value	90% probability interval	
M_0	16.08	0.7348	-	14.69	17.47
alpha	3.26E+07	1.48E+07	-	-	-
beta	5.78E+07	2.39E+07	-	-	-

DFOP model: χ^2-error = 8.76



France (21SGS76-06) - DFOP model

21SGS76-06	Model	χ^2-error	DT_{50} (d)	DT_{90} (d)
Mesotrione	DFOP	8.76	1.23	4.09

Parameter	value	st.dev	t-test p-value	90% probability interval	
M_0	16.07	0.6275	-	14.85	17.29
k1_Parent	0.5625	0.06002	4.19E-05	0.4459	0.6791
k2_Parent	0.01724	nd	nd	nd	nd
g	1	nd	-	nd	nd

Conclusions of the kinetic analysis

Table 9-6 shows the residue decline endpoints derived for mesotrione on maize. Mesotrione decline was consistently rapid in all four field trials and was best described by single first-order (SFO) kinetics. Based on visual and statistical assessment, each of the selected model descriptions and derived parameters were considered acceptable.

Table 9-6: Summary residue decline endpoints, mesotrione

Location	Model	Model parameter, k	χ^2-error	DT_{50} (d)	t-test p-value
Poland 01	SFO	0.4813	9.34	1.44	$p < 0.05$
Hungary	SFO	0.5078	7.74	1.37	$p < 0.05$
Poland 05	SFO	0.4921	4.04	1.41	$p < 0.05$
France	SFO	0.5625	7.89	1.23	$p < 0.05$
*the worst case			Geomean	1.36	

RMS comment: The data has not been evaluated by RMS in Core Dossier. The evaluation of new data should be considered at Ms level.

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

A 2.2 KCP 10.2 Effects on aquatic organisms

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

A.2.2.1.1 Study 1

Comments of zRMS:	Study was carried out according to appropriate OECD 202 and all validity criteria were met. Deviation from the study: ✓ The deviation in temperature level, exceeding 1ºC (above 19.5°C occurred on 17.02.2022 from 18:30 to 21:30 and 18.02.2022 from 10:30 to 11:00 (highest temperature was 19.6ºC). ✓ The deviation in temperature level exceeding 1ºC (above 19.5°C occurred on 23.02.2022 from 13:00 to 20:00 (highest temperature was 19.7ºC). ✓ Editorial mistake in the Amendment No. 2 to the Study Plan SOP/P/47 instead of SOP/P/85. ✓ Ineffective chromatogram integration for sample EMI-4-5-2021-R3-r003.d by algorithm Agile 2 manually integrated. The method is acceptable in section B5 in dRR of Juzan Extra 100 SC. In opinion zRMS, above deviations did not affect the study results. The validity criteria: 1. The immobilisation of *Daphnis magna* in the control should be not more than 10% (actual: 0%) 2. The dissolved oxygen concentrations at the and of the test should be ≥ 3 mg/l (actual: 7.5-8.9 mg/l) **Study limitation:** 1. The study summary lacks a table with numerical results of the study. RMS has added a table for evaluation transparency. 2. The 95% confidence limits was not provided for toxicity endpoints. 3. NOEC > 3200 mg formulation/L (no pH adjustment) is not reliable value (100% immobility). RMS proposes to set NOEC at 320 mg formulation/L (0% immobility) **The study is considered acceptable.** **Agreed endpoints:** 48h/LC_{50} = 1872.63 mg formulation/L $_{nom}$ (the worst case – no pH adjustment) 48h/LC_{50} = 186.90 mg as/L $_{nom}$ (the worst case – no pH adjustment) 48h/NOEC = 320 mg formulation/L $_{nom}$

Reference:	KCP 10.2.1
Report	Juzan Extra 100 SC - *Daphnia magna*, Acute Immobilization Test, Sonia Szlauer, 2022, STUDY CODE: EMI/4/5/2021, Ecomelius Institute sp. z o. o.
Guideline(s):	Yes. According to the OECD Guideline No. 202 (2004)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

For analytical method validation see section B5 of dRR

Materials and methods

A. MATERIALS

1. Test material

Lot/Batch #	1/2021
Appearance:	Beige to brown suspension
Content of active substance	Mesotrione: 103.8 g/L
Density:	1.040 g/mL
Expiry date	09.02.2023

2. Test organism

Species	*Daphnia magna*
Source	Toxkit ephippia, Belgium
Culturing	At 24 ± 2 °C under constant illumination in Elendt M7 medium.
Acclimation period	Culturing was done under test conditions.
Test units	100 mL glass beakers

3. Environmental conditions

Test water

Final solution - M7 culture medium	
Solution	ml/L
Basic solution II	50.0
$CaCl_2 * 2H_2O$	1.0
$MgSO_4 * 7H_2O$	0.5
KCl	0.1
$NaHCO_3$	1.0
$Na_2SiO_3 * 9H_2O$	0.2
$NaNO_3$	0.1
KH_2PO_4	0.1
K_2HPO_4	0.1
Vitamins solution	0.1

Water temperature The temperature was kept constant at 18 to 22ºC, controlled at ± 1ºC

pH The test was performed in two versions – with and without regulation of pH. The pH was measured on days 0 and 2 in each test vessels and controls The pH was in the range of 6-9 and hardness between 140-250 mg/L

Lighting In the experiment carried with **pH adjustment**, the average light intensity was 1295.92 lux.
In the experiment carried without **pH adjustment**), the average light intensity was 1441.1 lux.

B. STUDY DESIGNS AND METHODS

1. Experimental conditions

Test design

The impact of M-100SC-OR2-C on *Daphnia magna* was investigated during a 48-hour toxicity study. Five daphnids in four replicates were exposed to the test item solutions. The test was performed in two versions – with and without regulation of pH. Number of immobilized daphnids at 24 and 48 hours after the beginning of the test and any abnormal behavior or appearance were reported. In total, 9 concentrations of the test item was prepared, 4 replicates containing 5 daphnids each.

Concentrations tested

Based on the results of the preliminary experiment, concentrations for the main experiment were selected, i.e.:

a) 1 000, 1 800, 3 200, 5 600, 10 000 mg/L – experimental part with pH adjustment;
b) 320, 1 000, 1 800, 3 200 mg/L – experimental part with no pH adjustment.

Analytics

The concentration of Mesotrione in Elendt M7 medium was determined using a validated ultrahigh performance liquid chromatographic method with mass spectrometer detection. The analytical method was validated according to SANTE/2020/12830, Rev.

2. Sampling and measurements

The temperature was kept constant at 18 to 22ºC, controlled at ± 1ºC, by means of air conditioner. The measurement was carried out continuously throughout the test using the temperature and humidity recorder.
The pH was measured on days 0 and 2 in each test vessels and controls.
The light intensity was measured on days 0, 1 and 2.
Observations were carried out daily. Each test vessel was checked for immobilized daphnids at 24 and 48 hours after the beginning of the test. In addition to immobility, any abnormal behaviour or appearance were noted.

3. Calculation of toxicity

The test endpoint is the concentration estimated to immobilize 50 per cent of the daphnids within a stated exposure period. The percentages immobilized at 24 hours and 48 hours are plotted against test concentrations.
The endpoint values were determined on the basis of nominal concentrations of the test item [1]. The ToxRat Professional commercial software was used to make calculations and to conduct statistical analyses.

Results and discussions

A. ANALYTICAL RESULTS

The aim of the analytical part of the experiment was to determine the concentrations of the test item with a liquid chromatographic method with mass spectroscopy.
The analytical method has been validated during the study. A Standard Operating Procedure for the analytical test method has been developed based on the validation carried out during the study. The determination of concentration was performed by the analytical personnel.
Samples of the highest and the lowest test concentrations and the control collected at exposure initiation and at exposure termination of both experimental parts were analyzed. Each sample was analysed 3 times. Before and after the analysis, a series of samples, blank samples (ultrapure water), and quality control samples (QC - standard solutions) were additionally analysed to confirm proper operation of the LC-MS.

With pH adjustment

Nominal concentration of test item [mg/L]	Exposure initiation			Exposure termination		
	Expected concentration of active substance (µg/L)	Measured concentration of active substance (µg/L)	% of active substance measured based on expected concentration	Expected concentration of active substance (µg/L)	Measured concentration of active substance (µg/L)	% of active substance measured based on expected concentration
Control	<LoQ	<LoQ	-	<LoQ	<LoQ	-
1 000	100	113	112.7	100	105	105.4
1 800	180	166	92.4	180	156	86.8
3 200	319	303	95.0	319	303	95.0
5 600	559	492	88.1	559	547	97.9
10 000	998	1083	108.5	998	973	97.5

LoQ= 0.7 µg /L

No pH adjusment

Nominal concentration of test item [mg/L]	Exposure initiation			Exposure termination		
	Expected concentration of active substance (mg/L)	Measured concentration of active substance (mg/L)	% of active substance measured based on expected concentration	Expected concentration of active substance (mg/L)	Measured concentration of active substance (mg/L)	% of active substance measured based on expected concentration
Control	<LoQ	<LoQ	-	<LoQ	<LoQ	-
320	32	28	87.3	32	26	80.4
1000	100	89	89.3	100	90	89.5
1800	180	156	86.6	180	162	90.2
3200	319	313	98.2	319	279	87.6

LoQ= 0.7 µg /L

B. BIOLOGICAL RESULTS

In the experimental part of the study **with pH adjustment**, in the test item concentrations R1 (1 000 mg/L) and R2 (1 800 mg/L), daphnids had normal behavior and appearance similar to control group, both after 24 and 48 hours. In the test item concentration R3 (3 200 mg/L), immobilized daphnids were whiter compared to control group after 24 hours and after 48 hours, immobilized daphnids were white and were lying on the bottom. Not immobilized daphnids were also white. In the test item concentration R4 (5 600 mg/L), after 24 hours, immobilized daphnids were white and were lying at the bottom, whereas not immobilized daphnids were whiter than control group and slower than control group. After 48 hours, even not immobilized daphnids were white like these immobilized. In the test item concentration R5 (10 000 mg/L), all daphnids were white and were lying at the bottom of test vessels.

In the experimental part of the study **without pH adjustment,** in the test item concentrations R1 (320 mg/L) and R2 (1000 mg/L), not immobilized daphnids had normal behavior and appearance, both after 24 and 48 hours. The immobilized ones in R2 had normal color. In the test item concentration R3 (1 800 mg/L), daphnids were whiter compared to the control group after 24 hours, and after 48 hours, daphnids were also lying at the bottom. In the test item concentration R4 (3 200 mg/L), all daphnids were immobilized after 24 hours, were lying at the bottom and were completely white.

Calculations made on the basis of the nominal test item concentrations [mg/L] at the end of the experiment.

Endpoint	mg/L	no pH adjustment	pH adjustment
EC_{50}	Test item	1 872.63 (n.d)	2700.22 (n.d)
	Active substance	186.90	269.50
LOEC	Test item	> 3 200.00	3 200.00
	Active substance	-	319.38
NOEC	Test item	≥ 3 200.00	1 800.00
	Active substance	-	179.65

C. VALIDITY CRITERIA

For the test to be valid, the following performance criteria specified in OECD Guideline No. 202 (2004) were met for both experimental parts:

- In the control 0% of daphnids were immobilized (criterium: not more than 10%);
- The concentration of dissolved oxygen in the test and control vessels was ≥ 3 mg/L at the end of the test.

The effects on immobility in *Daphnia magna* (pH adjustment).

Treatment [mg/L]	Total number of daphnids	After 24 h		After 48 h	
		Immobile	% immobility	Immobile	% immobility
Control	20	0	0	0	0
1 000	20	0	0	0	0
1 800	20	1	5	1	5
3 200	20	15	75	19	95
5 600	20	15	75	19	95
10 000	20	19	95	20	100

The effects on immobility in *Daphnia magna* (no pH adjustment).

Treatment [mg/L]	Total number of daphnids	After 24 h		After 48 h	
		Immobile	% immobility	Immobile	% immobility
Control	20	0	0	0	0
320	20	0	0	0	0
1 000	20	5	25	6	30
1 800	20	4	20	4	20
3 200	20	20	100	20	100

A.2.2.1.2 Study 2

Comments of zRMS:	Study was carried out according to appropriate OECD 201 (2006) and all validity criteria were met. Deviation from the study: - Inaccurate automatic chromatogram integration for R7 (spent solution from 28.01.2022), R8 (spent solution from 31.01.2022) and R9 (spent solution from 31.01.2022). Chromatograms were manually integrated. - Study completion date February 2022 changed to March 2022. - Editorial mistake in the Amendment No. 1 to the Study Plan page 10 SOP/P/47 instead

	of SOP/P/85. The validity criteria: - The doubling time of frond number in the control was 1.8 d (criterion: less than 2.5 days) - The average specific growth rate in the control between day 0 and day 7 was 0.378 d^{-1} (minimum requirement higher than 0.275 d-1). The method is acceptable in section B5 in dRR of Juzan Extra 100 SC. The study is considered to be reliable and suitable for the risk assessment. Toxicity endpoints 7d ER_{50} = 1.44 mg formulation/L (95% CI: 0.961-2.499), equivalent to 0.144 mg s.a./L (95% CI: 0.096-0.249) was used in risk assessment. Agreed endpoints: *Results based on geometric mean concentrations:* *Frond number* E_rC_{50} = 2.224 mg formulation/L E_rC_{50} = 0.222 mg as/L E_yC_{50} = 0.225 mg formulation/L E_yC_{50} = 0.022 mg as/L *Dry weight* E_rC_{50} = 1.44 mg formulation/L E_rC_{50} = 0.144 mg as/L E_bC_{50} = 0.239 mg formulation/L E_bC_{50} = 0.024 mg as/L

Reference:	KCP 10.2.1
Report	Juzan Extra 100 SC – *Lemna sp.*, Growtj Inhibition Test, Sonia Szlauer, 2022, STUDY CODE: EMI/4/6/2021, Ecomelius Institute sp. z o. o.
Guideline(s):	Yes. According to the OECD Guideline No. 221 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

For analytical method validation see section B5 of dRR

Materials and methods

A. MATERIALS

1. Test material

Lot/Batch #	1/2021
Appearance:	Beige to brown suspension
Content of active substance	Mesotrione: 103.8 g/L
Density:	1.040 g/mL
Expiry date	09.02.2023

2. Test organism

Species	*Lemna gibba*
Source	The fronds *Lemna gibba* were obtained from the Landolt Duckweed Collection
Culturing	At 24 ± 2 °C under constant illumination in 20X AAP medium.
Acclimation period	Culturing was done under test conditions.
Test units	100 mL glass beakers

3. Environmental conditions

Test water

Substance	Concentration in the 20X AAP medium [mg/L]
$NaNO_3$	510
$MgCl_2 * 6 H_2O$	240
$CaCl_2$	90
$MgSO_4 * 7H_2O$	290
K_2HPO_4	30
$NaHCO_3$	300
Microelements	
H_3BO_3	3.7
$MnCl_2*4H_2O$	8.3
$FeCl_3*6H_2O$	3.2
$Na_2EDTA*2H_2O$	6.0
$ZnCl_2$	0.066
$CoCl_2*6H_2O$	0.029
$Na_2MoO_4*2H_2O$	0.145
$CuCl_2*2H_2O$	0.00024

Water temperature 24°C ± 2°C.
pH pH of the control not warried more than 1.5 unit during the test; pH of the control was in the range 7.47 – 7.71
Lighting Constant illumination in the range of 6500 to 10 000 lux
Test vessel Glass crystallizers containing 100 mL of a given test item concentration or control

B. STUDY DESIGNS AND METHODS

1. Experimental conditions

Test design

Semi-static; 7 days of exposure; three replicates of each test item concentration; six replicates of control.

Concentrations tested

Nine test item concentrations: 0.056, 0.1, 0.18, 0.32, 0.56, 1.0, 1.8, 3.2, 5.6 mg/L plus the control

Analytics

The concentration of Mesotrione in 20X AAP medium was determined using a validated ultrahigh performance liquid chromatographic method with mass spectrometer detection. The analytical method was validated according to SANTE/2020/12830, Rev.

2. Sampling and measurements

In order to quantify the test item-related effects on vegetative growth over a period of 7 days, the number of fronds in each replicate was counted twice during exposure (days 2 and 4) and at exposure termination. At the same time observations of plant development were performed, i.e. size of fronds, necroses, chloroses, colony break up, gibbosity, changes in appearance of roots.
The dry weight of the representative sample of the duckweed culture used as the inoculum was measured at exposure initiation. The dry weight of all plants from each test vessel was measured after exposure termination. All colonies (with roots) were transferred onto previously weighed microscopic slides and dried at 60°C in a laboratory oven for 72 hours.

3. Calculation of toxicity

The endpoint values were determined on the basis of the nominal test item concentrations.

Due to the fact, that the deviations from the nominal test item concentrations were not within ± 20%, analysis of the results were also based on the geometric mean concentrations during exposure.
The following values were determined:

- The growth rate based on the nominal test item concentrations
- The growth rate based on the geometric mean concentrations
- The yield based on the nominal test item concentrations.
- The yield based on geometric mean concentrations

Results and discussions

A. ANALYTICAL RESULTS

~~The aim of the analytical part of the experiment was to determine the concentrations of the test item with a liquid chromatographic method with mass spectroscopy.~~
~~The analytical method has been validated during the study. A Standard Operating Procedure for the analytical test method has been developed based on the validation carried out during the study. The determination of concentration was performed by the analytical personnel.~~
~~Each sample was analysed 3 times. Before and after the analysis, a series of samples, blank samples (ultrapure water), and quality control samples (QC – standard solutions) were additionally analysed to confirm proper operation of the LC-MS.~~

~~With pH adjustment~~

Nominal concentration of test item [mg/L]	Exposure initiation			Exposure termination		
	Expected concentration of active substance (µg/L)	Measured concentration of active substance (µg/L)	% of active substance measured based on expected concentration	Expected concentration of active substance (µg/L)	Measured concentration of active substance (µg/L)	% of active substance measured based on expected concentration
Control	<LoQ	<LoQ	-	<LoQ	<LoQ	-
1 000	100	113	112.7	100	105	105.4
1 800	180	166	92.4	180	156	86.8
3 200	319	303	95.0	319	303	95.0
5 600	559	492	88.1	559	547	97.9
10 000	998	1083	108.5	998	973	97.5

LoQ= 0.7 µg /L

~~No pH adjusment~~

Nominal concentration of test item [mg/L]	Exposure initiation			Exposure termination		
	Expected concentration of active substance (mg/L)	Measured concentration of active substance (mg/L)	% of active substance measured based on expected concentration	Expected concentration of active substance (mg/L)	Measured concentration of active substance (mg/L)	% of active substance measured based on expected concentration
Control	<LoQ	<LoQ	-	<LoQ	<LoQ	-
320	32	28	87.3	32	26	80.4
1000	100	89	89.3	100	90	89.5
1800	180	156	86.6	180	162	90.2
3200	319	313	98.2	319	279	87.6

$LoQ = 0.7\ \mu g\ /L$

B. BIOLOGICAL RESULTS

In the experimental part of the study **with pH adjustment**, in the test item concentrations R1 (1 000 mg/L) and R2 (1 800 mg/L), daphnids had normal behavior and appearance similar to control group, both after 24 and 48 hours. In the test item concentration R3 (3 200 mg/L), immobilized daphnids were whiter compared to control group after 24 hours and after 48 hours, immobilized daphnids were white and were lying on the bottom. Not immobilized daphnids were also white. In the test item concentration R4 (5 600 mg/L), after 24 hours, immobilized daphnids were white and were lying at the bottom, whereas not immobilized daphnids were whiter than control group and slower than control group. After 48 hours, even not immobilized daphnids were white like these immobilized. In the test item concentration R5 (10 000 mg/L), all daphnids were white and were lying at the bottom of test vessels.

In the experimental part of the study **without pH adjustment,** in the test item concentrations R1 (320 mg/L) and R2 (1000 mg/L), not immobilized daphnids had normal behavior and appearance, both after 24 and 48 hours. The immobilized ones in R2 had normal color. In the test item concentration R3 (1 800 mg/L), daphnids were whiter compared to the control group after 24 hours, and after 48 hours, daphnids were also lying at the bottom. In the test item concentration R4 (3 200 mg/L), all daphnids were immobilized after 24 hours, were lying at the bottom and were completely white.

Calculations made on the basis of the nominal test item concentrations [mg/L] at the end of the experiment.

Endpoint	mg/L	no pH adjustment	pH adjustment
EC_{50}	Test item	1 872.63 (n.d)	2700.22 (n.d)
	Active substance	186.90	269.50
LOEC	Test item	> 3 200.00	3 200.00
	Active substance	-	319.38
NOEC	Test item	≥ 3 200.00	1 800.00
	Active substance	-	179.65

The aim of analytical part of the experiment was to determine the concentration of active ingredient, mesotrione in test item (M-100SC-OR2-C) with a validated liquid chromatographic method with tandem spectrometry mass detection [SOP/P/85], [SOP/T/16]. Samples of all (fresh and spent) test item concentrations and the control at exposure initiation, day 2, day 4 and at exposure termination were chemically analysed. The determined test item

concentrations were in the range of 82.4 –91.7% of the nominal concentration at the exposure initiation. The determined test item concentrations were in the range of 74.9 – 82.1% for spent concentrations and 86.2 – 92.6% for fresh concentration at day 2 of the experiment. The determined test item concentrations were in the range of 69.9 –79.7% for spent concentrations and 80.4 – 105.2% for fresh concentration at day 4 of the experiment. The determined test item concentrations were in the range of 57.5 – 76.4% of the nominal concentration at the exposure termination.

Test with the reference item

The test with reference item, i.e. 3,5-dichlorophenol (batch number: MKCH 5211, purity: 97% provided by Sigma Aldrich), was performed under static conditions [SOP/B/4]. The test was performed between 27.09.2021 and 04.10.2021. The 3,5-dichlorophenol was dissolved in acetone (batch number: 11219, Protolab). For this reason, an additional control containing the solvent was introduced. Six concentrations of the reference item ranging from 1.8, 3.3, 6.0, 10.8, 19.4 and 34.9 mg/L were used. Total acetone content in each vessel was 10 µl (100 µl/L, 0.01%). Each concentration of the reference item was divided into three replicates, whereas the control into six replicates. The results were within the range given in the references [SOP/B/4].

RESULTS

Observations

After 2 days of exposure, in the test item concentrations of R1 (0.056 mg/L), R2 (0.1 mg/L), R3 (0.18 mg/L) and R4 (0.32 mg/L), no distinctive changes from the normal development of plants in the control were observed. In the test item concentrations of R5 (0.56 mg/L) and R6 (1.0 mg/L), the fronds were smaller and were discolored compared with control, few fronds also had symptoms of chlorosis. In the test item concentration R7 (1.8 mg/L), the fronds were smaller, discolored, a few fronds had symptoms of chlorosis and were gibbed, the roots were shorter. In test item concentrations R8 and R9 (respectively 3.2 mg/L and 5.6 mg/L) the frond were smaller and discolored, also few fronds had symptoms of chlorosis. Additionally, the roots were shorter than in the control group. After 4 days of exposure, in the test item concentrations of R1 (0.056 mg/L), no distinctive changes from the normal development of plants in the control were observed. In the test item concentration of R2 (0.1 mg/L), the fronds were smaller, discolored and had symptoms of chlorosis compared with control. In the test item concentration R3 (0.18 mg/L), the fronds were smaller and discolored compared with control. Also, the frond had symptoms of chlorosis and break-up of the colony was observed. In the test item concentration R4 (0.32 mg/L), half of the fronds were smaller and with chlorosis. Additional, 1 colony lost its buoyancy. In the test concentration R5 (0.56 mg/L) half of the fronds were smaller, with chlorosis and had gibbosity. In the concentrations R6 (1.0 mg/L), R7 (1.8 mg/L), R8 (3.2 mg/L) and R9 (5.6 mg/L) the fronds were much smaller, with chlorosis or symptoms of discoloration on its surface. Additionally, in R6 (1.0 mg/L) 3 colonies were broken-up. In test item concentrations R5-R9 roots were shorter compared to the control. At exposure termination, in the test item concentrations of R1 (0.056 mg/L) and R2 (0.1 mg/L) the fronds were smaller and discolored compared with the control. In R2 (0.1 mg/L) fronds had symptoms of chlorosis. In the test item concentrations R3 (0.18 mg/L), R4 (0.32 mg/L) and R5 (0.56 mg/L) half of the fronds were smaller and with chlorosis compared with the control. Also, break-up of the colony was observed. Additionally in these concentrations, roots were shorter compared to the control. In the test item concentrations R6 (1.0 mg/L), R7 (1.8 mg/L), R8 (3.2 mg/L) and R9 (5.6 mg/L) fronds were smaller and with chlorosis or discolored. The roots were much shorter than the roots in the control group. The dry weight of the plants (fronds and roots) was determined at exposure termination.

Growth of Lemna gibba after 7 days.

Nominal test item concentration [mg/L]	Geometric mean concentration [mg/L]	Based on the frond number		Based on the dry weight	
		Growth rate inhibition [%]	Yield inhibition [%]	Growth rate inhibition [%]	Yield inhibition [%]
Control		0.0	0.0	0.0	0.0
0.056	0.048	13.2	33.7	19.2	42.8
0.1	0.081	9.6	26.5	-3.2*	-1.6
0.18	0.144	20.9	47.5	14.3	37.3
0.32	0.253	22.3	49.4	31.1	61.3
0.56	0.454	31.4	61.9	29.6	57.1
1.0	0.829	39.2	70.2	55.9	82.2
1.8	1.395	42.8	73.7	52.9	81.3
3.2	2.601	54.8	82.9	58.2	84.6
5.6	4.480	56.1	83.7	56.8	83.7

*compared to control

Growth rate endpoint values and active substance content based on the **nominal** test item concentrations [mg/L].

Endpoint values	mg/L	Frond number			Dry weight
		2 d	4 d	7 d	7 d
E_rC_{10}	Test item	0.102 (0.013 – 0.250)*	0.029 (0.005 – 0.074)	0.044 (0.028 – 0.063)	0.031 (0.006 – 0.076)
	Active substance	0.010 (0.001 – 0.025)	0.003 (0.000 – 0.007)	0.004 (0.003 – 0.006)	0.003 (0.001 – 0.008)
E_rC_{20}	Test item	0.424 (0.139 – 0.748)	0.169 (0.062 – 0.302)	0.203 (0.155 – 0.254)	0.125 (0.044– 0.230)
	Active substance	0.042 (0.014 – 0.075)	0.017 (0.006 – 0.030)	0.020 (0.016 – 0.025)	0.012 (0.004 – 0.023)
E_rC_{50}	Test item	4.809 (2.750 – 14.146)	3.531 (2.189 – 7.518)	2.779 (2.346 – 3.375)	1.769 (1.165 – 3.076)
	Active substance	0.480 (0.274 – 1.412)	0.352 (0.218 – 0.750)	0.277 (0.234 – 0.337)	0.177 (0.116 – 0.307)

*95% confidence limit

(Continued) Growth rate endpoint values and active substance content based on the **nominal** test item concentrations [mg/L].

Endpoint value	mg/L	Frond number			Dry weight
		2d	4d	7d	7d
NOEC	Test item	0.320	0.100	≥ 5.600	0.180
	Active substance	0.032	0.010	-	0.018
LOEC	Test item	0.560	0.180	> 5.600	0.320
	Active substance	0.056	0.018	-	0.032

Growth rate endpoint values and active substance content based on the **geometric mean** concentrations [mg/L].

Endpoint values	mg/L	Frond number			Dry weight
		2 d	4 d	7 d	7 d
E_rC_{10}	Test item	0.084 (0.011 – 0.203)*	n.d	0.036 (0.023 – 0.051)	0.042 (0.010 – 0.092)
	Active substance	0.008 (0.001 – 0.020)	-	0.004 (0.002 – 0.005)	0.004 (0.001 – 0.009)
E_rC_{20}	Test item	0.344 (0.116 – 0.602)	0.138 (0.052 – 0.245)	0.165 (0.127 – 0.205)	0.142 (0.057 – 0.243)
	Active substance	0.034 (0.012 – 0.060)	0.014 (0.005 – 0.024)	0.016 (0.013 – 0.020)	0.014 (0.006 – 0.024)
E_rC_{50}	Test item	3.814 (2.201 – 10.883)	2.815 (1.755 – 5.916)	2.224 (1.886 – 2.685)	1.444 (0.961 – 2.499)
	Active substance	0.381 (0.220 – 1.086)	0.281 (0.175 – 0.590)	0.222 (0.188 – 0.268)	0.144 (0.096 – 0.249)
NOEC	Test item	0.253	0.081	≥4.480	0.144
	Active substance	0.025	0.008	-	0.014

*95% confidence limit; n.d – not determined

(continued) Growth rate endpoint values and active substance content based on the **geometric mean** concentrations [mg/L].

Endpoint values	mg/L	Frond number			Dry weight
		2 d	4 d	7 d	7 d
LOEC	Test item	0.454	0.144	>4.480	0.253
	Active substance	0.045	0.014	-	0.025

Yield endpoints values and active substance contents based on the **nominal** test item concentrations [mg/L].

Endpoint values	mg/L	Frond number			Dry weight
		2d	4d	7d	7 d
E_yC_{10}	Test item	0.056 (0.007 – 0.147)*	n.d	n.d	n.d
	Active substance	0.006 (0.001 – 0.015)	-	-	-
E_yC_{20}	Test item	0.222 (0.062 – 0.418)	0.041 (0.013 – 0.081)	0.027 (0.017 – 0.038)	0.023 (0.006 – 0.049)
	Active substance	0.022 (0.006 – 0.042)	0.004 (0.001 – 0.008)	0.003 (0.002 – 0.004)	0.002 (0.001 – 0.005)
E_yC_{50}	Test item	2.325 (1.415 – 5.032)	0.595 (0.402 – 0.885)	0.277 (0.230 – 0.328)	0.208 (0.118 – 0.328)
	Active substance	0.232 (0.141 – 0.502)	0.059 (0.040 – 0.088)	0.028 (0.023 – 0.033)	0.021 (0.012 – 0.033)
NOEC	Test item	1.800	n.d	0.100	0.180
	Active substance	0.180	-	0.010	0.018
LOEC	Test item	3.200	n.d	0.180	0.320
	Active substance	0.319	-	0.018	0.032

*95% confidence limit; n.d – not determined

Yield endpoint values and active substance content based on the **geometric mean** concentrations [mg/L].

Endpoint values	mg/L	Frond number			Dry weight
		2 d	4 d	7 d	7 d
E_yC_{10}	Test item	0.046 (0.006 – 0.119)*	n.d	n.d	n.d
	Active substance	0.005 (0.001 – 0.012)	-	-	-
E_yC_{20}	Test item	0.181 (0.053 – 0.338)	0.034 (0.011 – 0.066)	n.d	0.040 (0.010 – 0.079)
	Active substance	0.018 (0.005 – 0.034)	0.003 (0.001 – 0.007)	-	0.004 (0.001 – 0.008)
E_yC_{50}	Test item	1.855 (1.138 – 3.942)	0.481 (0.326 – 0.712)	0.225 (0.188 – 0.265)	0.239 (0.140 – 0.377)
	Active substance	0.185 (0.114 – 0.393)	0.048 (0.033 – 0.071)	0.022 (0.019 – 0.026)	0.024 (0.014 – 0.038)
NOEC	Test item	1.395	n.d	0.081	0.144
	Active substance	0.139	-	0.008	0.014
LOEC	Test item	2.601	n.d	0.144	0.253
	Active substance	0.260	-	0.014	0.025

*95% confidence limit; n.d. – not determined

C. VALIDITY CRITERIA

~~For the test to be valid, the following performance criteria specified in OECD Guideline No. 202 (2004) were met for both experimental parts:~~

- ~~In the control 0% of daphnids were immobilized (criterium: not more than 10%);~~
- ~~The concentration of dissolved oxygen in the test and control vessels was ≥ 3 mg/L at the end of the test.~~

A.2.2.1.4 Study 4

Comments of zRMS:	The study was conducted to OECD guideline 201 and according to the principles of GLP. In the definitive test the validity criteria were met. The measured concentrations of the test item varied between 92.0% and 108.6% of the nominal concentration during the experiment. The study is considered to be reliable and suitable for the risk assessment. All results refer to nominal concentrations. Deviation from the study: 1. Inoculated test vessels should be placed in a phytotron chamber. Instead of it, the test were conducted in appropriately prepared test room, where conditions were maintained within the required range. Conditions were checked before starting of the study using a temperature register, and lighting was checked using a lux meter. 2. During the experiment, according to the Study Plan, temperature should be measured be the means of the phytotron chamber. Instead, the temperature was measured by temperature recorder, because the experiment was carried in the test room, without using phytotron chamber.

3. Temperature during experiment rose above 24ºC for one hour (between May 10, 2022, 10:50 and May 10, 2022, 11:50).
4. Temperature dropped below 15°C in the cabinet in with test item was stored on March 31, 2022 between 1321-1324.

However, above deviation did not have any impact on study results, as the validity criteria were met.

The validity criteria:
• the biomass in the control increased by a factor of 60.4 within the 72-hour test period (criterion: at least a 16-fold growth).
• the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation - exposure termination) in the control culture was 1.6% (criterion: it must not exceed 10%).
• the mean coefficient of variation for the section-by-section growth rate in the control culture was 29.1% (criterion: it must not exceed 35%).

The method is acceptable in section B5 in dRR of Juzan Extra 100 SC.
Agreed endpoints:

Growth rate endpoint values based on the nominal test item concentrations with 95% confidence limits.

Endpoint value	[mg/L]	Time		
		24h	48h	72h
E_rC_{10}	Test item	0.321 (0.097 – 0.698)	2.634 (0.315 – 7.691)	8.911 (1.763 – 20.546)
	Active substance	0.032 (0.010 – 0.070)	0.263 (0.031 – 0.768)	0.889 (0.176 – 2.051)
E_rC_{20}	Test item	1.055 (0.441 – 1.898)	9.915 (2.340 – 21.959)	24.541 (7.991 – 43.084)
	Active substance	0.105 (0.044 – 0.189)	0.990 (0.234 – 2.192)	2.449 (0.798 – 4.600)
E_rC_{50}	Test item	10.281 (6.813 – 15.108)	125.201 (66.408 – 266.335)	170.448 (102.169 – 304.708)
	Active substance	1.026 (0.680 – 1.508)	12.496 (6.628 – 26.582)	17.012 (10.197 – 30.412)
LOEC	Test item	≤1.000	≤1.000	3.200
	Active substance	n.d	n.d	0.319
NOEC	Test item	<1.000	<1.000	1.000
	Active substance	n.d	n.d	0.100

n.d – not determined

Reference: KCP 10.2.1

Report: Juzan Extra 100 SC – *Freshwater Alga and Cyanobacetria*, Growth Inhibition Test, Sonia Szlauer, 2022, STUDY CODE: EMI/4/2/2021, Ecomelius Institute sp. z o. o.

Guideline(s): Yes. According to the OECD Guideline No. 201 (2011f)

Deviations: Yes*

GLP: Yes

Acceptability: Yes

*

For analytical method validation see section B5 of dRR

Materials and methods

A. MATERIALS

1. Test material

Lot/Batch #	1/2021
Appearance:	Beige to brown suspension
Content of active substance	Mesotrione: 103.8 g/L
Density:	1.040 g/mL
Expiry date	09.02.2023

2. Test organism

Species	non-green alga Navicula pelliculosa
Source	Culture Collection of Algae and protozoa (CCAP) Scottish Marine Institute
Culturing	At 21 - 24°C under constant illumination (4400 – 8800 lux) in AAP medium.
Acclimation period	Culturing was done under test conditions.
Test units	250 mL Erlenmeyer flasks

3. Environmental conditions

Test water

The component	Concentration in the AAP medium	
	mg/L	mM
$NaHCO_3$	15.0	0.179
$NaNO_3$	25.5	0.300
$MgCl_2{*}6(H_2O)$	12.16	0.0598
$CaCl_2 * 2(H_2O)$	4.41	0.0300
$MgSO_4 * 7(H_2O)$	14.6	0.0592
K_2HPO_4	1.044	0.00599
$FeCl_3 * 6(H_2O)$	0.160	0.000591
$Na_2EDTA * 2(H_2O)$	0.300	0.000806
H_3BO_3	0.186	0.00300
$MnCl_2 * 4(H_2O)$	0.415	0.00201
$ZnCl_2$	0.00327	0.000024
$CoCl_2 * 6(H_2O)$	0.00143	0.000006
$Na_2MoO_4{*}2(H_2O)$	0.00726	0.000030
$CuCl_2 * 2(H_2O)$	0.000012	0.00000007

Water temperature	Temperature in incubator was maintained between: 24 and 26°C, not varied more than ±2°C
Lighting	4400 – 8800 lux (not varied more than 15 %)

B. STUDY DESIGNS AND METHODS

1. Experimental conditions

Test design

The impact of M-100SC-OR2-C on non-green alga species *Navicula pelliculosa* was investigated during a 72-hour toxicity study. There were prepared 7 solutions and 3 replicates for each concentrations. The control samples were prepared in 6 test vessels. Algae from the culture were added to vessels in quantity of 1×10^4 cells/mL. During the test, the cell concentrations were determined after 24, 48 and 72 h in samples taken directl form the test vessels.

Concentrations tested

Seven exposure concentrations of 1.0 mg/L, 3.2 mg/L, 10.0 mg/L, 32.0 mg/L, 100.0 mg/L, 320.0 mg/L and 1000.0 mg/L in three replicates and one control in six replicates.

Analytics

The concentration of Mesotrione in AAP medium was determined using a validated ultrahigh performance liquid chromatographic method with mass spectrometer detection. The analytical method was validated according to SANTE/2020/12830, Rev. 1

2. Sampling and measurements

The pH was measured on days 0 and 3 in each concentration and control.
The light intensity was measured on days 0 and 3, in different parts of the chamber.
Morphology observations of algae cells were performed at each day of experiment. Any changes in the appearance of the concentrations or morphological changes in the cells of the organisms tested were noticed

3. Calculation of toxicity

The test endpoint is inhibition of growth, expressed as the logarithmic increase in biomass (average specific growth rate) during the exposure period. From the average specific growth rates recorded in a series of test solutions, the concentration bringing about a specified x% inhibition of growth rate (e.g. 50%) is determined and expressed as the ErCx (e.g. ErC50), the toxicity of the test item from algae causing 10% and 20% inhibition of growth rate is determined as the ErC10 and ErC20. The 50% inhibition of yield is determined and expressed as the EYC50 of an algae culture in relation to the control. The ToxRat Professional commercial software was used to make calculations and to conduct statistical analyses.

Results and discussions

A. ANALYTICAL RESULTS

The aim of the analytical part of the experiment was to determine the concentrations of the test item with a liquid chromatographic method with mass spectroscopy.
The analytical method has been validated during the study. A Standard Operating Procedure for the analytical test method has been developed based on the validation carried out during the study. The determination of concentration was performed by the analytical personnel.
Based on the chemical determination it can be concluded, that the concentrations of the test item were prepared correctly (mean recovery at the beginning of the experiment was in the range: 97.6% - 103.5%). At the end of the experiment, the test item concentrations mean recovery was in the range 92.0% – 108.6%. According to the Study Plan, if the deviation from the nominal concentration is within the range ±20%, analysis of the results could be based on the nominal concentrations, therefore the final endpoints were calculated based on the nominal test item concentrations.

Analytical measurements based on SOP/P/85						
Nominal concentration of test item [mg/L]	At exposure initiation			At exposure termination		
	Expected concentration of active substance (mg/L)	Measured concentration of active substance (mg/L)	% of active substance measured based on expected concentration	Expected concentration of active substance (mg/L)	Measured concentration of active substance (mg/L)	% of active substance measured based on expected concentration
Control	<LoQ	<LoQ	-	<LoQ	<LoQ	-
R1 [1.0]	0.10	0.10	102.2	0.10	0.11	108.6
R2 [3.2]	0.32	0.31	97.6	0.32	0.33	104.9
R3 [10.0]	1.0	1.0	102.5	1.0	1.0	103.8
R4 [32.0]	3.2	3.3	102.9	3.2	2.9	92.0
R5 [100.0]	10.0	10.3	103.5	10.0	10.4	104.4
R6 [320.0]	31.9	31.6	99.0	31.9	33.0	103.3
R7 [1000.0]	99.8	98.6	98.8	99.8	105.1	105.3

LoQ= 0.7 µg/L

B. BIOLOGICAL RESULTS

Calculations made on the basis of nominal test item concentrations [mg/L] at the end of the experiment(72 h) with 95% confidence limits.

Endpoint	mg/L	Growth rate	Yield
EC_{50}	Test item	170.448 (102.169 – 304.708)	11.563 (7.864 – 16.728)
	Active substance	17.012 (10.197 – 30.412)	1.154 (0.785 – 1.670)
LOEC	Test item	3.200	≤1.000
	Active substance	0.319	n.d
NOEC	Test item	1.000	<1.000
	Active substance	0.100	n.d

n.d – not determined

C. VALIDITY CRITERIA

The following validity criteria specified in OECD Guideline No. 201 (2006) were met:

- the biomass in the control increased by a factor of 254.3 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 1.1% (criterion: it must not exceed 7%).
- the mean coefficient of variation for the section-by-section growth rate in the control culture was 31.00% (criterion: it must not exceed 35%).

A.2.2.1.3 Study 5

Comments of zRMS:	The study was conducted to OECD guideline 201 and according to the principles of GLP. In the definitive test the validity criteria were met. At the end of the experiment mean recovery for concentration R10 (180.3 mg/L) of the test item fell below 80% (i.e. 73.9%), whereas remaining concentrations mean recovery were within the range 80% - 120% (i.e. 83.6% - 93.8%). The study is considered to be reliable and suitable for the risk assessment. All results refer to geometric mean concen-

trations.

Deviation from the study:

- ✓ Temperature on 27.01.2022 between 1:42 – 4:19 (max. temperature was 26.5ºC); 4:20-4:22 (max. temperature was 26.1ºC) and 4:24-4:26 a.m. (max. temperature was 26.1ºC) increased above 26ºC;
- ✓ Temperature on 27.01.2022 on 4:29, 4:34 and 4:37 a.m. (max. temperature each time was 26.1ºC) increased above 26ºC;
- ✓ Temperature on 27.01.2022 at 12:18-12:22 (max. temperature each time was 26.3ºC) increased above 26 ºC.
- ✓ Study completion date February 2022 –changed to March 2022.
- ✓ Editorial mistake in the Amendment No. 1 to the Study Plan –page 9 –SOP/P/47 instead of SOP/P/85

However, above deviation did not have any impact on study results, as the validity criteria were met.

The validity criteria:
• the biomass in the control increased by a factor of 254.3 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 1.1% (criterion: it must not exceed 7%).
• the mean coefficient of variation for the section-by-section growth rate in the control culture was 31.00% (criterion: it must not exceed 35%).

The method is acceptable in section B5 in dRR of Juzan Extra 100 SC.
Agreed endpoints:

Growth rate endpoint values based on the geometric mean test item concentrations.

Endpoint value	[mg/L]	Time		
		24h	48h	72h
ErC_{50}	Test item	69.01 (50.69 – 99.68)	141.92 (117.97 – 176.13)	74.71 (69.34 – 80.66)
	Active substance	6.89 (5.06 – 9.95)	14.16 (11.77 – 17.58)	7.46 (6.92 – 8.05)
ErC_{20}	Test item	4.94 (2.78 – 7.51)	18.48 (14.20 – 22.93)	19.65 (17.35 – 21.94)
	Active substance	0.49 (0.28 – 0.75)	1.84 (1.42 – 2.29)	1.96 (1.73 – 2.19)
E_rC_{10}	Test item	1.25 (0.52 – 2.29)	6.37 (4.24 – 8.75)	9.78 (8.22 – 11.38)
	Active substance	0.12 (0.05 – 0.23)	0.64 (0.42 – 0.87)	0.98 (0.82 – 1.14)
LOEC	Test item	≤0.90	≤0.90	≤0.90
	Active substance	-	-	-
NOEC	Test item	<0.90	<0.90	<0.90
	Active substance	-	-	-

Yield endpoint values based on the geometric mean test item concentrations.

Endpoint value	[mg/L]	Time		
		24h	48h	72h
ErC_{50}	Test item	23.10 (16.79 – 32.28)	16.51 (14.04 – 19.40)	10.65 (9.55 – 11.86)
	Active substance	2.31 (1.68 – 3.22)	1.65 (1.40 – 1.94)	1.06 (0.95 – 1.18)
ErC_{20}	Test item	1.38 (0.66 – 2.33)	1.77 (1.28 – 2.30)	2.95 (2.45 – 3.46)
	Active substance	0.14 (0.07 – 0.23)	0.18 (0.13 – 0.23)	0.29 (0.24 – 0.35)
E_rC_{10}	Test item	n.d	0.55 (0.35 – 0.79)	1.51 (1.18 – 1.86)
	Active substance	-	0.05 (0.04 – 0.08)	0.15 (0.12 – 0.19)
LOEC	Test item	≤0.90	≤0.90	≤0.90
	Active substance	-	-	-
NOEC	Test item	<0.90	<0.90	<0.90
	Active substance	-	-	-

Reference: KCP 10.2.1

Report: Juzan Extra 100 SC – *Freshwater Alga and Cyanobacetria*, Growth Inhibition Test, Sonia Szlauer, 2022, STUDY CODE: EMI/4/3/2021, Ecomelius Institute sp. z o. o.

Guideline(s): Yes. According to the OECD Guideline No. 201 (2011f)

Deviations: Yes

GLP: Yes

Acceptability: Yes

For analytical method validation see section B5 of dRR

Materials and methods

A. MATERIALS

1. Test material

Lot/Batch # 1/2021
Appearance: Beige to brown suspension
Content of active substance Mesotrione: 103.8 g/L
Density: 1.040 g/mL
Expiry date 09.02.2023

2. Test organism

Species Green alga *Raphidocelis subcapitata*
Source Georg-August-Universität Göttingen Stiftungöffentlichen Rechts
Culturing At 21 - 24°C under constant illumination (4400 – 8800 lux) in AAP medium.
Acclimation period Culturing was done under test conditions.
Test units 250 mL Erlenmeyer flasks

3. Environmental conditions

Test water

The component	Concentration in the AAP medium	
	mg/L	mM
$NaHCO_3$	15.0	0.179
$NaNO_3$	25.5	0.300
$MgCl_2{*}6(H_2O)$	12.16	0.0598
$CaCl_2 * 2(H_2O)$	4.41	0.0300
$MgSO_4 * 7(H_2O)$	14.6	0.0592
K_2HPO_4	1.044	0.00599
$FeCl_3 * 6(H_2O)$	0.160	0.000591
$Na_2EDTA * 2(H_2O)$	0.300	0.000806
H_3BO_3	0.186	0.00300
$MnCl_2 * 4(H_2O)$	0.415	0.00201
$ZnCl_2$	0.00327	0.000024
$CoCl_2 * 6(H_2O)$	0.00143	0.000006
$Na_2MoO_4{*}2(H_2O)$	0.00726	0.000030
$CuCl_2 * 2(H_2O)$	0.000012	0.00000007

Water temperature Temperature in incubator was maintained between: 24 and 26°C, not varied more than ±2°C

Lighting 4400 – 8800 lux (not varied more than 15 %)

B. STUDY DESIGNS AND METHODS

1. Experimental conditions

Test design

The impact of M-100SC-OR2-C on Green freshwater alga species *Raphidocelis subcapitata* was investigated during a 72-hour toxicity study. There were prepared 11 solutions and 3 replicates for each concentrations. The control samples were prepared in 6 test vessels. Algae from the culture were added to vessels in quantity of 1×10^4 cells/mL. During the test, the cell concentrations were determined after 24, 48 and 72 h in samples taken directl form the test vessels.

Concentrations tested

Eleven exposure concentrations of 1.0 mg/L, 1.8 mg/L, 3.2 mg/L, 5.6 mg/L, 10.0 mg/L, 18.0 mg/L. 32.1 mg/L, 56.1 mg/L, 100.2 mg/L, 180.3 mg/L, 320.6 mg/L in three replicates and one control in six replicates.

Analytics

The concentration of Mesotrione in AAP medium was determined using a validated ultrahigh performance liquid chromatographic method with mass spectrometer detection. The analytical method was validated according to SANTE/2020/12830, Rev. 1

2. Sampling and measurements

The pH was measured on days 0 and 3 in each concentration and control.
The light intensity was measured on days 0 and 3, in different parts of the chamber.
Morphology observations of algae cells were performed at each day of experiment. Any changes in the appearance of the concentrations or morphological changes in the cells of the organisms tested were noticed

3. Calculation of toxicity

The test endpoint is inhibition of growth, expressed as the logarithmic increase in biomass (average specific growth rate) during the exposure period. From the average specific growth rates recorded in a series of test solutions, the concentration bringing about a specified x% inhibition of growth rate (e.g. 50%) is determined and expressed as the ErCx (e.g. ErC50), the toxicity of the test item from algae causing 10% and 20% inhibition of growth rate is determined as the ErC10 and ErC20. The 50% inhibition of yield is determined and expressed as the EYC50 of an algae culture in relation to the control. The ToxRat Professional commercial software was used to make calculations and to conduct statistical analyses.

Results and discussions

A. ANALYTICAL RESULTS

The aim of the analytical part of the experiment was to determine the concentrations of the test item with a liquid chromatographic method with mass spectroscopy.
The analytical method has been validated during the study. A Standard Operating Procedure for the analytical test method has been developed based on the validation carried out during the study. The determination of concentration was performed by the analytical personnel.
Based on the chemical determination it can be concluded, that the concentrations of the test item were prepared correctly (mean recovery at the beginning of the experiment was in the range: 89.7% - 104.7%). At the end of the experiment mean recovery for concentration R10 (180.3 mg/L) of the test item fell below 80% (i.e. 73.9%), whereas remaining concentrations mean recovery were within the range 80% - 120% (i.e. 83.6% - 93.8%). According to the Study Plan, if the deviation from the nominal concentration is not within the range ±20%, analysis of the results should be based on the geometric mean concentration during exposure. The final endpoints were calculated based on the nominal test item concentrations and geometric mean concentrations.

Analytical measurements based on SOP/P/85						
Nominal concentration of test item [mg/L]	At exposure initiation			At exposure termination		
	Expected concentration of active substance (mg/L)	Measured concentration of active substance (mg/L)	% of active substance measured based on expected concentration	Expected concentration of active substance (mg/L)	Measured concentration of active substance (mg/L)	% of active substance measured based on expected concentration
Control	<LoQ	<LoQ	-	<LoQ	<LoQ	-
R1 [1.0]	0.10	0.09	89.7	0.10	0.09	87.9
R2 [1.8]	0.18	0.19	104.7	0.18	0.16	91.6
R3 [3.2]	0.32	0.30	92.6	0.32	0.29	90.9
R4 [5.6]	0.56	0.51	90.2	0.56	0.52	93.1
R5 [10.0]	1.00	0.95	94.9	1.00	0.91	91.2
R6 [18.0]	1.80	1.73	95.4	1.80	1.70	92.8
R7 [32.1]	3.20	3.10	97.1	3.20	2.83	88.0
R8 [56.1]	5.60	5.23	93.5	5.60	5.23	93.8
R9 [100.2]	10.00	9.63	96.4	10.00	9.13	91.2
R10 [180.3]	18.00	17.23	95.8	18.00	13.30	73.9
R11 [320.6]	32.00	30.97	96.8	32.00	26.77	83.6

LoQ= 0.7 µg/L

B. BIOLOGICAL RESULTS

Calculations made on the basis of the geometric mean test item concentrations [mg/L] at the end of the experiment.

Endpoint	mg/L	Growth rate	Yield
EC_{50}	Test item	74.71 (69.34 – 80.66)	10.65 (9.55 – 11.86)
	Active substance	7.46 (6.92 – 8.05)	1.06 (0.95 – 1.18)
NOEC	Test item	<0.9	<0.9
	Active substance	-	-
LOEC	Test item	≤0.9	≤0.9
	Active substance	-	-

C. VALIDITY CRITERIA

The following validity criteria specified in OECD Guideline No. 201 (2006) were met:

- the biomass in the control increased by a factor of 254.3 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 1.1% (criterion: it must not exceed 7%).
  the mean coefficient of variation for the section-by-section growth rate in the control culture was 31.00% (criterion: it must not exceed 35%).

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:	Study was carried out according to appropriate OECD 213 (1998) and all validity criteria were met. Deviation from the study: none. The validity criteria: • 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 0.29 µg a.i./bee (criterion: 0.10 – 0.30 µg a.i./bee). **Agreed endpoints:** The median lethal doses LD_{50}/24h and LD_{50}/48h are higher than 200.0 µg formulation/honeybee (19.35 µg s.a./honeybee). **The study is considered acceptable.**

Reference:	KCP 10.3.1.1/01
Report	M-100SC-OR2-C Honeybees (*Apis mellifera* L.), Acute Oral Toxicity Test, Knapik M., 2020, B-86-20
Guideline(s):	OECD 213
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and Methods

Test item:	M-100SC-OR2-C content: 101.1 g/L of mezotrione batch no.: 1/2020 production date: 03.06.2020 expiry date: 03.06.2022
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 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 and a control (0.0 µg/bee)
Test conditions:	
– temperature :	24 − 26°C (required: 25 ± 2°C)
– relative air humidity:	54 − 56% (required: 50 – 70%)
Photoperiod:	24h darkness, except during application and assessments
Statistical analysis:	regression analysis using the log-probit method (ToxRat Proffesional software, version 3.3.0 [9],
Endpoints:	– honeybee mortality after 24 and 48 hours of the exposure, – the oral LD50/24 h and LD50/48 h of the test item, – the oral LD50/24 h of the reference item (dimethoate).

Test design:
The study was divided into a preliminary non-GLP range-finding test and a definitive test. The preliminary non-GLP test was conducted to determine the range of doses to be used in the definitive test.
In the preliminary test the doses of 8.0, 40.0 and 200.0 µg/bee (with a separation factor of 5) and the control were used. There was one replicate of each dose containing 10 bees.

In the definitive test, five doses of the test item i.e.: 12.5, 25.0, 50.0, 100.0 and 200.0 µg/bee (1.21, 2.42, 4.84, 9.67 and 19.35 µg a.s./bee) were used (with a separation factor of 2) plus the control.
In the definitive test, three doses of the reference item, Bi 58 Top 400 EC were used. These were 0.1, 0.2, and 0.4 µg a.i./bee (with a separation factor of 2). The doses included the one expected to be the LD50 value mentioned in the OECD Guideline for the Testing of Chemicals No. 213. On average, each insect from a test item group received 12.5, 25.0, 50.0, 100.0 and 200.0 µg M-100SC-OR2-C in 10 µL of the 50% sucrose solution. While each insect from the reference item group received 0.1, 0.2 or 0.4 µg of the dimethoate in 10 µL of the 50% sucrose solution.
In the definitive test, there were three replicates of each dose of the test item, reference item and the control containing 10 bees.

Results:

After 4 hours of exposure, mortality of the control group was 0.0% and for the treated groups' mortality percentages at the doses of 12.5, 25.0, 50.0, 100.0 and 200.0 µg/bee (1.21, 2.42, 4.84, 9.67 and 19.35 µg a.s./bee), were 0.0, 0.0, 0.0, 0.0 and 6.7%, respectively.
After 24 hours of exposure, mortality of the control group was 3.3% and for the treated groups' mortality percentages (corrected using the formula of Abbott) at the doses of 12.5, 25.0, 50.0, 100.0 and 200.0 µg/bee (1.21, 2.42, 4.84, 9.67 and 19.35 µg a.s./bee), were -3.5, 3.5, -3.5, -3.5 and 3.5%, respectively. The negative value indicates lower mortality in group treated with the test item compared to the control group.
After 48 hours of exposure, mortality of the control group was 3.3% and for the treated groups' mortality percentages (corrected using the formula of Abbott) at the doses of 12.5, 25.0, 50.0, 100.0 and 200.0 µg/bee (1.21, 2.42, 4.84, 9.67 and 19.35 µg a.s./bee), were 6.9, 6.9, 0.0, 3.5 and 6.9%, respectively.
The median lethal dose LD_{50}/24 h, LD_{50}/48 h are higher than 200.0 µg/honeybee of the test item.
No abnormal behavioral effects were observed during the test.

Dose [µg/bee]	Dose [µg a.s./bee]	Number of tested bees [no.]	Mortality after 48h after the beginning of the treatment			LD_{50} [µg/bee]	LD_{50} [µg a.s./bee]
			Total				
			[no.]	[%]	[%][c]		
0.0	**0.0**	30	1	3.3	-	**>200.0***	**>19.35**
12.5	**1.21**	30	3	10.0	6.9		
25.0	**2.42**	30	3	10.0	6.9		
50.0	**4.84**	30	1	3.3	0.0		
100.0	**9.67**	30	2	6.7	3.5		
200.0	**19.35**	30	3	10.0	6.9		

[c]: mortality corrected according formula of Abbott's [6]

*: oral LD_{50} value was estimated with the log-probit method (ToxRat Proffesional 3.3.0 computer software), [9]

Conclusion:

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 (19.35 µg a.s./honeybee).

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

Comments of zRMS:	Study was carried out according to appropriate OECD 214 (1998) and all validity criteria were met. Deviation from the study: The validity criteria: • the mortality for the control was 0.0% after 48 h (criterion: it must not exceed 10.0%), • the LD_{50}/24 h of the reference item (dimethoate) was 0.19 µg a.i./bee (criterion: 0.10 – 0.30 µg a.i./bee). **Agreed endpoints:** The median lethal doses LD_{50}/24h and LD_{50}/48h are higher than 200.0 µg formula-

	tion/honeybee. **The study is considered acceptable.**

Reference:	KCP 10.3.1.1/02
Report	M-100SC-OR2-C Honeybees (Apis mellifera L.), Acute Contact Toxicity Test, Knapik M., 2020, B-87-20
Guideline(s):	OECD 213
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods:

Test item:	M-100SC-OR2-C content: 101.1 g/L of mezotrione batch no.: 1/2020 production date: 03.06.2020 expiry date: 03.06.2022
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 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 and a control (0.0 µg/bee)
Test conditions:	
– temperature:	24 − 25°C (required: 25 ± 2°C)
– relative air humidity:	57 − 61% (required: 50 – 70%)
16 hours light : 8 hours dark	
Place:	Dark room
Statistical analysis:	regression analysis using the log-probit method (ToxRat Proffesional software, version 3.3.0)
Endpoints:	– honeybee mortality after 24 and 48 hours of the exposure, – the contact LD50 of the test item after 24 and 48 hours of the exposure, – the contact LD50/24 h of the reference item (dimethoate).

Test design:
In the preliminary non-GLP range-finding test the doses of 8.0, 40.0 and 200.0 µg/bee (with a separation factor of 5) and the control were used. There was one replicate of each dose containing 10 bees.

In the definitive test, five doses of the test item i.e.: 12.5, 25.0, 50.0, 100.0 and 200.0 µg/bee (1.21, 2.42, 4.84, 9.67 and 19.35 µg a.s./bee) were used (with a separation factor of 2) plus the control.
In the definitive test, three doses of the reference item, Bi 58 Top 400 EC were used. These were 0.1, 0.2, and 0.4 µg a.i./bee (with a separation factor of 2). The doses included the one expected to be the LD50 value mentioned in the OECD Guideline for the Testing of Chemicals No. 214. On average, each insect from a test item group received 12.5, 25.0, 50.0, 100.0 and 200.0 µg of M-100SC-OR2-C in 1 µL the solution was applied to the dorsal part of the thorax of each bee. While each insect from the reference item group received 0.1, 0.2 or 0.4 µg of the dimethoate in 1 µL the solution was applied to the dorsal part of the thorax of each bee.
Results:
Mortality in the definitive test of the control group after 4, 24 and 48 hours of the test were 0.0%.
After 4, 24 and 48 hours the percentages of mortality of the bees treated with the test item at the doses of 12.5, 25.0, 50.0, 100.0 and 200.0 µg/honeybee were 0.0%.
The median lethal doses (LD_{50}/24 h and LD_{50}/48 h contact) are ≥ 200.0 µg/honeybee. No abnormal behavioural effects were observed during the test.

Dose [µg/bee]	Dose [µg a.s./bee]	Number of tested bees [no.]	Mortality after 96 h after the beginning of the treatment		LD_{50} [µg/bee]	LD_{50} [µg a.s./bee]
			Total			
			[no.]	[%]		
0.0 (control)	**0.0**	30	0	**0.0**	**≥200.0**	**>19.35**
12.5	**1.21**	30	0	**0.0**		
25.0	**2.42**	30	0	**0.0**		
50.0	**4.84**	30	0	**0.0**		
100.0	**9.67**	30	0	**0.0**		
200.0	**19.35**	30	0	**0.0**		

Conclusion:
The median lethal doses LD50/24 h and LD50/48 h is higher than the highest dose used in the test i.e. 200.0 µg/honeybee (19.35 µg a.s./honeybee).

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

Comments of zRMS:	Study was carried out according to appropriate OECD 245 and all validity criteria were met. Deviation from the study: none The validity criteria: • At the end of the experiment average mortality of the control groups was 0.0% (criterion: it must not exceed 15%). • mean mortality of bees in the reference test: 73.3% at the end of the test (after 10 days) (required: ≥50%). **Agreed endpoints:** In the course of this test, the tested item did not affect the mortality of bees after 10 days of the test in the concentration range of 64 mg/kg of food-2500 mg/kg of food. The LOEC and NOEC values were determined as above the highest tested concentration of the tested item taken in the test, i.e. 2500 mg of test item/kg of food. The LOEDD and NOEDD values were determined to be above the highest tested dose of test item taken in the study, i.e. 58.248 µg of test item/bee/day. The values of LC_{10}, LC_{20}, LC_{50} have not been determined, they are outside the range of concentrations used in the study 64 mg of the test item/kg food - 2500 mg of the test item/kg food. LDD_{10}, LDD_{20}, LDD_{50} values have not been determined, they are outside the dose range of 1,338 µg of the test item/bee/day - 58.248 µg of the test item/bee/day.

	The study is considered acceptable. NOED >58.248 µg forulation/bee/day

Reference:	KCP 10.3.1.2/01
Report	Honey bee chronic oral toxicity test according to OECD 245 guideline, Woźniak A., 2020, 0016/0093/E
Guideline(s):	OECD 245
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods:

Test design

stability test:
- tested concentrations and control in one repetition

range-finding, definitive and reference test:
-tested concentrations and control in three repetitions, 10 bees per repetition

Test cages

stability test:
- storage conditions: plastic containers of 100 mL volume
-test conditions: plastic syringe of 2 mL volume

range-finding, definitive and reference test:
- cages 20 cm × 20 cm × 20 cm

Duration time

stability test:
- storage conditions: 72 hours
- test conditions: 24 hours

range-finding, definitive and reference test:
- 10 days

Tested concentrations

stability test:
- control; 2.5 mg/kg of food; 2500 mg/kg of food

range-finding test:
- control; 2.5 mg/kg of food; 25 mg/kg of food; 250 mg/kg of food; 2500 mg/kg of food

definitive test:
- control;
- 64.0 mg/kg of food
the dose of the tested item taken by bees: 1.338 µg/bee/day
- 160.0 mg/kg of food
the dose of the tested item taken by bees: 3,611 µg/bee/day
- 400.0 mg/kg of food
the dose of the tested item taken by bees:10.234 µg/bee/day
- 1000.0 mg/kg of food
the dose of the tested item taken by bees: 24.232 µg/bee/day
- 2500 mg/kg of food
the dose of the tested item taken by bees: 58.248 µg/bee/day
reference test:
- control; dimethoate: 0.5 mg/kg of food

Study conditions

stability test:
- storage conditions: average temperature 5.144°C (minimal tem-

perature 4.50°C; maximal temperature 6.70°C); darkness
- test conditions: average temperature 34.912°C (minimal temperature 34.6°C; maximal temperature 35.40°C); average humidity: 79.600% (minimal humidity 79.10%; maximal humidity 80.10%); darkness

range-finding test:
-average temperature 33.864°C (minimal temperature 32.80°C; maximal temperature 35.10°C); average humidity: 80.833% (minimal humidity 71.40%; maximal humidity 91.20%); darkness

definitive test and reference test:
- average temperature 34.922°C (minimal temperature 33.70°C; maximal temperature 35.80°C); average humidity: 79.419% (minimal humidity 51.40%; maximal humidity 93.20%); darkness

Test item

Name	M-100SC-OR2-C
Test item description	a beige to brown suspension with a faint odor
Packaging material appearance	HDPE container
Date of delivery	10.06.2020
Batch No.	1/2020
Name of active substance	mesotrione
Content of active substance	101,10 g/L; purity 920 g/kg
CAS of active substance	104206-82-8
Production date	03.06.2020
Expiration date	03.06.2022
Solubility in water or other solvents, ability to form emulsions, solutions, suspensions	the agent disperses in water; solubility in organic solvents (values for mesotrione at 20 ° C): in acetone 93.3 g/L, in xylene 1.6 g/L, in toluene 3.1 g/L, in ethyl acetate 18.6 g/L
Storage conditions	temperature: 0-30°C; <70% RH

The study was carried out on honeybee (*Apis mellifera* L.) coming from a registered breeding. Quarantine of bees was not carried out, because in the month before the start of test insects were not treated with chemical compounds, including antibiotics or anti-varroa treatment. The study used young honeybees (2-day-old) derived from healthy, well-maintained breeding. The bees were placed in the test room under the conditions of the experiment one day before the experiment.
The weight of the test item was dissolved in a 50% aqueous sucrose solution (w/v). Next, final concentrations of the test item were prepared with dilution method in 50% sucrose solution. In the course of the range-finding test, solutions of the test item were prepared daily during the course of the test. During the definitive test, fresh test and reference substance solutions were prepared on day 1, 5 and 8 of the test. The test solutions were stored in a closed vessel in the dark at a temperature of 6±2°C.

Definitive test

The definitive test was performed for control and five concentrations of test item:
- 64.0 mg/kg of food; the dose of the tested item taken by bees: 1.338 µg/bee/day
- 160.0 mg/kg of food; the dose of the tested item taken by bees: 3,611 µg/bee/day
- 400.0 mg/kg of food; the dose of the tested item taken by bees:10.234 µg/bee/day
- 1000.0 mg/kg of food; the dose of the tested item taken by bees: 24.232 µg/bee/day
- 2500 mg/kg of food; the dose of the tested item taken by bees: 58.248 µg/bee/day.

Each concentration and control were prepared in three repetitions, 10 bees per repetition.
Feeders were changed daily, every 24±2 hours.

In the course of the test, the amount of food consumed by the bees was determined daily by weighing the feeders before and after feeding. Then the amount of food consumed was corrected by the amount of evaporated solution. For this purpose, an additional feeder (previously weighed) filled with food was placed in an empty cage under the test conditions (three replicates were used). During the daily feeder replacement, it was reweighed and replaced with a new feeder.

Final results:

In the course of this test, the tested item did not affect the mortality of bees after 10 days of the test in the concentration range of 64 mg/kg of food-2500 mg/kg of food.
The LOEC and NOEC values were determined as above the highest tested concentration of the tested item taken in the test, i.e. 2500 mg of test item/kg of food. The LOEDD and NOEDD values were determined to be above the highest tested dose of test item taken in the study, i.e. 58.248 µg of test item/bee/day. The values of LC_{10}, LC_{20}, LC_{50} have not been determined, they are outside the range of concentrations used in the study 64 mg of the test item/kg food - 2500 mg of the test item/kg food. LDD_{10}, LDD_{20}, LDD_{50} values have not been determined, they are outside the dose range of 1,338 µg of the test item/bee/day - 58.248 µg of the test item/bee/day.

Mortality of the bees – range-finding test

Test item concentration [mg/kg of food]	*)	1 day		2 day		3 day		4 day		5 day		6 day		7 day		8 day		9 day		10 day	
		Dead individuals [pcs.]	Symptoms of intoxication **)	Dead individuals [pcs.]	Symptoms of intoxication **)	Dead individuals [pcs.]	Symptoms of intoxication **)	Dead individuals [pcs.]	Symptoms of intoxication **)	Dead individuals [pcs.]	Symptoms of intoxication **)	Dead individuals [pcs.]	Symptoms of intoxication **)	Dead individuals [pcs.]	Symptoms of intoxication **)	Dead individuals [pcs.]	Symptoms of intoxication **)	Dead individuals [pcs.]	Symptoms of intoxication **)	Dead individuals [pcs.]	Symptoms of intoxication **)
Control	1	0	none	0	none	0	none	0	none	0	none	0	none	1	none	1	none	1	none	1	none
	2	0		0		0		0		0		0		0		0		0		0	
	3	0		0		0		0		0		1		1		1		1		1	
2.5	1	0	none	0	none	0	none	0	none	0	none	0	none	0	v	0	v	0	v	0	v
	2	0		0		0		0		0		0		0		1		1		1	
	3	0		0		0		0		0		0		0		0		0		0	
25.0	1	0	none	0	none	0	none	0	none	0	none	0	none	0	v	0	v	0	v	0	v
	2	0		0		0		0		0		2		2		2		2		2	
	3	0		0		0		0		0		0		0		0		0		0	
250.0	1	0	none	0	none	0	none	0	none	0	none	0	v	2	v	2	v	2	v	3	v; a
	2	0		0		0		0		0		0		0		0		2		2	
	3	0		0		0		0		0		0		0		0		0		1	
2500.0	1	0	none	0	none	0	none	0	none	0	none	0	v	0	v	0	v	0	v; a	0	v; a
	2	0		0		0		0		0		0		0		0		0		0	
	3	0		0		0		0		1		1		1		3		3		3	

*) repetition

**) v (vomiting) - vomiting; a (affected) - visible impact (bees are standing, trying to walk, there are signs of decreased coordination; overactivity; aggressiveness; increased self-cleaning behavior; rotations; shivering); the markings are further explained in clause 4.2.3.3..

Final bee mortality results – definitive test

Test item concentration [mg/kg of food]	Introduced individuals [pcs.]	Daead individuals [pcs.]	Mortality [%]	Statistical significance*)
control	30	0	0.0	not applicable
64	30	1	3.3	-
160	30	2	6.7	-
400	30	3	10.0	-
1000	30	3	10.0	-
2500	30	2	6.7	-

- not statistically significant

*) values calculated by ToxRat Professional using Fisher's test after Bonferroni correction at significance level p> 0.05

Food consumption by bees – definitive test

Test item concentration [mg/kg of food]	Average daily food consumption [mg/bee/day]	Average daily consumption of the tested item [µg/bee/day]	Average consumption of the tested item [µg/bee]
Control	26.09	0	0.0000
64	20.91	1.338	13.3823
160	22.57	3.611	36.1099
400	25.59	10.234	102.3402
1000	24.23	24.232	242.3186
2500	23.30	58.248	582.4840

Bees mortality – reference test

Reference item concentration [mg/kg of food]	p*)	1 day		2 day		3 day		4 day		5 day		6 day		7 day		8 day		9 day		10 day	
		Dead individuals [pcs.]	Symptoms of intoxication **)	Dead individuals [pcs.]	Symptoms of intoxication **)	Dead individuals [pcs.]	Symptoms of intoxication **)	Dead individuals [pcs.]	Symptoms of intoxication **)	Dead individuals [pcs.]	Symptoms of intoxication **)	Dead individuals [pcs.]	Symptoms of intoxication **)	Dead individuals [pcs.]	Symptoms of intoxication **)	Dead individuals [pcs.]	Symptoms of intoxication **)	Dead individuals [pcs.]	Symptoms of intoxication **)	Dead individuals [pcs.]	Symptoms of intoxication **)
Control	1	0	none	0	none	0	none	0	none	0	none	0	none	0	none	0	none	0	none	0	none
	2	0		0		0		0		0		0		0		0		0		0	
	3	0		0		0		0		0		0		0		0		0		0	
0.5	1	0	none	0	none	0	none	0	none	0	none	2	a	4	a	4	a	6	a	6	a
	2	0		0		0		0		0		2		3		6		7		9	
	3	0		0		0		0		0		1		4		5		6		7	

*) repetition

**) a (affected) - visible impact (bees are standing, trying to walk, there are signs of decreased coordination; overactivity; aggressiveness; increased self-cleaning behavior; rotations; shivering); the markings are further explained in clause 4.2.3.3..

Final results calculated using ToxRat Professional			
Parameter	**Concentration [mg of the test item/kg of food]**	**Parameter**	**Dose [µg of the test item/bee/day]**
LC_{10}	n.d.** (n.d. – n.d.)*	**LDD_{10}**	n.d.*** (n.d. – n.d.)*
LC_{20}	n.d.** (n.d. – n.d.)*	**LDD_{20}**	n.d.*** (n.d. – n.d.)*
LC_{50}	n.d.** (n.d. – n.d.)*	**LDD_{50}**	n.d.*** (n.d. – n.d.)*
NOEC	>2500.0	**NOEDD**	>58.248
LOEC	≥2500.0	**LOEDD**	≥58.248

n.d impossible to determine for mathematical reasons
LC_{10} concentration of test item causing mortality in 10% of individuals
LC_{20} concentration of test item causing mortality in 20% of individuals
LC_{50} concentration of the test item causing 50% mortality of individuals
NOEC the highest concentration of the tested item that did not cause statistically significant differences compared to the control
LOEC the lowest concentration of the tested item causing statistically significant differences from the control
LDD_{10} the daily dose of the tested item causing mortality in 10% of individuals
LDD_{20} the daily dose of the tested item causing mortality in 20% of individuals
LDD_{50} the daily dose of the tested item causing mortality in 50% of individuals
NOEDD the highest daily dose of the tested item, showing no statistically significant differences compared to the control
LOEDD the lowest daily dose of the tested item inducing statistically significant differences compared to the control
*) lower and upper 95% confidence interval
**) based on the analysis of the results, the values were determined as >2500.0 mg of the test item/kg of food
***) based on the analysis of the results, the values were determined as >58.248 µg of the test item/bee/day

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

Comments of zRMS:	The chronic study on honeybees larvae is GLP compliant and was conducted in accordance with the current OECD guideline 239. The validity criteria were met: ✓ cumulative larval mortality in control on days 3-8 was 0.0% (required: ≤15%), ✓ the adults emergence rate in control on day 22 was 80.6 % (required: ≥70%), ✓ the adults emergence rate for fenoxycarb as a reference item on day 22 was 13.9

	% (required: ≤20%). Deviations from OECD 239 have been noted: • On day 8 of the test, desiccator with larvae was transferred to test room what poses a deviation from Standard Experimental Procedure and Study Plan. The alteration was introduced due to observable improvement of larvae development outside incubator. • During the range-finding and definitive test were cyclical drops in temperature and humidity. They resulted from daily feedings and observations. These were short-term declines which did not affect the condition of the research system. • Chemical analysis during the definitive test was subjected to the test item solutions only at the beginning of the validity period, due to the conducted stability test, which confirmed the stability of the test item solutions at the end of the validity period. The above deviations did not affect the test result. The study met the validity criteria. **Agreed endpoints:** The tested item in the course of this test did not show any apitoxic effect on larvae mortality on days 3-8 in the concentration range of 16.64 mg/kg food - 650 mg/kg food. The tested item did not show any statistically significant apitoxic effect in the mortality of pupae on days 8-22 in the concentration range of 16.64 mg/kg food - 650 mg/kg food. The tested item did not show any statistically significant apitoxic effect on adult mortality on days 3-22 in the concentration range of 16.64 mg of the test item/kg food - 650 mg of test item/kg food. The values of LC_{10}, LC_{20}, LC_{50} have not been determined, they are outside the range of concentrations used in the test of 16.64 mg of the test item/kg of food - 650 mg of the test item/kg of food. LD_{10}, LD_{20}, LD_{50} values have not been determined, they are outside the range of doses used in the test 0.1 µg of the test item/larvae - 100 µg of the test item/larva. The NOEC value was defined as ≥650.0 mg of the test item/kg food, NOED value ≥100.0 µg of the test item/larvae.

Reference:	KCP 10.3.1.2/02
Report	Chronic toxicity test for honey bee larvae according to OECD GD 239, Woźniak A., 2020, 0016/0091/E
Guideline(s):	OECD GD 239
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods:
The aim of the study was determination of the concentration causing 50% mortality of population (LC_{50} value) and the dose causing mortality of 50% of the population after 22 days (LD_{50} value). Values NOEC and NOED were calculated for development stages of honeybees.

Test design	stability test: - tested concentrations and control in one repetition range-finding test, definitive test and reference test: - tested concentrations and control in one replicate; 36 larvae per replicate
Test cages	stability test: - volumetric flask of 250 mL volume range-finding test, definitive test and reference test: - 48-well breeding plates with queen-cell cups placed in the dissector and placed in incubator; from day 8 dissectors placed in the

	test room; from day 15 of the test – transparent plastic boxes placed in test room
Duration of the test	stability test: - 72 h range-finding, definitive and reference test: - 22 days
Tested concentrations	stability test: - control - 0.05 g/L, corresponding to 0.65 mg/kg of food - 50 g/L, corresponding to 650 mg/kg of food range-finding test: - control - 0.65 mg/kg of food, corresponding to 0.1 µg/larvae - 6.5 mg/kg of food, corresponding to 1.0 µg/larvae - 65 mg/kg of food, corresponding to 10.0 µg/larvae - 650 mg/kg of food, corresponding to 100.0 µg/larvae preliminary reference test: - control - dimethoate - 48 mg/kg of food, corresponding to 7.39 µg/larvae - fenoxycarb - 0.32 mg/kg of food, corresponding to 49.28 ng/larvae definitive test: - control - 40.625 mg/kg of food, corresponding to 6.25 µg/larvae - 81.25 mg/kg of food, corresponding to 12.5 µg/larvae - 162.5 mg/kg of food, corresponding to 25.0 µg/larvae - 325.0 mg/kg of food, corresponding to 50.0 µg/larvae - 650 mg/kg of food, corresponding to 100.0 µg/larvae definitive reference test - control - fenoxycarb - 0.32 mg/kg of food, corresponding to 49.28 ng/larvae
Test conditions	stability test: - average temperature 5.069°C (minimal temperature 4.7°C; maximal temperature 6.2°C); darkness range-finding test and reference test during range-finding test: - for larval stage (day 1-8): average temperature 34.099°C (minimal temperature 32.20°C; maximal temperature 34.30°C); average relative humidity 95.610% (minimal humidity 63.50%; maximal humidity 99.90%) - for pupal stage (day 8-15): average temperature 34.704°C (minimal temperature 33.80°C; maximal temperature 35.20°C); average relative humidity 80.278% (minimal humidity 78.50%; maximal humidity 93.30%) - for imago stage (day 15-22): average temperature 34.421°C (minimal temperature 33.10°C; maximal temperature 35.50°C); average relative humidity 69.453% (minimal humidity 56.40%; maximal humidity 81.20%). all stages were tested in the dark definitive test and reference test during definitive test: - larval stage (day 1-8): average temperature 35.0001°C (minimal temperature 33.60°C; maximal temperature 36.20°C); average

relative humidity 78.920% (minimal humidity 76.80%; maximal humidity 93.20%)
- pupal stage (day 8-15): average temperature 34.107°C (minimal temperature 33.00°C; maximal temperature 34.40°C); average relative humidity 96.489% (minimal humidity 79.10%; maximal humidity 99.90%)
- imago stage (day 15-22): average temperature 34.765°C (minimal temperature 33.90°C; maximal temperature 35.60°C); average relative humidity 81.074% (minimal humidity 56.90%; maximal humidity 91.80%).
all stages were tested in the dark

Test item

Name	M-100SC-OR2-C
Test item description	a beige to brown suspension with a faint odor
The appearance of the original packaging	HDPE container
Date of delivery	10.06.2020
Batch No.	1/2020
Name of active substance	mesotrione
Content of active substance	101,10 g/L; purity 920 g/kg
CAS of active substance	104206-82-8
Production date	03.06.2020
Expiration date	03.06.2022
Solubility in water or other solvents, ability to form emulsions, solutions, suspensions	the agent disperses in water; solubility in organic solvents (values for mesotrione in 20°C): - in acetone 93,3 g/L - in xylene 1,6 g/L - in toluene 3,1 g/L - in ethyl acetate 18,6 g/L
Storage conditions	temperature: 0-30°C; humidity<70 %RH

The study was carried out on larvae of honey bee *Apis mellifera L*. coming from a registered breeding. Quarantine of bees was not carried out, because in the month before the start of test insects were not treated with chemical compounds, including antibiotics or anti-mite agents. The study used 1-day larvae of honey bee originated from 3 different, healthy, well-maintained breeding.
3 days before the beginning of the test, in each family, queen bee was isolated using one-frame isolator. After max. 30 hours, queens were released from the isolator (after conforming the presence of freshly laid eggs). The frame containing the eggs remains in the isolator, placed next to the frame containing brood, for 3 days, until the hatching.
On day 1, the frames with freshly brooded larvae are transferred from the hive to laboratory in the temperature optimal for larvae (above 20°C). For the study, larvae, which has not yet formed C-shape or the ones laying on the top of royal jelly are chosen. The larvae were carefully placed in the same position at the bottom of queen-cell cup filled with diet A placed in breeding plate's well.
On day 8, the larvae plates were placed in a desiccator which was kept at 80%±5% humidity suitable for pupae development, maintained by placing a vessel filled with saturated NaCl at the bottom of the desiccator. A piece of filter paper was placed at the bottom of each well to absorb larvae droppings. The desiccators were placed in the test room.
On day 15, the plates were transferred to a plastic box. A 50% aqueous solution of sucrose was given as food (w/v) and pine pollen, The boxes were placed in the test room at 50-80% humidity maintained with humidifiers.
The temperature in the incubator or test room was kept in the range of 34°C-35°C throughout the test; minimum temperature 23°C; maximum temperature 40°C (fluctuations last no longer than 30 minutes, once within 24 hours).

Larval diets were adjusted depending on the developmental stage (all solutions were prepared in weight percentage):
Food A: 50% fresh royal jelly + 50% aqueous solution containing 2% yeast extract/ 12% glucose/ 12% fructose.
Food B: 50% fresh royal jelly + 50% aqueous solution containing 3% yeast extract / 15% glucose / 15% fructose.
Food C: 50% fresh royal jelly + 50% aqueous solution containing 4% yeast extract / 18% glucose / 18% fructose.
Following the above, prepared food should have density around 1.1 mg/µL (20 µL of food corresponds to 22 mg of food).
Before administration, food was warmed to 35°C. It was provided using automatic pipette, with caution to

avoid touching a larva or drowning it in food liquid.
From the emergence phase (day 15 - day 22) as food was used:
-50% aqueous solution of sucrose
-pine pollen.

Definitive test

In the definitive test, were used following concentration (ratio: 2)
Control, 40.625 mg/kg of food, corresponding to 6.25 µg/larvae, 81.25 mg/kg of food, corresponding to 12.5 µg/larvae, 162.5 mg/kg of food, corresponding to 25.0 µg/larvae, 325.0 mg/kg of food, corresponding to 50.0 µg/larvae, 650 mg/kg of food, corresponding to 100.0 µg/larvae.
Parallel to definitive test, reference test was performed using fenoxycarb as reference item.

Duration of the test: 22 days (duration of exposure to the test item 4 days - from day 3 to day 6).
- Test cages: 48-well breeding plates with queen-cell cups placed in the dissector and placed in incubator. From day 8 dissectors placed in the test room. From day 15 of the test transparent plastic boxes placed in test room.
Each concentration and control were prepared in one replicate of 36 larvae (12 larvae from 3 colonies) on one culture plate.
- Feeding: food for larvae and adult bees
- Lighting: darkness
- Temperature and humidity:
larval stage (day 1-8): average temperature 35.001°C (minimal temperature 33.60°C; maximal temperature 36.20°C); average relative humidity 78.920% (minimal humidity 76.80%; maximal humidity 93.20%)
pupal stage (day 8-15): average temperature 34.107°C (minimal temperature 33.00°C; maximal temperature 34.40°C); average relative humidity 96.489% (minimal humidity 79.10%; maximal humidity 99.90%)
imago stage (day 15-22): average temperature 34.765°C (minimal temperature 33.90°C; maximal temperature 35.60°C); average relative humidity 81.074% (minimal humidity 56.90%; maximal humidity 91.80%).

Conclusions

The tested item in the course of this test did not show any apitoxic effect on larvae mortality on days 3-8 in the concentration range of 16.64 mg/kg food - 650 mg/kg food.
The tested item did not show any statistically significant apitoxic effect in the mortality of pupae on days 8-22 in the concentration range of 16.64 mg/kg food - 650 mg/kg food.
The tested item did not show any statistically significant apitoxic effect on adult mortality on days 3-22 in the concentration range of 16.64 mg of the test item/kg food - 650 mg of test item/kg food.
The values of LC_{10}, LC_{20}, LC_{50} have not been determined, they are outside the range of concentrations used in the test of 16.64 mg of the test item/kg of food - 650 mg of the test item/kg of food. LD_{10}, LD_{20}, LD_{50} values have not been determined, they are outside the range of doses used in the test 0.1 µg of the test item/larvae - 100 µg of the test item/larva. The NOEC value was defined as ≥650.0 mg of the test item/kg food, NOED value ≥100.0 µg of the test item/larvae.

Final results of larval mortality – definitive test

Concentration [mg/kg of food]	Time [day]									
	4		5		6		7		8	
	Mortality[1] [%]	Statistical significance*	Mortality[1] [%]	Statistical significance*	Mortality[1] [%]	Statistical significance*	Mortality[1] [%]	Statistical significance*	Mortality[1] [%]	Statistical significance*
Control	0.0	not appplicable	0.0	not appplicable	0.0	not appplicable	0.0	not appplicable	0.0	not appplicable
40.625	0.0	-	0.0	-	0.0	-	0.0	-	0.0	-
81.250	0.0	-	0.0	-	0.0	-	0.0	-	0.0	-
162.500	0.0	-	0.0	-	0.0	-	0.0	-	0.0	-
325.000	0.0	-	0.0	-	0.0	-	0.0	-	0.0	-
650.000	0.0	-	0.0	-	0.0	-	0.0	-	2.8	-

- statistically insignificant
*) values calculated using ToxRat Professional using Fisher's test after Bonferroni-Holm correction at a significance level p=0.05
1) percentage calculated by comparing the number of dead larvae on days 4-8 with the number of larvae on day 3

Adult mortality and adult mortality final results – definitive test

Concentration [mg/kg of food]	Time [day]				
	3	22			
	Introduced larvae [pcs.]	Unemerged adults[1)] [pcs.]	Emerged adults [%]	Mortality[2)] [%]	Statistical significance*)
Control	36	7	80.6	19.4	not applicable
40.625	36	7	80.6	19.4	-
81.250	36	12	66.7	33.3	-
162.500	36	11	69.4	30.6	-
325.000	36	13	63.9	36.1	-
650.000	36	12	63.9	36.1	-

- statistically insignificant
1) dead larvae, pupae and adults on days 3-22
2) the percentage calculated by comparing the number of adults emerged on day 22 with the number of larvae on day 3
*) values calculated using ToxRat Professional using Chi2 2x2 Table after Bonferroni correction at a significance level p>0.05

Pupal and adult mortality – definitive reference test

Reference item	Time [day]				
	Pupal mortality			Adult mortality	
	3	15		22	
	Introduced larvae [pcs.]	Dead larvae and pupae[1)] [pcs.]	Symptoms of intoxication	Unemerged adults[2)] [pcs.]	Adult survival [%]
Control	36	3	developmental delay – 2 pupae	9	80.6
Fenoxycarb 0.32 [mg/kg of food]	36	8	developmental delay – 10 pupae	32	13.9

1) dead larvae and pupae compared to day 3
2) dead larvae, pupae and adults compared to day 3

Reference item	Time [day]											
	4		5		6		7		8		22	
	Mortality [%]	Statistical significance*)	Mortality [%]	Statistical significance*)	Mortality [%]	Statistical significance*)	Mortality [%]	Statistical significance*)	Mortality [%]	Statistical significance*)	Mortality[1)] [%]	Statistical significance*)
Control	0.0	not applicable	0.0	not applicable	0.0	not applicable	0.0	not applicable	0.0	not applicable	19.4	not applicable
Fenokaykarb 0.32 [mg/kg of food]	not applicable	not applicable	not applicable	not applicable	not applicable	not applicable	not applicable	not applicable	not applicable	not applicable	86.1	+

+ statistically significant
*) values calculated by ToxRat Professional using Fisher's test with significance level p> 0.05
1) percentage calculated by comparing the number of adults emerged on day 22 with the number of larvae introduced on day 3

Final results calculated by ToxRat Professional			
Parameter	Concentration [mg of the test item/kg of food]	Parameter	Dose [µg of the test item/larvae]
LC_{10}	n.d.*	LD_{10}	n.d.**
LC_{20}	n.d.*	LD_{20}	n.d.**
LC_{50}	n.d.*	LD_{50}	n.d.**
NOEC	≥650.0	NOED	≥100.0

NOEC the highest concentration of the tested item that did not cause statistically significant differences compared to the control
NOED the highest dose of the tested item that did not cause statistically significant differences compared to the control
LC_{10} test item concentration causing reduction by 10%
LC_{20} test item concentration causing reduction by 20%
LC_{50} test item concentration causing reduction by 50%
LD_{10} test item dose causing reduction by 10%
LD_{20} test item dose causing reduction by 20%
LD_{50} test item dose causing reduction by 50%
n.d. impossible to determine for mathematical reasons
*) **based on the analysis of the results, the values were determined as >650.0 mg of the test item/kg of food**
) **based on the analysis of the results, the values were determined as >100 µg of the test item/larvae

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

No data submitted.

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

No data submitted.

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

No data submitted.

A 2.3.2 KCP 10.3.2 Effects on arthropods other than bees

A 2.3.2.1 KCP 10.3.2.1 Standard laboratory testing

No standard labolatory tests on arthropods.

A 2.3.2.2 KCP 10.3.2.2 Extended labolatory testing

A 2.3.2.2.1 Study 1: Toxicity to *Aphidius rhopalosiphi*

Comments of zRMS: *Aphidius rhopalosiphi*
The study is considered as acceptable. All validity criteria were met.

The following validity criteria were met during the study:
– after 48 hours, mortality of the control group was 0.0% (criterion: a maximum of 10.0%),
– after 48 hours, mortality of the group treated with the reference item at the rate of 4.0 g/ha was 73.3% (criterion: a minimum of 50%),
– all wasps survived the 24-hour oviposition period (criterion: only wasps that survive oviposition can be examined for fecundity),
– the mean number of mummies per female in the control group was 21.1 (criterion: a minimum of 5.0 mummies/female),
– all wasps in the control group gave offspring (criterion: a maximum of 2 females giving no offspring).

Agreed endpoints:

Parameter (endpoint)						
Mortality			**Fecundity**			
Test item [L/ha]	**Total [%]**	**LR_{50} [L/ha]**	**Test item [L/ha]**	**Mean no. of mummies /female**	**Fecundity reduction Pr [%]**	**ER_{50} [L/ha]**
Control	0.0	–	**Control**	21.1	–	–
Test item: M-100SC-OR2-C						
0.375	0.0	**> 3.0** > 303.6 [g a.i./ha]	**0.375⁺**	16.9	19.9	**> 3.0** > 303.6 [g a.i./ha]
0.75	0.0		**0.75⁺**	15.6	26.2	
1.5	0.0		**1.5⁺**	13.7	35.3	
3.0	0.0		**3.0⁺**	11.1	47.6	
$NOER_{mortality}$	**≥ 3.0 [L/ha]** ≥ 303.6 [g a.i./ha]		**$NOER_{fecundity}$**	**< 0.375 [L/ha]** < 37.95 [g a.i./ha]		
Reference item: technical dimethoate						
[g/ha]	**Mortality**		**Fecundity**			
	Total [%]					
4.0	73.3		not assessed			

⁺: statistically significant differences between control and groups exposed to test item; ToxRat Professional 3.3.0. software [3], [SOP/B/67, SOP/OG/7].

Reference:	KCP 10.3.2.2/01
Report	An extended laboratory test for evaluating the effects of M-100SC-OR2-C on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez), Kulec-Płoszczyca E., 2021, B-52-21
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., 2010)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Duplication (if vertebrate study) No

Materials and Methods

Test item:
Name: M-100SC-OR2-C
Active substance: 101.20 g/L of mesotrione
Batch number: 2/2021
Production date: 12.03.2021
Expiry date: 12.03.20231
Active substance: 101.20 g/L of mesotrione
CAS number: 104206-82-8
Density: 1.045 g/cm3

Biological test system: the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez); Hymenoptera: Braconidae, Aphiidinae
– age: imago (24 – 48 hours after emerging from mummies)
– source: the culture was obtained from a commercial breeder (Katz Biotech AG)

Experimental design: 6 study groups: a control group (0.0 L/ha), 0.375 L/ha, 0.75 L/ha, 1.5 L/ha, 3.0 L/ha
− Reference item: dimethoate at the rate of 4.0 g/ha
mortality assessment: 6 replicates/group; 5 females/replicate
fecundity assessment: 15 replicates/group; 1 females/replicate

Test conditions: temperature: 19 – 21°C, relative air humidity: 63 – 76%, photoperiod: 16 hours light : 8 hours dark, light intensity: mortality and oviposition assessment: 2129 lx, fecundity phase: 5405 lx
Statistical analyses: Fecundity: Estimated parameters of the 3-param. normal CDF, Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity, Williams Multiple Sequential t-test Procedure, Repellency: Shapiro-Wilk´s Test on Normal Distribution, Levene´s Test on Variance Homogeneity, One-way Analysis of Variance.

Endpoints:
– wasp mortality after 48 hours of exposure,
– determination of the LR50 and the NOERmortality,
– determination of the ER50 and the NOERfecundity.
– reduction in fecundity (Pr) of the surviving female wasps exposed to test item, 12 days after the oviposition period

Reference item
Technical dimethoate in the form of colourless crystals, sample number: IPO 146; serial number: 2L/21; expiry date: December 2022; purity: 97.3% ± 0.6% (CAS number: 60-51-5).

Test design

The study was divided into a preliminary range-finding test and a definitive test. The preliminary test was conducted to determine the range of rates to be used in the definitive test and involved only the assessment of the impact of M-100SC-OR2-C on wasp mortality. It was performed on one control group and three test item groups. There were two replicates of each group (5 wasps/replicate).
The definitive test were performed on one control group, four test item group, and one reference item group. There were six replicates of each group (5 wasps/replicate).
In the preliminary test three test item application rates were used i.e. 0.33, 1.0 and 3.0 L/ha of the test item.
In the definitive experiment four test item application rates were used i.e. 0.375, 0.75, 1.5 and 3.0 L/ha of test item. The rate of the reference item, dimethoate was 4.0 g/ha.

Final results:

Parameter (endpoint)						
Mortality			Fecundity			
Test item [L/ha]	Total [%]	LR_{50} [L/ha]	Test item [L/ha]	Mean no. of mummies /female	Fecundity reduction Pr [%]	ER_{50} [L/ha]
Control	0.0	–	Control	21.1	–	–
Test item: M-100SC-OR2-C						
0.375	0.0	> 3.0 > 303.6 [g a.i./ha]	0.375+	16.9	19.9	> 3.0 > 303.6 [g a.i./ha]
0.75	0.0		0.75+	15.6	26.2	
1.5	0.0		1.5+	13.7	35.3	
3.0	0.0		3.0+	11.1	47.6	
$NOER_{mortality}$	≥ 3.0 [L/ha] ≥ 303.6 [g a.i./ha]		$NOER_{fecundity}$	< 0.375 [L/ha] < 37.95 [g a.i./ha]		
Reference item: technical dimethoate						
[g/ha]	Mortality Total [%]		Fecundity			
4.0	73.3		not assessed			

+: statistically significant differences between control and groups exposed to test item; ToxRat Professional

On the basis of the obtained mortality results it can be concluded that M-100SC-OR2-C at the rates of 0.375, 0.75, 1.5 and 3.0 L/ha has no adverse effect on the mortality of the wasps.
On the basis of the obtained fecundity results it can be concluded that M-100SC-OR2-C at all the tested rates, i.e. of 0.375, 0.75, 1.5 and 3.0 L/ha has an adverse effect on the fecundity of the wasps.

A 2.3.2.2.2 Study 2: Toxicity to *Typhlodromus pyri*

Comments of zRMS:	*Typhlodromus pyri* The study is considered as acceptable. All validity criteria were met. The following validity criteria were met during the study: - mean mortality in the untreated control was 18 %. For the test to be considered valid the mean mortality in the negative control should not exceed 20 % on the 7th day of mite exposure - the cumulative mean number of eggs per female in the control (from the 7th to 14th day following mite exposure) was 4.4. For the test to be considered valid it should be ≥4 eggs/female. - the cumulative mean mortality (control corrected) of mites exposed to the fresh toxic reference treatment was 98.8%. For the test to be considered valid it should range between 50 and 100%. **Agreed endpoints:** Application of M-100SC-OR2-C did not increase mortality of *T. pyri* in any of the tested application rates (3.84–150 g a.i./ha). The decrease of reproductive output was detected in the data reaching 36.6% in the highest rate tested (150 g a.i./ha) as compared with the untreated control (this decrease was not statistically significant). zRMS pointed out that LR_{50} for mortality and ER_{50} on reproductive output

	should be established on 150 g a.i./ha.
Reference:	KCP 10.3.2.2/02
Report	Extended GLP laboratory test for evaluating the effects of a test item on the predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae), Šklíba J., 2020, 20/201
Guideline(s):	Blümel et al. (2000) and Candolfi et al. (2001)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item name: M-100SC-OR2-C content: Mesotrione 101.1 g/L (CoA)
batch number: 1/2020, expiry date: 06.2022, storage: ambient room temperature (i2L chemical store)
Biological test system predatory mite *Typhlodromus pyri* (Phytoseiidae), age: 24 hours old protonymphs, source synchonized eggs obtained from a laboratory culture (Katz Biotech AG)
Experimental design
Range-finding test: - M-100SC-OR2-C, 0.15, 1.5, 15, 150 and 1500 g a.i./ha
- Dimethoate 40% EC, 24 g a.i./Ha (toxic reference)
- Untreated (water only) control
- application volume 200 L/Ha for all
- 3 replicates/treatment, 20 protonymphs/replicate
Definitive test: M-100SC-OR2-C, 3.84, 9.6, 24, 60, 150 g a.i./ha
- Dimethoate 40% EC, 48 g a.i./Ha (toxic reference)
- Untreated (water only) control
- application volume 200 L/Ha for all
- 5 replicates/treatment, 20 protonymphs/replicate
Test phases:
- mortality phase: Mortality of Typhlodromus pyri evaluated following 7 days of exposure to the aged spray residues.
- fecundity phase (Definitive test only): Fecundity of surviving females calculated following Blümel et al. (2000) based on assessments conducted at 9th, 11th and 14 th day of the exposure to the aged spray residues.
Test conditions:
Laboratory conditions – Range-finding test:
- temperature 23.3–27.8 °C
- relative humidity 52–84 %
- photoperiod: 16 hours light (1000–1300 lux), 8 hours dark
Laboratory conditions – Definitive test:
- temperature 22.3–24.1°C
- relative humidity 61–79 %
- photoperiod: 16 hours light (700–900 lux), 8 hours dark
Endpoints: - mortality of T. pyri after 7 days
- reproductive output per female T. pyri surviving the mortality phase calculated according to Blümel et al. (2000)
Statistical analyses:
Mortality phase - Probit analysis using linear max. likelihood regression (LD50)
- Chi2 2x2 table wih Bonferroni correction (NOER/LOER)
Fecundity phase - - 3-param. normal CDF (ER50)
- Dunnett's multiple t-test procedure (NOER/LOER)

Test design

Treatments were applied on segments of mature leaves of corn (Zea mays) distributed on a 25x35cm glass sitting on a tarred balance. The leaf segments were laid on water-saturated cotton wool pads placed in plastic Petri dishes with the treated leaf surfaces facing upwards. In the Range-finding test, 3 replicates were made for each treatment and control. This was increased to 5 in the Definitive test. 20 *T. pyri* protonymphs (24h old) were transferred into each test unit using a fine artist brush. To avoid contamination, a new brush was used for each treatment. During the

whole experiment, apple pollen was supplied every 2–3 days and the water level in the dishes was topped up every day. The mites were exposed to the test arenas for 7 days before continuing to the fecundity phase.
Statistical analyses were performed following the requirements outlined in Blümel et al. 2000 with ToxRat Professional 3.3.0.

Final results
Application of M-100SC-OR2-C did not increase mortality of *T. pyri* in any of the tested application rates (3.84–150 g a.i./ha). Slight decrease of reproductive output was detected in the data reaching 36.6% in the highest rate tested (150 g a.i./ha) as compared with the untreated control, however this decrease was not statistically significant. Data therefore suggest NOER of both mortality and reproductive output to be higher than or equal to 150 g a.i./ha. Concurrently, LR50 and ER50 on reproductive output are above 150 g a.i./ha.

Treatment ID	Substance	Application rate (g a.i./Ha)	Mean ± SD % Mortality	Corrected % mortality after Abbott (1925)	Mean ± SD Reproductive output per female	Mean % decrease of Reproductive output
Untreated control	water only	-	18 ± 7.6	0.0	4.4 ± 2.1	0
T5	M-100SC-OR2-C	3.84	7 ± 9.7	-13.4	3.49 ± 1.34	20.7
T4		9.6	19 ± 10.4	1.2	3.08 ± 1.19	30
T3		24	17 ± 10.8	-1.2	3.34 ± 1.82	24.1
T2		60	28 ± 10.8	12.2	3.04 ± 1.75	30.9
T1		150	26 ± 10.4	9.8	2.79 ± 0.69	36.6
Ref	Dimethoate	48	99 ± 10.8	98.8	n/a	n/a

A 2.3.2.2.3 Study 3: Toxicity to *Coccinella septempunctata*

Comments of zRMS:	*Coccinella septempunctata* The study is considered as acceptable. All validity criteria were met. The following validity criteria were met during the study: • Mean pre-imaginal mortality in the water treated control was 6.67 %. For the test to be considered valid, the mean pre-imaginal mortality in the water treated control should not exceed 30 %. Mean pre-imaginal mortality of larvae exposed to the toxic reference treatment was 100%. For the test to be considered valid, it should be ≥ 40 %. • The number of fertile eggs per female in control was 4.49. For the test to be considered valid, the number should be above 2 fertile eggs per viable female per day. The beetles from the reference item and the highest rate (T1) did not proceed to the reproduction part of the test. There were not any differences among UTC and treatments that proceeded to the fecundity phase. Application of **Juzan Extra 100 SC** did not affect female fecundity nor egg fertility in range of the test item (38.4 – 600 g a.i/ha). **Agreed endpoints:** Application of M-100SC-OR2-C had 64.3 % (Abbott`s corrected) effect on survival of *Coccinella septempunctata* larvae at the highest rate. LR_{50} was established to be 1107 g a.i./ha. RMS pointed that in the study report shows that the dose for which the effect on reproduction was tested is 600 g a.s./ha. Therefore, ER_{50} > 600 g a.i./ha should be used for risk assessment.

Reference: KCP 10.3.2.2/03

Report	Extended GLP laboratory test for evaluating the effects of a test item on the plant dwelling insect *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), Nácarová J., 2020, 20/199
Guideline(s):	Schmuck et al. (2000) in Candolfi (2000) guidelines.
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test system
- species: ladybird beetle (*Coccinella septempunctata*), Coleoptera
- age: 4-5 days old larvae
- source: Synchronized eggs were obtained from a laboratory culture (Katz Biotech AG) and stored at 20°C until the start of the larvae hatching.
- rearing: adults and hatched larvae were kept at 25°C ± 2°C, 60 – 90% RH, 16L:8D

Test item

M-100SC-OR2-C, content: Mesotrione 101.1 g/L (CoA), batch number: 1/2020, expiry date: 06.2022, storage: ambient room temperature (i2L chemical store)

Test system

3-5 days old larvae of ladybird (Coccinella septempunctata). Ladybird beetles will be obtained from a specified commercial supplier, laboratory culture, or field collected. All control and treatment beetles used in the test will be from the same source.

Test design

To initiate the test, the bean leaf discs were treated with the test item, water control or reference item and left to dry. Then larvae were enclosed into the safety cylinders (one larva per cylinder = 1 replicate). In the range finder, ten replicates were used for each of the five test item rates, and negative control and toxic reference treatment. In the definitive test, 30 replicates were used for each of the five test item rates and negative control and the toxic reference treatment.

Treatment	Substance	Application rate (g a.i./ha)	% Mortality	% Abbott`s corrected mortality
UTC	water only	n/a	6.7	-
T5	M-100SC-OR2-C	38.4	10.0	3.6
T4		96	17.2	11.3
T3		240	26.7	21.4
T2		600	30.0	25.0
T1		1500	66.7	64.3
Tox. Ref	Dimethoate	40	100	100

Treatment	Substance	Application rate (g a.i./ha)	Female fecundity: Mean number of eggs per female per day	Female fertility: % of fertile eggs
UTC	water only	n/a	5.3 ± 1.99	85 ± 7
T5	M-100SC-OR2-C	38.4	5.6 ± 2.55	84 ± 10
T4		96	5.3 ± 2.38	85 ± 8
T3		240	5.4 ± 1.97	82 ± 6
T2		600	4.8 ± 4.59	78 ± 21
T1		1500	n/a	n/a
Tox. Ref	Dimethoate	40	n/a	n/a

Conclusion:

Application of M-100SC-OR2-C had 64.3 % (Abbott`s corrected) effect on survival of *Coccinella septempunctata* larvae at the highest rate. LR50 was established to be 1107 g a.i./ha. NOER was established to be 96.0 g a.i./ha larva and LOER to be 240 g a.i./ha. Reproductive performance of surviving beetles was not affected in a tested range of M-100SC-OR2-C in terms of female fecundity nor egg fertility. No other adverse effect was observed in any treatment.

A 2.3.2.2.4 Study 4: Toxicity to *Chrysoperla carnea*

Comments of zRMS:	*Chrysoperla carnea* The study is considered as acceptable. All validity criteria were met. The following validity criteria were met during the study: Mean mortality in the untreated control was 19.2% which is lower than 20 %. Mean number of eggs per female per day in the control was 24.5 which is higher than 15. Mean hatching rate in the untreated control was 79.1%, which is higher than 70%. Mortality in the toxic reference treatment was 100 % which is higher than 50%. The test is therefore considered valid. **Agreed endpoints:** Application of M-100SC-OR2-C did not increase mortality of *C. carnea* in any of the tested application rates (38.4–1500 g a.i./ha). LR50 and NOER are therefore higher or equal to 1500 g a.i./ha. Decrease of fecundity was detected in higher rates reaching 59% in the highest rate tested (1500 g a.i./ha), as compared to the untreated control. ER50 for fecundity was set to 559.4 g a.i./ha. There was no negative effect of the test item on the egg fertility.
Reference:	KCP 10.3.2.2/04
Report	Extended GLP laboratory test for evaluating the effects of a test item on *Chrysoperla carnea* (Neuroptera: Chrysopidae) Šklíba J., 2020, 20/200
Guideline(s):	Vogt et al. (2000) guideline
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods:
Test item name: M-100SC-OR2-C , content: Mesotrione 101.1 g/L (CoA) , batch number: 1/2020 , expiry date: 06.2022 , storage: ambient room temperature (i2L chemical store)
Biological test system *Chrysoperla carnea* (Neuroptera: Chrysopidae)
- age: 2–3 days old larvae
- source: synchonized eggs obtained from a laboratory culture (Katz Biotech AG)
Experimental design
- Range-finding test: - M-100SC-OR2-C, 0.15, 1.5, 15, 150 and 1500 g a.i./ha
- Dimethoate 40% EC, 24 g a.i./Ha (toxic reference)
- Untreated (water only) control
- application volume 200 L/Ha for all
- 10 larvae per treatment
- Definitive test: - M-100SC-OR2-C, 38.4, 96, 240, 600, 1500 g a.i./ha
- Dimethoate 40% EC, 156 g a.i./Ha (toxic reference)
- Untreated (water only) control
- application volume 200 L/Ha for all
- 30 larvae per treatment
Test phases: - mortality phase: Mortality of *Chrysoperla carnea* evaluated up to adult emergence.
- fecundity phase (Definitive test only): Fecundity of surviving females (number of eggs per female and day). Fertility (mean % hatching rate).
Test conditions:
Laboratory conditions – Range-finding test:
- temperature 23.0–27.4°C
- relative humidity 55–86 %
- photoperiod: 16 hours light (2500 lx), 8 hours dark
Laboratory conditions – Definitive test:
- temperature 23.1–26.5°C
- relative humidity 56–85 %
- photoperiod: 16 hours light (2000 lx), 8 hours dark
Endpoints: - mortality of Chrysoperla carnea until adults
- fecundity (number of eggs per female and day)
- fertility (hatching rate)

Test design
Treatments were applied on segments of mature leaves of corn (Zea mays) distributed on a 25x35cm glass sitting on a tarred balance. Once the spray deposits had dried, leave segments were placed on wet cotton pads with the treated surface facing upwards and covered with polypropylene rings forming a 4cm-diameter arenas. Inner surface of the rings was treated by fluon to prevent climbing of the larvae. Rings were covered by a netting and secured by rubber bands to be gently pressed against the leaf segments. One 2–3 days old lacewing larvae was released to each arena within one hour from the treatment application. UV-sterilised *Sitotroga cerealella* eggs were provided as food every 2–3 days until pupation. In the Range-finding test, 10 test units were made for each treatment and control. This was increased to 30 in the Definitive test.

Results
Mortality: No statistically significant rate/response was found ($p(F) > 0.05$; i.e. slope of the relationship was not significantly different from zero. LR50 is therefore above the highest rate tested (1500 g a.i./ha). Concurrently, the NOER appeared to be higher than or equal 1500 g a.i./ha.
Fecundity: Amount of variance explained by the regression model was not significant, however, ER50 was set to 559.4 g a.i./ha.

Treatment ID	Substance	Application rate (g a.i./Ha)	% Mortality	Corrected % mortality after Abbott (1925)	Mean fecundity (No eggs/female per day)	Mean fertility (% hatching rate)
Untreated control	water only	-	19.2	0.0	24.5	79.1
T5	M-100SC-OR2-C	38.4	29.6	12.9	42.0	88.1
T4		96	20.0	1.0	21.7	83.4
T3		240	23.1	4.8	14.8	87.3
T2		600	16.0	-4.0	17.9	82.1
T1		1500	20.0	1.0	10.0	85.5
Ref	Dimethoate	156	100.0	100.0	n/a	n/a

Conclusions:
Application of M-100SC-OR2-C did not increase mortality of *Chrysoperla carnea* in any of the tested application rates (38.4–1500 g a.i./ha). NOER for survival is therefore ≥ 1500 g a.i./ha. Decrease of fecundity was detected in higher application rates, reaching ER50 at 559.4 g a.i./Ha. No effect on egg fertility was detected.

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

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

Comments of zRMS:	The study is accepted by zRMS. All validity criteria were met. The following validity criteria were met during the study: 1. mortality of adult worms over initial 28 days of the experiment was 1.25% (criterion: it have not to exceed 10%); 2. the lowest number of offspring produced in replicate was 61 (criterion: a minimum of 30 offspring are produced in each replicate containing 10 adults); 3. the coefficient of variation of reproduction was equal to 14.9% (criterion: it should not exceed 30%). Deviations in the study: 1. According to Study Plan lost soil moisture had to be refilled by adding deionized water to the soil surface by hand-held spryer. An automatic pipette was used to fill up the water loss. This deviations did not affected the study results. 2. Deviation from the Study Plan, Guidelines and SOP/B/16/1 concerning the incorrect test room temperature occurred. OECD 222 states that the test temperature is 20±2°C. During the test, temperature out the range specified in the guideline were recorded six times and minimum measured temperature was 17.8°C while the maximum measured temperature was 23.1°C. These deviations did not, affect the test result.

All mentioned deviations did not affect the study results. All validity criteria of the study were met.

Agreed endpoints:

Endpoint values determined for earthworm reproduction after 8 weeks and for mortality after 4 weeks – Test item.

ENDPOINT	VALUE [mg test item/kg dry soil]	VALUE [mg active substance/kg dry soil]
EC_{10}	38.25 (13.39 – 69.13)	3.82 (1.34 – 6.90)
EC_{20}	127.42 (71.02 – 182.34)	12.72 (7.09 – 18.20)
EC_{50}	784.31 (589.44 – 1181.99)	78.28 (58.83 – 117.97)
NOEC (offspring number)	41.00	4.09
LOEC (offspring number)	70.00	6.99
LC_{10}	n. d.	n. d.
LC_{20}	n. d.	n. d.
LC_{50}	n. d.	n. d.
NOEC (survival)	>=1000.00	>=99.81
LOEC (survival)	>1000.00	>99.81

n.d. – no determined due to mathematical reasons or inappropriate data

In the 56 - day Earthworm reproduction study with **Juzan Extra 100 EC**, the lowest endpoint (EC_{10}) of 38.25 mg of test item/kg dry soil (equal to 3.82 mg active substance/kg dry soil) was obtained and thus, it should be used in the risk assessment.

Reference: KCP 10.4.1/01

Report: Earthworm Reproduction Test (*Eisenia andrei*) according to the OECD Guideline for the Testing of Chemicals No. 222 (July 29, 2016), STUDY CODE: EMI/4/7/2021, Ecomelius Institute Sp. z o. o.; Swoboda T., 2021

Guideline(s): OECD Guideline for the Testing of Chemicals No. 222 (2016): "Earthworm Reproduction Test *(Eisenia fetida/Eisenia andrei)"*.

Deviations:
• According to Study Plan lost soil moisture had to be refilled by adding deionized water to the soil surface by hand-held spryer. An automatic pipette was used to fill up the water loss. This deviations did not affected the study results.
• Deviation from the Study Plan, Guidelines and SOP/B/16/1 concerning the incorrect test room temperature occurred. OECD 222 states that the test temperature is 20±2°C. During the test, temperature out the range specified in the guideline were recorded six times and minimum measured temperature was 17.8°C while the maximum measured temperature was 23.1°C. These deviations did not, affect the test result.

All mentioned deviations did not affect the study results. All validity criteria of the study were met.

GLP: Yes

Acceptability: Yes

Material and Methods

Test item: name: M-100SC-OR2-C; active ingredients content (analysed): mesotrione: 103.80 g/L, batch number: 1/2021; manufacturing date: 09.02.2021; expiry date: 09.02.2023

Test organism: A biological systems used in the study were earthworms *Eisenia andrei*. The source of the test system was laboratory-bred synchronized culture (originating from Wurmwelten, Inch. Jasper Rimpau, Warteweg 50, Stadtoldendorf, Germany) at the Test Facility according to [SOP/P/6/1]. The culture was maintained in peat moss mixed with cattle manure (50:50) and fed with alfalfa, cardboard and potatoes.
Adult worms with clitella and with fresh weight of 10 individuals between 4025-5249 mg were used in this study. Age of organisms used in the study was 6 months and didn't differ in age by more than 4 weeks. The animals selected for the experiment were acclimatized in untreated artificial soil for 24 hours prior to the start of the experiment.

Artificial soil Components:
- 10% sphagnum peat (pH = 5.87)
- 20% kaolin clay (kaolinite content >30%)
- 70 % quartz sand (air dried, particles between 50-200 microns >50%)

Test conditions: – temperature: 17.8 – 23.1°C
- controlled light – dark cycles (16h : 8h)

Tested concentrations: Eight concentrations of the test item were used in the experiment (24, 41, 70, 120, 204, 346, 588 and 1000 mg test item/kg soil dry weight).

There were four replicates of each test concentration.

At the same time, an untreated control group (eight replicates) was introduced to the soil without the test item.

The test item in the form of a aqueous suspension was mixed with a suitable amounts of the artificial soil.

Study duration: 8 weeks

Observations: After 4 weeks: mortality, behavioral and morphological changes
After 8 weeks: number of juveniles hatched from the cocoons

Endpoints: NOEC, LOEC, EC_{10}, EC_{20}, EC_{50}, LC_{10}, LC_{20}, LC_{50}

Results
Observations of burrowing behavior were conducted at the beginning of the experiment (Day 0). When compared with the control group, there were no apparent effects upon burrowing behavior in all treatments group. Earthworm were reported as having showed no aversion to test item treated soils at Day 0.

After 28 days of exposure to M-100SC-OR2-C, the percent mortalities of adult earthworms in the control and concentrations: 24, 41, 70, 120, 204, 346, 588 and 1000 mg test item/kg dry soil weight in treatment groups were 1.25; 0.0; 0.0; 2.5; 0.0; 0.0; 2.5; 2.5 and 0.0% respectively. Mortalities in the treatment groups of 24, 41, 70, 120, 204, 346, 588 and 1000 mg test item/kg dry soil were not statistically different from these in the control group. The treatment-related mortality of adult earthworm did not occurred and the LC_{10}, LC_{20} and LC_{50} values were not determined due to mathematical reasons. All surviving earthworms in the control and the treatment groups were normal in appearance and behavior.

After 28-day exposure period, the mean body weights change of the survived adult worms in the control and concentrations: 24, 40, 70, 120, 204, 346, 588 and 1000 mg test item/kg dry soil treatment groups were 28.9; 31.3; 30.8; 36.5; 27.4; 34.9; 26.2; 27.0 and 25.5 % respectively.

On Day 56, the impact of the test item on reproduction of the worms was assessed by counting the number of juveniles hatched from the cocoons in the test soils. The mean number of juveniles in the control and 24, 40, 70, 120, 204, 346, 588 and 1000 mg test item/kg dry soil treatment groups were 76.8; 81.3; 71.5; 63.3; 60.0; 53.0; 52.5; 45.5 and 33.3 respectively. The mean numbers of juveniles produced in 70, 120, 204, 346, 588 and 1000 mg test item/kg dry soil groups were significantly different when compared to the mean value of control group.

Number of juvenile earthworms after 8 weeks of the reference test with Carbendazim.

Concentration [mg/kg dry soil]	Replicate	Number of juveniles	Mean ± SD	Coefficient of variation [%]
Control	A	41	48.00±6.41	13.4
	B	50		
	C	48		
	D	47		
	E	55		
	F	46		
	G	58		
	H	39		
Acetone control	A	60	50.63±7.80	15.4
	B	61		
	C	52		
	D	43		
	E	43		
	F	41		
	G	50		
	H	55		
0.31	A	58	52.25±6.95	13.3
	B	49		
	C	44		
	D	58		
0.63	A	47	50.00±3.56	7.1
	B	52		
	C	47		
	D	54		
1.25	A	46	39.75±5.32	13.4
	B	37		
	C	34		
	D	42		
2.5	A	13	17.75±3.77	21.3
	B	19		
	C	22		
	D	17		
5.0	A	0	0.75±0.96	127.7
	B	1		
	C	2		
	D	0		

Earthworms reproduction and morphological observations after 8 weeks of the experiment.

Concentration [mg/kg dry soil]	Replicate	Number of juveniles	Mean ± SD	Coefficient of variation [%]	Morphological observations
Control	A B C D E F G H	81 64 88 70 61 92 85 73	76.8±11.44	14.9	nc
24	A B C D	99 74 73 79	81.3±12.12	14.9	nc
41	A B C D	75 66 72 73	71.5±3.87	5.4	nc
70	A B C D	66 66 76 45	63.3±13.05+	20.6	nc
120	A B C D	56 71 51 62	60.0±8.60+	14.3	nc
204	A B C D	51 57 61 43	53.0±7.83+	14.8	nc
346	A B C D	57 38 58 57	52.5±9.68+	18.4	nc
588	A B C D	49 55 40 38	45.5±7.94+	17.4	nc
1000	A B C D	29 47 26 31	33.3±9.39+	28.3	nc

nc – no changes + - statistically significant;

Evaluation of endpoints results are shown in below table:

ENDPOINT	VALUE [mg test item/kg dry soil]	VALUE [mg active substance/kg dry soil]
EC_{10}	38.25 (13.39 – 69.13)	3.82 (1.34 – 6.90)
EC_{20}	127.42 (71.02 – 182.34)	12.72 (7.09 – 18.20)
EC_{50}	784.31 (589.44 – 1181.99)	78.28 (58.83 – 117.97)
NOEC (offspring number)	41.00	4.09
LOEC (offspring number)	70.00	6.99
LC_{10}	n. d.	n. d.
LC_{20}	n. d.	n. d.
LC_{50}	n. d.	n. d.
NOEC (survival)	>=1000.00	>=99.81
LOEC (survival)	>1000.00	>99.81

n.d. – no determined due to mathematical reasons or inappropriate data

Validity criteria:

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

- mortality of adult worms over initial 28 days of the experiment was 1.25% (criterion: it have not to exceed 10%);
- the lowest number of offspring produced in replicate was 61 (criterion: a minimum of 30 offspring are produced in each replicate containing 10 adults);
- the coefficient of variation of reproduction was equal to 14.9% (criterion: it should not exceed 30%).

Conclusions

In the 56 - day Earthworm reproduction study with M-100SC-OR2-C, the lowest endpoint (EC_{10}) of 38.25 mg of test item/kg dry soil (equal to 3.82 mg active substance/kg dry soil) was obtained and thus, it is proposed to be used in the risk assessment.

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

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

A 2.4.2.1 KCP 10.4.2.1 Species level testing

A 2.4.2.1.1 Study 1

Comments of zRMS:

All validity criteria were met.

The following validity criteria were met during the study:
- mean adult mortality: 7.5% (criterion: ≤ 20%),
- the mean number of juveniles per vessel at the end of the test: 247.25 (criterion: ≥ 100 juveniles at the end of the test),
- the coefficient of variation calculated for the number of juveniles: 12.2% (criterion: ≤ 30%).

Deviations in the study:
1.The Study Plan states that springtails will be fed with dried yeast at quantity between 2-5 mg per container. In the study, the amount of food per container was 10 mg.
2. Deviation from the Study Plan, Guidelines and SOP/B/16/1 concerning the incorrect test room temperature occurred. OECD 232 states that the test temperature is 20±2°C. During the test, temperature out the range specified in the guideline were recorded five times and minimum measured temperature was 17.8°C while the maximum measured temperature was 23.1°C.

All mentioned deviations did not affect the study results. All validity criteria of the study were met.

Agreed endpoints:

Endpoint values - the impact of the test item on reproduction of collembolans (Folsomia candida).

Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg pendimethalin/kg dry weight of the artificial soil]
EC_{10}	**507.94** (459.97 – 547.39)	**50.70** (45.91 –54.63)
EC_{20}	**651.30** (613.12 – 683.89)	**65.00** (61.19 – 68.26)
EC_{50}	**1047.93** (995.68 – 1117.32)	**104.59** (99.38 – 111.52)
NOEC	**510**	**50.90**
LOEC	714	71.26

Reference: KCP 10.4.2./01

Report: Collembolan (*Folsomia candida*) Reproduction Test according to OECD Guideline No. 232 (2016), STUDY CODE: EMI/4/1/2021, Ecomelius Institute Sp. z o. o.; Swoboda T., 2021

Guideline(s): OECD Guideline for the Testing of Chemicals No. 232 (2016): "Collembolan reproduction test in soil" [1] and the Standard Operating Procedure SOP/G/87: "Collembolan *(Folsomia candida)* reproduction test".

Deviations: Yes.
1.The Study Plan states that springtails will be fed with dried yeast at quantity between 2-5 mg per container. In the study, the amount of food per container was 10 mg.

2. Deviation from the Study Plan, Guidelines and SOP/B/16/1 concerning the incorrect test room temperature occurred. OECD 232 states that the test temperature is 20±2°C. During the test, temperature out the range specified in the guideline were recorded five times and minimum measured temperature was 17.8°C while the maximum measured temperature was 23.1°C.

All mentioned deviations did not affect the study results. All validity criteria of the study were met.

GLP: Yes

Acceptability: Yes

Material and Methods

Test item: name: M-100SC-OR2-C; active ingredients content (analysed): mesotrione: 103.80 g/L, batch number: 1/2021; manufacturing date: 09.02.2021; expiry date: 09.02.2023

Test organism: The collembolan, *Folsomia candida* obtained from a standard laboratory culture at the Test Facility. The collembolans used in the study were 9 – 12 days old

Artificial soil Components:
- 5% sphagnum peat
- 20% kaolin clay
- 75% air-dried quartz sand

maximum water holding capacity: 34.20%
pH: 5.57.
soil dry weight content: 92.48%

Test conditions: – temperature: 17.8-23.1 °C
- controlled light – dark cycles (16h : 8h)

Tested concentrations: Eight concentrations of the test item were used in the experiment (95, 133, 186, 260, 364, 510, 714 and 1000 mg of the test item/kg of dry weight of the artificial soil).

There were four replicates of each test concentration.

At the same time, an untreated control group (eight replicates) was introduced to the soil without the test item.

The test item in the form of a aqueous suspension was mixed with a suitable amounts of the artificial soil.

Study duration: 28 days

Observations: After 28 days: mortality, number of juveniles

Endpoints: NOEC, LOEC, EC_{10}, EC_{20}, EC_{50}, LC_{10} LC_{20}, LC_{50}

The aims of the study were to assess the impact of the test item on reproduction of the collembolan, *Folsomia candida* and to determine the EC_{10}, EC_{20}, EC_{50}, and NOEC.

Results

Mortality at the concentrations ranging from 95 to 1000 mg/kg dry weight of the artificial soil ranged from 0.0 to 15.0 %. As for the control group, it was equal to 7.5%.

The concentration of the test item causing a 10% and 50% mortality of adults within the exposure period (LC_{10} and LC_{50}) were not determined due to mathematical reasons or inappropriate data.

After 28 days of the exposure of collembolans to the test item at the concentrations ranging from 95 to 1000 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 274.75 – 132.25 per replicate. As for the control group, the number of juveniles was equal to 247.25 per replicate.

Number of juvenile collembolans (Folsomia candida) after 28 days of the experiment

Concentration [mg/kg dry weight of the artificial soil]	Replicate	Number of juveniles	Mean ±SD	Reduction [%]	CV* [%]
0 (control)	1 2 3 4 5 6 7 8	289 214 253 221 236 224 248 293	247.25 ± 30.07	-	12.2
95	1 2 3 4	320 281 229 244	268.50 ± 40.70	**-8.6**	15.2
133	1 2 3 4	288 288 252 271	274.75 ± 17.15	**-11.1**	6.2
186	1 2 3 4	298 209 232 251	247.50 ± 37.79	**-0.1**	15.3
260	1 2 3 4	295 284 208 286	268.25 ± 40.45	**-8.5**	15.1
364	1 2 3 4	234 299 253 260	261.50 ± 27.31	**-5.8**	10.4
510	1 2 3 4	211 205 180 271	216.75 ± 38.58	**12.3**	17.8
714	1 2 3 4	190 158 191 207	186.50+ ± 20.53	**24.6**	11.0
1000	1 2 3 4	159 109 146 115	132.25+ ± 24.10	**46.5**	18.2

*CV – coefficient of variation
+ - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure, significance level = 0.05, one-sided smaller)
'-' not determined

Mortality of adult collembolans (Folsomia candida) after 28 days of the experiment.

Concentration [mg/kg dry weight of the artificial soil]	Replicate	Number of tested collembolans	Number of living collembolans after 28 days [no.]	Total mortality	
				No.	%
0 (control)	1 2 3 4 5 6 7 8	10 10 10 10 10 10 10 10	10 8 10 8 9 9 10 10	6	7.5
95	1 2 3 4	10 10 10 10	10 8 9 9	4	10.0
133	1 2 3 4	10 10 10 10	10 9 8 10	3	7.5
186	1 2 3 4	10 10 10 10	10 9 10 9	2	5.0
260	1 2 3 4	10 10 10 10	10 10 10 10	0	0.0
364	1 2 3 4	10 10 10 10	10 9 10 10	1	2.5
510	1 2 3 4	10 10 10 10	10 9 10 10	1	2.5
714	1 2 3 4	10 10 10 10	10 9 8 9	4	10.0
1000	1 2 3 4	10 10 10 10	9 9 7 9	6	15.0

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 active ingredient/kg dry weight of the artificial soil]
EC_{10}	**507.94** (459.97 – 547.39)	**50.70** (45.91 –54.63)
EC_{20}	**651.30** (613.12 – 683.89)	**65.00** (61.19 – 68.26)
EC_{50}	**1047.93** (995.68 – 1117.32)	**104.59** (99.38 – 111.52)
NOEC	**510**	**50.90**
LOEC	714	71.26

Validity criteria:

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

- mean adult mortality: 7.5% (criterion: ≤ 20%),
- the mean number of juveniles per vessel at the end of the test: 247.25 (criterion: ≥ 100 juveniles at the end of the test),
- the coefficient of variation calculated for the number of juveniles: 12.2 (criterion: ≤ 30%).

Conclusions

In the 28 - day collembolan reproduction study with M-100SC-OR2-C, the lowest endpoint EC_{10} of 507.94 mg/kg dry weight of the artificial soil (50.70 mg of mesotrione/kg dry weight of the artificial soil) reproduction was obtained and thus, it is proposed to be used in the risk assessment.

A 2.4.2.1.2 Study 2

Comments of zRMS:	All validity criteria were met. The following validity criteria were met during the study: • mean adult mortality: 0.0% (criterion: ≤ 20%), • the mean number of juveniles per vessel at the end of the test: 183.3 (criterion: ≥ 50 juveniles at the end of the test, • the coefficient of variation for the number of juveniles: 15.3 (criterion: ≤ 30%). Deviations in the study: 1. According to the OECD Guideline No. 226 (2016) the water content of the soil substrate should be maintained throughout the test by weighing and if needed rewatering the vessels periodically. In the study to maintain proper moisture content, a small sample (about 10 g) of soil has been dried at 105°C and re-weighing at the beginning, after 7 days of the test and the end of the experiment. The soil was collected from additional containers (abiotic controls) (point 3.6.8.). The moisture content during the test not differed by more than 10% from the start value. The

moisture of the soil ranged from 54.63% to 51.33% of the maximum water holding capacity and thus was within required 40-60% range.
2. Due to the use of the temperature extraction method, there was no need for euthanasia of the extracted organisms since the mites are fixed in a 70% ethanol solution.
3. Due to the use of the temperature extraction method, there was no possibility to record the symptoms with behavioral and morphology changes of the extracted predatory mites.
4. According to the Study Plan, the concentrations of boric acid (reference test) should be 15, 22, 32, 46, 68, 100, 150, 220, 320, 460, 680 and 1000 mg/kg dry weight of the artificial soil. In reference test the following concentrations of boric acid were used: 11.56, 17.34, 26.01, 39.02, 58.53, 87.79, 131.69, 197.53, 296.30, 444.44, 666.67, and 1000.00 mg/kg dry weight of the artificial soil. The obtained results from the reference test allowed for the determination of the EC_{50} value. The concentration of boric acid causing a 50% reduction in the number of juveniles produced within the exposure period (EC_{50}) was equal to 177.58 mg/kg dry weight of the artificial soil. According to OECD Guideline No. 226, the EC_{50} should fall in the range between 100 and 500 mg / kg dry weight of the artificial soil; hence, it may be concluded that the sensitivity of the test organisms was proper.

All mentioned deviations did not affect the study results. All validity criteria of the study were met.

Agreed endpoints:
In the 14 - day *Hypoaspis* reproduction study with Juzan Extra 100 SC, NOEC of 714.29 mg of test item/ kg dry weight of the artificial soil (equal to 71.29 mg of active substance/ kg dry weight of the artificial soil) for reproduction was obtained and thus, it is proposed to be used in the risk assessment (the worst case).

The endpoint values showing the impact of the test item on reproduction of *Hypoaspis (Geolaelaps) aculeifer* are presented in the table given below.

Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg active ingredient/kg dry weight of the artificial soil]
EC_{10}	**907.22** (843.52 – 975.73)	**90.55** (84.19 – 97.39)
EC_{20}	**970.66** (949.20 – 992.60)	**96.88** (94.74 – 99.07)
EC_{50}	**> 1000.00**	**> 99.81**
NOEC	**714.29**	**71.29**
LOEC	**1000.00**	**99.81**

Reference: KCP 10.4.2/02

Report: Predatory mite (*Hypoaspis* (*Geolaelaps*) *aculeifer*) Reproduction Test according to the OECD Guideline No. 226 (2016), STUDY CODE: EMI/4/8/2021, Ecomelius Institute Sp. z o. o.; Dec W., 2021

Guideline(s):	OECD Guideline for the Testing of Chemicals No. 226 (2016): "Predatory mite *(Hypoaspis (Geolaelaps) aculeifer)* reproduction test in soil"
Deviations:	Yes. 1. According to the OECD Guideline No. 226 (2016) the water content of the soil substrate should be maintained throughout the test by weighing and if needed rewatering the vessels periodically. In the study to maintain proper moisture content, a small sample (about 10 g) of soil has been dried at 105°C and re-weighing at the beginning, after 7 days of the test and the end of the experiment. The soil was collected from additional containers (abiotic controls) (point 3.6.8.). The moisture content during the test not differed by more than 10% from the start value. The moisture of the soil ranged from 54.63% to 51.33% of the maximum water holding capacity and thus was within required 40-60% range. 2. Due to the use of the temperature extraction method, there was no need for euthanasia of the extracted organisms since the mites are fixed in a 70% ethanol solution. 3. Due to the use of the temperature extraction method, there was no possibility to record the symptoms with behavioral and morphology changes of the extracted predatory mites. 4. According to the Study Plan, the concentrations of boric acid (reference test) should be 15, 22, 32, 46, 68, 100, 150, 220, 320, 460, 680 and 1000 mg/kg dry weight of the artificial soil. In reference test the following concentrations of boric acid were used: 11.56, 17.34, 26.01, 39.02, 58.53, 87.79, 131.69, 197.53, 296.30, 444.44, 666.67, and 1000.00 mg/kg dry weight of the artificial soil. The obtained results from the reference test allowed for the determination of the EC_{50} value. The concentration of boric acid causing a 50% reduction in the number of juveniles produced within the exposure period (EC_{50}) was equal to 177.58 mg/kg dry weight of the artificial soil. According to OECD Guideline No. 226, the EC_{50} should fall in the range between 100 and 500 mg / kg dry weight of the artificial soil; hence, it may be concluded that the sensitivity of the test organisms was proper. All above deviations did not affect the results of the study. The all-validity criteria were satisfied in the control group.
GLP:	Yes
Acceptability:	Yes

Material and Methods

Test item:	name: M-100SC-OR2-C; active ingredients content (analysed): mesotrione: 103.80 g/L, batch number: 1/2021; manufacturing date: 09.02.2021; expiry date: 09.02.2023
Test organism:	the predatory mites, Hypoaspis (Geolaelaps) aculeifer (adult female mites from a synchronized cohort) obtained from a standard laboratory culture at the Test Facility. The mites were introduced 7-14 days after becoming adult (i.e. 28 – 35 days after the start of the egg laying in the synchronisation).
Artificial soil	Components: - 5% sphagnum peat - 20% kaolin clay

- 75% air-dried quartz sand
maximum water holding capacity: 39.30%
pH: 6.19.
soil dry weight content: 91.18%

Test conditions: – temperature: 18.9-21.6ºC
- controlled light – dark cycles (16h : 8h)

Tested concentrations: Eight concentrations of the test item were used in the experiment (94.86, 132.81, 185.93, 260.31, 364.43, 510.20, 714.29, and 1000.00 mg of the test item/kg of dry weight of the artificial soil).

There were four replicates of each test concentration.

At the same time, an untreated control group (eight replicates) was introduced to the soil without the test item.

The test item in the form of a aqueous suspension was mixed with a suitable amounts of the artificial soil.

Study duration: 14 days

Observations: After 14 days: mortality, number of juveniles

Endpoints: NOEC, LOEC, EC_{10}, EC_{20}, EC_{50}, LC_{10}, LC_{20}, LC_{50}

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

Results

After the application of the test item at the concentrations ranging from 94.86 to 1000.00 mg/kg dry weight of the artificial soil, mortality was between 0.0 to 5.0%. As for the control group, it was equal to 0.0%. The concentration of the test item causing a 50% mortality of adults within the exposure period (LC_{50}) was not determined due to mathematical reasons.

After the application of the test item at the concentrations ranging from 94.86 to 1000.00 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 135.00 – 216.75 per replicate. As for the control group, the mean number of juveniles was equal to 183.25 per replicate.

Mortality of adult females (Hypoaspis (Geolaelaps) aculeifer) at the end of the experiment.

Concentration [mg/kg dry weight of the artificial soil]	Replicate	Number of tested mites	Number of living mites after 14 days [no.]	Total mortality	
				No.	%
0.00	1	10	10	0	0.00
	2	10	10		
	3	10	10		
	4	10	10		
	5	10	10		
	6	10	10		
	7	10	10		
	8	10	10		
94.86	1	10	10	0	0.00
	2	10	10		
	3	10	10		
	4	10	10		
132.81	1	10	10	2	5.00
	2	10	10		
	3	10	8		
	4	10	10		
185.93	1	10	10	0	0.00
	2	10	10		
	3	10	10		
	4	10	10		
260.31	1	10	10	1	2.50
	2	10	9		
	3	10	10		
	4	10	10		
364.43	1	10	10	1	2.50
	2	10	9		
	3	10	10		
	4	10	10		
510.20	1	10	10	0	0.00
	2	10	10		
	3	10	10		
	4	10	10		
714.29	1	10	9	2	5.00
	2	10	10		
	3	10	9		
	4	10	10		
1000.00	1	10	10	1	2.50
	2	10	9		
	3	10	10		
	4	10	10		

Number of juvenile mites (Hypoaspis (Geolaelaps) aculeifer) at the end of the experiment

Concentration [mg/kg dry weight of the artificial soil]	Replicate	Number of juveniles	Mean ±SD	Reduction [%]	CV* [%]
0.00	1 2 3 4 5 6 7 8	189 237 173 161 152 160 202 192	183.3 ± 27.95	-	15.25
94.86	1 2 3 4	198 233 210 226	216.8 ± 15.78	-18.28	7.28
132.81	1 2 3 4	139 213 224 187	190.8 ± 37.83	-4.09	19.83
185.93	1 2 3 4	217 197 220 182	204.0 ± 17.87	-11.32	8.76
260.31	1 2 3 4	226 201 229 108	191.0 ± 56.74	-4.23	29.71
364.43	1 2 3 4	200 123 217 208	187.0 ± 43.23	-2.05	23.12
510.20	1 2 3 4	203 210 193 160	191.5 ± 22.13	-4.50	11.56
714.29	1 2 3 4	195 150 226 231	200.5 ± 37.24	-9.41	18.57
1000.00	1 2 3 4	136 108 129 167	135.0* ± 24.43	26.33	18.09

*CV – coefficient of variation

+ - statistically significant difference between the control and the treatment group (Multiple Sequentially-rejective t-test After Bonferroni-Holm, significance level = 0.05, one-sided smaller)

*not determined

The endpoint values showing the impact of the test item on mortality of *Predatory mite* are presented in the table given below.

Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg active substance/kg dry weight of the artificial soil]
LC_{10}	n. d.	n. d.
LC_{20}	n. d.	n. d.
LC_{50}	n. d.	n. d.
NOEC	≥ 1000.00	≥ 99.81
LOEC	> 1000.00	> 99.81

n. d. – not determined due to mathematical reasons

The endpoint values showing the impact of the test item on reproduction of *Predatory mite* are presented in the table given below.

Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg active ingredient/kg dry weight of the artificial soil]
EC_{10}	**907.22** (843.52 – 975.73)	**90.55** (84.19 – 97.39)
EC_{20}	**970.66** (949.20 – 992.60)	**96.88** (94.74 – 99.07)
EC_{50}	**> 1000.00**	**> 99.81**
NOEC	**714.29**	**71.29**
LOEC	**1000.00**	**99.81**

Validity criteria:

The results are considered valid because the following criteria were satisfied in the control:
• mean adult mortality: 0.0% (criterion: ≤ 20%),
• the mean number of juveniles per vessel at the end of the test: 183.3 (criterion: ≥ 50 juveniles at the end of the test,
• the coefficient of variation for the number of juveniles: 15.3 (criterion: ≤ 30%).

Conclusions

~~In the 14 - day *Hypoaspis* reproduction study with M-100SC-OR2-C, EC_{10} of 907.22 mg of test item/ kg dry weight of the artificial soil (equal to 90.55 mg of active substance/ kg dry weight of the artificial soil) for reproduction was obtained and thus, it is proposed to be used in the risk assessment.~~

In the 14 - day *Hypoaspis* reproduction study with Juzan Extra 100 SC, NOEC of 714.29 mg of test item/ kg dry weight of the artificial soil (equal to 71.29 mg of active substance/ kg dry weight of the artificial soil) for reproduction was obtained and thus, it is proposed to be used in the risk assessment (the worst case).

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

Not relevant.

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

Comments of zRMS:	The study is considered as acceptable. All validity criteria were met.

The following validity criteria were met during the study:
The coefficients of variation (CV) in the control group were 2.0; 3.5; 6.3; 4.8; 11.1; 2.4; and 4.9 %, after 0, 7, 14, 28, 42, 56 and 70 days of incubation. The validity criterion was met, because the variation between replicate control samples is less than ± 15%.

Deviation from the study:

1. Deviation from the Study Plan, Guidelines and SOP/B/13 concerning the incorrect test room temperature occurred. OECD 216 states that the test temperature is 20±2°C. During the test, temperature out the range specified in the guideline were recorded six times and minimum measured temperature was 17.8°C while the maximum measured temperature was 23.1°C.
Described deviations did not affect the study results. All validity criteria were met.

Agreed endpoints:

Nitrate formation rate* [mg nitrate/kg dry weight of soil/day] for selected time intervals

Interval of sampling days (X-Y)	Control					PEC 2.08 mg of the test item/kg dry weight of soil (0.21 mg of mesotrione/kg dry weight of soil)					5xPEC 10.40 mg of the test item/kg dry weight of soil (1.04 of mesotrione /kg dry weight of soil)				
	Replicate			Mean		Replicate			Mean		Replicate			Mean	
0-7	0.546	2.079	1.364	1.330	± 0.77	-2.360	-3.046	-1.176	-2.194	± 0.95*	-2.784	-4.243	-2.956	-3.328	± 0.80*
7-14	10.203	6.700	7.274	8.059	± 1.88	10.436	14.729	12.437	12.534	± 2.15	11.237	10.023	10.880	10.713	± 0.62
14-28	4.814	3.145	3.402	3.787	± 0.90	2.649	3.279	3.601	3.176	± 0.48	5.414	5.501	5.164	5.360	± 0.17
28-42	2.348	-1.420	-0.964	-0.012	± 2.06	-2.521	-0.010	1.634	-0.299	± 2.09	0.515	-0.986	-0.757	-0.409	± 0.81
42-56	5.609	4.497	4.803	4.970	± 0.57	4.656	3.926	3.337	3.973	± 0.66*	3.604	3.461	3.605	3.557	± 0.08*
56-70	7.386	5.006	4.824	5.739	± 1.43	6.981	5.872	7.529	6.794	± 0.84	6.304	5.566	6.043	5.971	± 0.37
Additional calculation at the request of the Sponsor:															
0-7*	0.546	2.079	1.364	1.330	± 0.77	-2.360	-3.046	-1.176	-2.194	± 0.95*	-2.784	-4.243	-2.956	-3.328	± 0.80*
0-14*	5.766	4.015	4.302	4.694	± 0.94	4.121	6.268	5.122	5.170	± 1.07	3.955	3.348	3.776	3.693	± 0.31
0-28*	4.754	3.920	4.048	4.241	± 0.45	3.910	4.225	4.386	4.174	± 0.24	4.553	4.597	4.428	4.526	± 0.09
0-42*	3.610	2.354	2.506	2.823	± 0.69	1.942	2.779	3.327	2.683	± 0.70	3.189	2.689	2.765	2.881	± 0.27
0-56*	3.519	3.242	3.318	3.360	± 0.14	3.176	2.993	2.846	3.01	± 0.17*	3.062	3.026	3.062	3.050	± 0.02*
0-70*	4.165	3.689	3.653	3.836	± 0.29	3.801	3.579	3.910	3.763	± 0.17	3.701	3.553	3.648	3.634	± 0.08

*Nitrate formation rates were calculated as follows:

$$\text{Nitrate formation rate} = \frac{\text{Concentration of nitrate on day Y} - \text{Concentration of nitrate on day X}}{Y - X \text{ days}} \text{ [mg/kg dry weight of soil/day]}$$

* statistically significant differences in nitrate concentrations between the control soil and the soil treated with the test item (Levene's test for homogeneous variances, Alpha = 0.05, one-sided smaller)
Mean, SD based on the data obtained from ToxRat statistical analysis (Appendix No. 6).
*Mean, SD based on the data obtained from ToxRat statistical analysis (Appendix No. 7).

The difference in the nitrate formation rate between control soil and treated soils exceed 25% on 28 day of analysis and measurements were continued until the difference between PEC, 5xPEC and the control soil was lower than 25% which occurred on day 70.

No negative effect > 25% at 70 d at 10.40 mg product/kg dry weight of soil (1.04 mg a.s./ kg dry weight of soil)

Reference: KCP 10.5

Report: Soil Microorganisms: Nitrogen Transformation Test according to the OECD Guideline No. 216 (2000), STUDY CODE: EMI/4/11/2021, Ecomelius Institute Sp. z o. o., Swoboda T. 2021

Guideline(s): Yes. According to the OECD Guideline for the Testing of Chemicals No. 216 (2000)

Deviations: Yes.
1. Deviation from the Study Plan, Guidelines and SOP/B/13 concerning the incorrect test room temperature occurred. OECD 216 states that the test temperature is 20±2°C. During the test, temperature out the range specified in the guideline were recorded six times and minimum measured temperature was 17.8°C while the maximum measured temperature was 23.1°C.

	Described deviations did not affect the study results. All validity criteria were met.
GLP:	Yes
Acceptability:	Yes

Materials and methods

1. Test material: M-100SC-OR2-C

2. Batch number: 1/2021
   Concentration of mesotrione 103.8 g/L

3. Soil: Agricultural soil (Type 5M) purchased from LUFA Speyer Obere Langgasse 40, 67346 Speyer.

4. Test design: Three portions of soil with lucerne weighing 1808.5 g each: one control group and two groups containing the test item. Every portion was divided into three replicates weighing about 600 g each. Test duration: 70 days.

5. Concentrations of the test item:

   PEC: 2.08 mg of the test item/kg dry weight of soil (0.21 mg of mesotrione/kg dry weight of soil), 5xPEC: 10.4 mg of the test item/kg dry weight of soil (1.04 mg of mesotrione/kg of dry weight soil).

The aim of this study was to detect long-term adverse effects of the test item (M-100SC-OR2- C) on the process of nitrogen transformation in aerobic surface soils.
The soil was divided into three portions – two treated portions and control soil; each of them was divided into three replicates.
On certain days of experiment soil samples from each test vessel were collected and soil extracts with 0.1M KCl were prepared. Particle-free soil extracts were frozen from -20.4 to -22.8°C and after defrosting quantities of nitrate were determined in it. The method was based on spectrophotometrical measurement. The nitrate formation rate in each treated group was compared to that in the control and the percent deviation of the treated from the control was calculated.

Results:
The difference in the nitrate formation rate between control soil and treated soils exceed 25% on 28 day of analysis and measurements were continued until the difference between PEC, 5xPEC and the control soil was lower than 25% which occurred on day 70.

The nitrate formation rate [mg/kg dry weight of soil/day] for selected time intervals of soil incubation, i.e. 0 - 7, 7 – 14, 14 – 28, 28 – 42, 42 – 56, 56 – 70 days and at 0-7, 0-14, 0-28, 0- 42, 0-56 and 0-70 days.

Interval of sampling days (X-Y)	Control						PEC 2.08 mg of the test item/kg dry weight of soil (0.21 mg of mesotrione/kg dry weight of soil)						5xPEC 10.40 mg of the test item/kg dry weight of soil (1.04 of mesotrione /kg dry weight of soil)					
	Replicate			Mean			Replicate			Mean			Replicate			Mean		
0-7	0.546	2.079	1.364	1.330	±	0.77	-2.360	-3.046	-1.176	-2.194	±	0.95+	-2.784	-4.243	-2.956	-3.328	±	0.80+
7-14	10.203	6.700	7.274	8.059	±	1.88	10.436	14.729	12.437	12.534	±	2.15	11.237	10.023	10.880	10.713	±	0.62
14-28	4.814	3.145	3.402	3.787	±	0.90	2.649	3.279	3.601	3.176	±	0.48	5.414	5.501	5.164	5.360	±	0.17
28-42	2.348	-1.420	-0.964	-0.012	±	2.06	-2.521	-0.010	1.634	-0.299	±	2.09	0.515	-0.986	-0.757	-0.409	±	0.81
42-56	5.609	4.497	4.803	4.970	±	0.57	4.656	3.926	3.337	3.973	±	0.66+	3.604	3.461	3.605	3.557	±	0.08+
56-70	7.386	5.006	4.824	5.739	±	1.43	6.981	5.872	7.529	6.794	±	0.84	6.304	5.566	6.043	5.971	±	0.37
Additional calculation at the request of the Sponsor:																		
0-7*	0.546	2.079	1.364	1.330	±	0.77	-2.360	-3.046	-1.176	-2.194	±	0.95+	-2.784	-4.243	-2.956	-3.328	±	0.80+
0-14*	5.766	4.015	4.302	4.694	±	0.94	4.121	6.268	5.122	5.170	±	1.07	3.955	3.348	3.776	3.693	±	0.31
0-28*	4.754	3.920	4.048	4.241	±	0.45	3.910	4.225	4.386	4.174	±	0.24	4.553	4.597	4.428	4.526	±	0.09
0-42*	3.610	2.354	2.506	2.823	±	0.69	1.942	2.779	3.327	2.683	±	0.70	3.189	2.689	2.765	2.881	±	0.27
0-56*	3.519	3.242	3.318	3.360	±	0.14	3.176	2.993	2.846	3.01	±	0.17+	3.062	3.026	3.062	3.050	±	0.02+
0-70*	4.165	3.689	3.653	3.836	±	0.29	3.801	3.579	3.910	3.763	±	0.17	3.701	3.553	3.648	3.634	±	0.08

*Nitrate formation rates were calculated as follows:

Percent deviation from the control in nitrate formation rate calculated for selected time intervals i.e. 0 - 7, 7 – 14, 14 – 28, 28 – 42, 42 – 56, 56 – 70 days and additionaly: 0-7, 0-14, 0-28, 0-42, 0-56 and 0-70 days.

Day of incubation	PEC 2.08 mg of the test item/kg dry weight of soil (0.21 mg of mesotrione/kg dry weight of soil)	5xPEC 10.40 mg of the test item/kg dry weight of soil (1.04 of mesotrione /kg dry weight of soil)
0-7	265.0	350.3
7-14	-55.5	-32.9
14-28	16.1	-41.5
28-42	-2391.7	-3311.1
42-56	20.1	28.4
56-70	-18.4	-4.0
Additional calculation at the request of the Sponsor:		
0-7*	265.0	350.3
0-14*	-10.1	21.3
0-28*	1.6	-6.7
0-42*	5.0	-2.0
0-56*	10.6	9.2
0-70*	1.9	5.3

The data obtained from ToxRat statistical analysis (Appendix No. 6).

* The data obtained from ToxRat statistical analysis (Appendix No. 7).

"-" higher formation rate of nitrate as compared to control

Validity criteria:
The coefficients of variation (CV) in the control group were 2.0; 3.5; 6.3; 4.8; 11.1; 2.4; and 4.9 %, after 0, 7, 14, 28, 42, 56 and 70 days of incubation. The validity criterion was met, because the variation between replicate control samples is less than ± 15%.

Conclusions:

The difference in the nitrate formation rate between control soil and treated soils exceed 25% on 28 day of analysis and measurements were continued until the difference between PEC, 5xPEC and the control soil was lower than 25% which occurred on day 70.

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 is considered as acceptable. All validity criteria were met. The following validity criteria were met during the study: • Seedling emergence in the control was at least 70% • the control seedlings did not exhibit any visible phytotoxic symptoms, • Mean survival of plants in control was 100% for every species (required at least 90%) • Environmental conditions and soil were identical for all used in the experiment plants species Deviations in the study: 1. A deviation from the Study Plan concerning decreases air humidity in the plant growth chamber. According to the Study Plan, the air humidity should very from 65 to 95%. A deviation from the Study Plan, OECD Guideline No. 208, SOP/B/9 concerning increases air humidity in the plant growth chamber. However, it did not have any influence on the results of the study. According to OECD Guideline No. 208, the air humidity between 45 – 95% is recommended to maintain good plant vigour. Moreover, the plants exposed good vigour throughout the experiment and the validity criteria were met. The values above 95% were caused by condensation of water and deposition on the humidity recorder sensor. The deviation did not affect the results of the study. All validity criteria were met. **Agreed endpoints:**

	Cabbage *Brassica oleracea var. capitata*	Tomato *Solanum lycopersicon*	Soybean *Glycine max. (G. soja)*	Lettuce *Lactuca sativa*	Onion *Allium cepa*	Oats *Avena sativa*
Plant emergence at the end of the experiment						
ER_{10}	> 1500.00	126.62 (27.43 – 259.41)	> 1500.00	32.73 (11.10 – 96.57)	194.25 (96.61 – 279.58)	> 1500.00
ER_{25}	> 1500.00	535.58 (261.81 – 1319.93)	> 1500.00	168.09 (86.48 – 326.72)	380.49 (258.88 – 491.25)	> 1500.00
ER_{50}	> 1500.00	> 1500.00 (1001.20 – > 1500.00)	> 1500.00	863.17 (379.15 – > 1500.00)	745.30 (581.66 – 1029.49)	> 1500.00
NOER	≥ 1500.00	≥ 1500.00	≥ 1500.00	240.00	131.69	≥ 1500.00
LOER	> 1500.00	> 1500.00	> 1500.00	600.00	197.53	> 1500.00
Plant number at the end of the experiment						
LR_{10}	> 1500.00	689.15 (367.10 – 1293.70)	> 1500.00	472.10 (100.88 – 806.75)	825.16 (593.61 – 1147.02)	> 1500.00
LR_{25}	> 1500.00	1308.62 (739.00 – > 1500.00)	> 1500.00	888.35 (468.04 – > 1500.00)	1068.72 (841.57 – 1357.17)	> 1500.00
LR_{50}	> 1500.00	> 1500.00 (999.90 – > 1500.00)	> 1500.00	> 1500.00 (1013.92 – > 1500.00)	1384.17 (1056.07 – >1500.00)	> 1500.00
NOER	≥ 1500.00	600.00	≥ 1500.00	240.00	666.67	≥ 1500.00
LOER	> 1500.00	1500.00	> 1500.00	600.00	1000.00	> 1500.00
Shoot length (plants without roots)						
ER_{10}	22.08 (16.17 – 28.61)	20.36 (15.39 – 25.72)	128.58 (61.42 – 196.48)	16.31 (10.35 – 25.69)	107.01 (87.71 – 125.38)	849.20 (235.47 – >1500.00)
ER_{25}	94.87 (79.62 – 110.56)	70.09 (59.42 – 81.00)	642.01 (511.14 – 794.96)	69.06 (52.79 – 90.34)	231.08 (207.70 – 252.89)	> 1500.00
ER_{50}	407.71 (360.30 – 464.89)	241.32 (214.77 – 272.50)	> 1500.00	343.30 (266.74– 441.85)	499.03 (463.24 – 541.53)	> 1500.00
NOER	15.36	15.36	197.53	15.36	131.69	≥ 1500.00
LOER	38.40	38.40	296.30	38.40	197.53	> 1500.00

Plant dry weight (plants without roots)						
ER_{10}	21.21 (13.82 – 29.72)	20.33 (15.05– 26.05)	104.35 (23.52 – 194.21)	18.97 (9.71 – 30.41)	91.26 (52.97 – 129.48)	210.39 (10.29 – 397.17)
ER_{25}	83.96 (65.17 – 103.79)	66.20 (55.19 – 77.40)	747.14 (536.16 – 1106.26)	72.42 (48.64 – 98.22)	220.18 (162.57 – 271.65)	> 1500.00 (1067.29 – >1500.00)
ER_{50}	387.36 (325.77 – 466.81)	215.57 (191.38 – 243.20)	> 1500.00	320.86 (249.65 – 421.79)	531.24 (453.83 – 626.59)	> 1500.00
NOER	15.36	15.36	197.53	38.40	131.69	296.30
LOER	38.40	38.40	296.30	96.00	197.53	444.44
Phytotoxic effects						
ER_{10}	51.68 (40.70 – 65.62)	35.29 (28.72 – 43.36)	560.64 (510.76 – 615.38)	64.19 (50.95 – 80.88)	313.28 (281.04 – 349.22)	1204.29 (1096.35 – 1322.86)
ER_{25}	187.62 (163.93 – 214.73)	125.53 (111.19 – 141.71)	> 1500.00	187.99 (163.93 – 215.57)	544.68 (511.32 – 580.21)	> 1500.00
ER_{50}	681.11 (611.11 – 759.13)	446.53 (408.04 – 488.66)	> 1500.00	550.50 (497.33 – 609.34)	947.00 (902.66 – 993.52)	> 1500.00
NOER	15.36	15.36	197.53	15.36	197.53	296.30
LOER	38.40	38.40	296.30	38.40	296.30	444.44

The lowest endpoints from the study:

Species	Substance	Exposure System	Results	Reference
Juzan Extra 100 SC				
Brassica oleracea var. capitata$_d$ *Solanum lycopersicon*$_d$ *Glycine max*$_d$ *Lactuca sativa*$_d$ *Allium cepa*$_m$ *Avena sativa*$_m$	Juzan Extra 100 SC (M-100SC-OR2-C)	14 d Seedling emergence	ER_{50} emergence = 745.30 ml product/ha (*Allium cepa*) ER_{50} plant number = 1384.17 ml product/ha (*Allium cepa*) **ER_{50} plant dry weight = 215.57**	Dec W, 2021 Study Code: EMI/4/10/2021

				ml product/ha (*Solanum lycopersicon*) ER_{50} plant height = 241.32 ml product/ha (*Solanum lycopersicon*) ER_{50} phytotoxicity = 446.53 ml product/ha (*Solanum lycopersicon*)	

Reference:	KCP 10.6.2/01
Report	Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test according to OECD Guideline No. 208 (2006), Dec W, 2021 STUDY CODE: EMI/4/10/2021 Ecomelius Institute Sp. z o. o.
Guideline(s):	Yes. According to the OECD Guideline for the Testing of Chemicals No. 208 "Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test"
Deviations:	Yes. Deviation from the OECD Guideline No. 208 (2006): 1. A deviation from the Study Plan concerning decreases air humidity in the plant growth chamber. According to the Study Plan, the air humidity should very from 65 to 95%. A deviation from the Study Plan, OECD Guideline No. 208, SOP/B/9 concerning increases air humidity in the plant growth chamber. However, it did not have any influence on the results of the study. According to OECD Guideline No. 208, the air humidity between 45 – 95% is recommended to maintain good plant vigour. Moreover, the plants exposed good vigour throughout the experiment and the validity criteria were met. The values above 95% were caused by condensation of water and deposition on the humidity recorder sensor. The deviation did not affect the results of the study. All validity criteria were met.
GLP:	Yes
Acceptability:	Yes

Materials and methods

1. Test material: M-100SC-OR2-C

2. Batch number: 1/2021
   Concentration of mesotrione 103.8 g/

3. Test organism: Test organism: Six plant species were used. These were: Cabbage (*Brassica oleracea var. capitata*), Tomato (*Solanum lycopersicon*), Soybean (*Glycine max. (G. soja)*), Lettuce (*Lactuca sativa*), Onion (*Allium cepa*), Oats (*Avena sativa*).

4. Test design:

The study, aimed at evaluating the effect of M-100SC-OR2-C on seedling emergence and seedling growth of 6 terrestrial plants, was conducted on 4 dicotyledonous and 2 monocotyledonous species.

The test item was sprayed onto the soil surface. Seven application rates (1500.00, 1000.00, 666.67, 444.44, 296.30, 197.53, 131.69 mL test item/ha) were used for oats, soybean, onion and seven application rates (1500.00, 600.00, 240.00, 96.00, 38.40, 15.36, 6.14 mL test item/ha) for tomato, lettuce, cabbage. There was also a concurrent control group. Selected number of plants per pot provide the adequate growth conditions and avoid overcrowding during the experiment.

The number of seeds per pot as well as the total number of seeds per concentration for each of the tested species is presented below:
- cabbage: 3 plants/pot – 21 plants/rate (7 pots/rate)
- tomato: 2 plants/pot – 20 plants/rate (10 pots/rate)
- soybean: 2 plants/pot – 20 plants/rate (10 pots/rate)
- lettuce - 5 plants/pot – 20 seeds/rate (4 pots/rate)
- onion: 5 plants/pot – 20 plants/rate (4 pots/rate)
- oats - 5 plants/pot – 20 plants/rate (4 pots/rate).

The experiment was conducted in a special plant growth chamber where suitable environmental conditions were provided. During the study, the plants were observed for emergence (every day before the emergence of 50% of the control seedlings and then every 2 – 3 days) and visual phytotoxicity (7 and 14 day after the emergence of 50% of the control seedlings). Phytotoxic effects and plant damage were recorded. The experiment finished 14 days after the germination of 50% of the control seedlings. 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.

Results and discussions:

The results concerning the emergence, the shoot length, and the dry weight were statistically analyzed to determine the ER_{10}, ER_{25}, ER_{50}, NOER, and LOER.

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

	Cabbage *Brassica oleracea var. capitata*	Tomato *Solanum lycopersicon*	Soybean *Glycine max. (G. soja)*	Lettuce *Lactuca sativa*	Onion *Allium cepa*	Oats *Avena sativa*
Plant emergence at the end of the experiment						
ER_{10}	> 1500.00	126.62 (27.43 – 259.41)	> 1500.00	32.73 (11.10 – 96.57)	194.25 (96.61 – 279.58)	> 1500.00
ER_{25}	> 1500.00	535.58 (261.81 – 1319.93)	> 1500.00	168.09 (86.48 – 326.72)	380.49 (258.88 – 491.25)	> 1500.00
ER_{50}	> 1500.00	> 1500.00 (1001.20 – > 1500.00)	> 1500.00	863.17 (379.15 – > 1500.00)	745.30 (581.66 – 1029.49)	> 1500.00
NOER	≥ 1500.00	≥ 1500.00	≥ 1500.00	240.00	131.69	≥ 1500.00
LOER	> 1500.00	> 1500.00	> 1500.00	600.00	197.53	> 1500.00
Plant number at the end of the experiment						
LR_{10}	> 1500.00	689.15 (367.10 – 1293.70)	> 1500.00	472.10 (100.88 – 806.75)	825.16 (593.61 – 1147.02)	> 1500.00
LR_{25}	> 1500.00	1308.62 (739.00 – > 1500.00)	> 1500.00	888.35 (468.04 – > 1500.00)	1068.72 (841.57 – 1357.17)	> 1500.00
LR_{50}	> 1500.00	> 1500.00 (999.90 – > 1500.00)	> 1500.00	> 1500.00 (1013.92 – > 1500.00)	1384.17 (1056.07 – >1500.00)	> 1500.00
NOER	≥ 1500.00	600.00	≥ 1500.00	240.00	666.67	≥ 1500.00
LOER	> 1500.00	1500.00	> 1500.00	600.00	1000.00	> 1500.00
Shoot length (plants without roots)						
ER_{10}	22.08 (16.17 – 28.61)	20.36 (15.39 – 25.72)	128.58 (61.42 – 196.48)	16.31 (10.35 – 25.69)	107.01 (87.71 – 125.38)	849.20 (235.47 – >1500.00)
ER_{25}	94.87 (79.62 – 110.56)	70.09 (59.42 – 81.00)	642.01 (511.14 – 794.96)	69.06 (52.79 – 90.34)	231.08 (207.70 – 252.89)	> 1500.00
ER_{50}	407.71 (360.30 – 464.89)	241.32 (214.77 – 272.50)	> 1500.00	343.30 (266.74– 441.85)	499.03 (463.24 – 541.53)	> 1500.00
NOER	15.36	15.36	197.53	15.36	131.69	≥ 1500.00
LOER	38.40	38.40	296.30	38.40	197.53	> 1500.00
Plant dry weight (plants without roots)						
ER_{10}	21.21 (13.82 – 29.72)	20.33 (15.05– 26.05)	104.35 (23.52 – 194.21)	18.97 (9.71 – 30.41)	91.26 (52.97 – 129.48)	210.39 (10.29 – 397.17)
ER_{25}	83.96 (65.17 – 103.79)	66.20 (55.19 – 77.40)	747.14 (536.16 – 1106.26)	72.42 (48.64 – 98.22)	220.18 (162.57 – 271.65)	> 1500.00 (1067.29 – >1500.00)
ER_{50}	387.36 (325.77 – 466.81)	215.57 (191.38 – 243.20)	> 1500.00	320.86 (249.65 – 421.79)	531.24 (453.83 – 626.59)	> 1500.00
NOER	15.36	15.36	197.53	38.40	131.69	296.30
LOER	38.40	38.40	296.30	96.00	197.53	444.44
Phytotoxic effects						
ER_{10}	51.68 (40.70 – 65.62)	35.29 (28.72 – 43.36)	560.64 (510.76 – 615.38)	64.19 (50.95 – 80.88)	313.28 (281.04 – 349.22)	1204.29 (1096.35 – 1322.86)
ER_{25}	187.62 (163.93 – 214.73)	125.53 (111.19 – 141.71)	> 1500.00	187.99 (163.93 – 215.57)	544.68 (511.32 – 580.21)	> 1500.00
ER_{50}	681.11 (611.11 – 759.13)	446.53 (408.04 – 488.66)	> 1500.00	550.50 (497.33 – 609.34)	947.00 (902.66 – 993.52)	> 1500.00
NOER	15.36	15.36	197.53	15.36	197.53	296.30
LOER	38.40	38.40	296.30	38.40	296.30	444.44

1. The test item, i.e., M-100SC-OR2-C applied at rates ranging from 6.14 to 1500.00 mL/ha had a varied impact on seedling emergence and early growth of selected plant species. The impact depended on the rate and test species.
2. The test item caused mortality of tomato and lettuce at the rates equal to 600.00 and 1500.00 mL/ha.

Plant mortality of onion at the rates equal to 1000.00 and 1500.00 mL/ha was also observed. LR_{50} values determined for cabbage, soybean, and oats based on the plant number at the end of the experiment were above 1500.00 mL/ha. The most sensitive plant species was onion with LR_{50} equal to 745.30 ml/ha.
3. Based on NOER, ER_{10}, ER_{25} and ER_{50} values determined from the shoot length it was proved that the test item does not inhibit the process of growth of oats. The most sensitive plant was tomato with ER_{50} of 241.32 ml/ha.
4. Based on NOER, ER_{10}, ER_{25} and ER_{50} values determined from the dry shoot weight it was proved that the test item slightly inhibited the process of growth of oats. The most sensitive plant was tomato, with ER_{50} values of 215.57 mL/ha.
5. Phototoxic symptoms, i.e., stunted growth, wilting, spots, chlorosis, necrosis, and dead plants were observed. Based on NOER, ER_{10}, ER_{25} and ER_{50} values determined from the phytotoxic symptoms it was proved that the test item slightly affected the oats. The most sensitive species was tomato with ER_{50} of 446.53 mL/ha.

Validity of the test:

- Seedling emergence in the control was at least 70%
- the control seedlings did not exhibit any visible phytotoxic symptoms,
- Mean survival of plants in control was 100% for every species (required at least 90%)
- Environmental conditions and soil were identical for all used in the experiment plants species

Conclusion:
The lowest ER_{50} was determined for tomato (plant dry weight) and is equal to 215.57 mL of the test item/ ha.

Comments of zRMS:	The study is considered as acceptable. All validity criteria were met. The following validity criteria were met during the study: • Seedling emergence in the control was at least 70% • In none of the control replications of any plants species there were any signs of intoxications visible • Mean survival of plants in control was 100% for every species (required at least 90%) • Environmental conditions and soil were identical for all used in the experiment plants species Deviations in the study: 1. A deviation from the Study Plan concerning short-term decreases in air temperature in the plant growth chamber. The air temperature was between 15.6 – 26.2ºC. 2. A deviation from the Study Plan concerning decreases air humidity in the plant growth chamber. According to the Study Plan, the air humidity should very from 65 to 95%. A deviation from the Study Plan, OECD Guideline No. 227 concerning increases air humidity in the plant growth chamber. The deviation did not affect the results of the study. All validity criteria were met. Agreed endpoints:

	Cabbage *Brassica oleracea var. capitata*	Tomato *Solanum lycopersicon*	Soybean *Glycine max. (G. soja)*	Lettuce *Lactuca sativa*	Onion *Allium cepa*	Oats *Avena sativa*
Plant number at the end of the experiment						
LR_{10}	>1500.00	95.91 (56.48 – 128.72)	>1500.00	131.52 (98.24 – 176.08)	103.94 (50.41 – 156.30)	>1500.00
LR_{25}	>1500.00	137.38 (94.86 – 175.78)	>1500.00	181.24 (146.84 – 223.70)	191.19 (119.12 – 264.71)	>1500.00
LR_{50}	>1500.00	204.78 (158.21 – 265.12)	>1500.00	239.97 (199.88 – 288.11)	351.67 (253.01 – 498.81)	>1500.00
NOER	≥1500.00	38.40	≥1500.00	96.00	96.00	≥1500.00
LOER	>1500.00	96.00	>1500.00	240.00	240.00	>1500.00
Shoot length (plants without roots)						
ER_{10}	83.02 (45.34 – 125.80)	2.78 (1.93 – 4.02)	8.62 (5.81 – 12.78)	4.98 (3.66 – 6.78)	80.16 (53.84 – 119.35)	>1500.00
ER_{25}	808.14 (598.15 – 1165.38)	17.53 (14.37 – 21.38)	66.89 (54.07 – 82.76)	16.61 (13.81– 19.99)	233.24 (180.76 – 300.96)	>1500.00
ER_{50}	>1500.00	135.39 (105.34 – 174.01)	651.74 (532.22 – 798.10)	63.37 (54.22 – 74.06)	764.15 (530.35 – 1101.02)	>1500.00
NOER	96.00	6.14	15.36	6.14	96.00	≥1500.00
LOER	240.00	15.36	38.40	15.36	240.00	>1500.00

	Cabbage *Brassica oleracea var. capitata*	Tomato *Solanum lycopersicon*	Soybean *Glycine max. (G. soja)*	Lettuce *Lactuca sativa*	Onion *Allium cepa*	Oats *Avena sativa*
Plant dry weight (plants without roots)						
ER_{10}	5.64 (2.70 – 9.68)	3.21 (1.99 – 5.19)	12.17 (7.57 – 19.57)	3.74 (2.55 – 5.48)	16.93 (6.34 – 45.19)	>1500.00
ER_{25}	31.62 (20.55 – 44.26)	10.59 (7.80 – 14.39)	66.77 (50.69 – 87.94)	11.28 (8.74 – 14.55)	63.07 (34.66 – 114.74)	>1500.00
ER_{50}	214.70 (165.52 – 284.02)	39.85 (31.84 – 49.87)	442.40 (352.35 – 555.47)	38.44 (31.97 – 46.23)	271.94 (170.14 – 434.67)	>1500.00
NOER	15.36	2.46	15.36	2.46	38.40	≥1500.00
LOER	38.40	6.14	38.40	6.14	96.00	>1500.00
Phytotoxic effects						
ER_{10}	110.44 (96.43 – 126.49)	13.37 (11.05 – 16.19)	29.98 (22.51 – 39.92)	20.20 (15.19 – 26.85)	248.33 (185.59 – 332.28)	>1500.00
ER_{25}	329.62 (305.36 – 355.80)	35.18 (30.98 – 39.95)	168.68 (144.63 – 196.73)	47.58 (39.36 – 57.52)	406.61 (335.68 – 492.54)	>1500.00
ER_{50}	1110.75 (1030.25 – 1197.55)	103.07 (94.02 – 112.98)	1149.95 (962.02 – 1374.58)	123.28 (107.49 – 141.40)	703.26 (612.44 – 807.55)	>1500.00
NOER	38.40	2.46	15.36	2.46	96.00	≥1500.00
LOER	96.00	6.14	38.40	6.14	240.00	>1500.00

The lowest endpoints from the study:

Species	Substance	Exposure System	Results	Reference
Juzan Extra 100 SC				
Brassica oleracea var. capitata$_d$ *Solanum lycopersicon* *Glycine max* *Lactuca sativa* *Allium cepa* *Avena sativa*	Juzan Extra 100 SC (M-100SC-OR2-C)	21 d Vegetative vigour	LR_{50} plant number = 204.78 ml product/ha (*Solanum lycopersicon*) **ER_{50} plant dry weight = 38.44 ml product/ha (*Lactuca sativa*)**	Dec W, 2021 Study Code: EMI/4/10/2021

				ER_{50} plant height = 63.37 ml product/ha (*Lactuca sativa*) ER_{50} phytotoxi-city = 103.07 ml product/ha (*Solanum lycopersicon*)	

Reference:	KCP 10.6.2/02
Report	Terrestrial Plant Test: Vegetative Vigour Test according to OECD Guideline No. 227 (2006), Dec W, 2021 STUDY CODE: EMI/4/9/2021 Ecomelius Institute Sp. z o. o.
Guideline(s):	Yes. According to the OECD Guideline for the Testing of Chemicals No. 227 "Terrestrial Plant Test: Vegetative Vigour Test".
Deviations:	Yes. Deviation from the OECD Guideline No. 208 (2006): 1. A deviation from the Study Plan concerning short-term decreases in air temperature in the plant growth chamber. The air temperature was between 15.6 – 26.2ºC. 2. A deviation from the Study Plan concerning decreases air humidity in the plant growth chamber. According to the Study Plan, the air humidity should very from 65 to 95%. A deviation from the Study Plan, OECD Guideline No. 227 concerning increases air humidity in the plant growth chamber. The deviation did not affect the results of the study. All validity criteria were met.
GLP:	Yes
Acceptability:	Yes

Materials and methods

1. Test material: M-100SC-OR2-C

2. Batch number: 1/2021
   Concentration of mesotrione 103.8 g/

3. Test organism: Test organism: Six plant species were used. These were: Cabbage (*Brassica oleracea var. capitata*), Tomato (*Solanum lycopersicon*), Soybean (*Glycine max. (G. soja)*), Lettuce (*Lactuca sativa*), Onion (*Allium cepa*), Oats (*Avena sativa*).

4. Test design:

The study, aimed at evaluating the effect of M-100SC-OR2-C on vegetative vigour of 6 terrestrial plants, was conducted on 4 dicotyledonous and 2 monocotyledonous species.

Seeds of the test plant species were sown in plastic pots (10 seeds/pot for carrot, oats, perennial ryegrass and 6 seeds/pot for cabbage, pea, sunflower). 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:
- cabbage: 3 plants/pot – 21 plants/rate (7 pots/rate)
- tomato: 2 plants/pot – 20 plants/rate (10 pots/rate)
- soybean: 2 plants/pot – 20 plants/rate (10 pots/rate)
- lettuce - 5 plants/pot – 20 seeds/rate (4 pots/rate)
- onion: 5 plants/pot – 20 plants/rate (4 pots/rate)
- oats - 5 plants/pot – 20 plants/rate (4 pots/rate).

The pot is defined as a replicate. The test item was sprayed onto the plants. Depending on the test species, ten or seven or six rates of the test item were used in the experiment. Untreated control group was conducted simultaneously. The treated and the control groups were divided into four replicates for lettuce, oats, and onion, 7 replicates for cabbage and 10 for tomato and soybean.

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 phytotoxic effects, the shoot length, the dry weight, and the number of plants at the end of the experiment were statistically analyzed to determine the ER_{10}, ER_{25}, ER_{50}, NOER, and LOER.

Results and discussions:

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 ER10, ER25, ER50, NOER, and LOER.

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

	Cabbage *Brassica oleracea var. capitata*	**Tomato** *Solanum lycopersicon*	**Soybean** *Glycine max. (G. soja)*	**Lettuce** *Lactuca sativa*	**Onion** *Allium cepa*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
LR_{10}	>1500.00	95.91 (56.48 – 128.72)	>1500.00	131.52 (98.24 – 176.08)	103.94 (50.41 – 156.30)	>1500.00
LR_{25}	>1500.00	137.38 (94.86 – 175.78)	>1500.00	181.24 (146.84 – 223.70)	191.19 (119.12 – 264.71)	>1500.00
LR_{50}	>1500.00	204.78 (158.21 – 265.12)	>1500.00	239.97 (199.88 – 288.11)	351.67 (253.01 – 498.81)	>1500.00
NOER	≥1500.00	38.40	≥1500.00	96.00	96.00	≥1500.00
LOER	>1500.00	96.00	>1500.00	240.00	240.00	>1500.00
Shoot length (plants without roots)						
ER_{10}	83.02 (45.34 – 125.80)	2.78 (1.93 – 4.02)	8.62 (5.81 – 12.78)	4.98 (3.66 – 6.78)	80.16 (53.84 – 119.35)	>1500.00
ER_{25}	808.14 (598.15 – 1165.38)	17.53 (14.37 – 21.38)	66.89 (54.07 – 82.76)	16.61 (13.81– 19.99)	233.24 (180.76 – 300.96)	>1500.00
ER_{50}	>1500.00	135.39 (105.34 – 174.01)	651.74 (532.22 – 798.10)	63.37 (54.22 – 74.06)	764.15 (530.35 – 1101.02)	>1500.00
NOER	96.00	6.14	15.36	6.14	96.00	≥1500.00
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	Cabbage *Brassica oleracea var. capitata*	**Tomato** *Solanum lycopersicon*	**Soybean** *Glycine max. (G. soja)*	**Lettuce** *Lactuca sativa*	**Onion** *Allium cepa*	**Oats** *Avena sativa*
Plant dry weight (plants without roots)						
ER_{10}	5.64 (2.70 – 9.68)	3.21 (1.99 – 5.19)	12.17 (7.57 – 19.57)	3.74 (2.55 – 5.48)	16.93 (6.34 – 45.19)	>1500.00
ER_{25}	31.62 (20.55 – 44.26)	10.59 (7.80 – 14.39)	66.77 (50.69 – 87.94)	11.28 (8.74 – 14.55)	63.07 (34.66 – 114.74)	>1500.00
ER_{50}	214.70 (165.52 – 284.02)	39.85 (31.84 – 49.87)	442.40 (352.35 – 555.47)	38.44 (31.97 – 46.23)	271.94 (170.14 – 434.67)	>1500.00
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ER_{25}	329.62 (305.36 – 355.80)	35.18 (30.98 – 39.95)	168.68 (144.63 – 196.73)	47.58 (39.36 – 57.52)	406.61 (335.68 – 492.54)	>1500.00
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NOER	38.40	2.46	15.36	2.46	96.00	≥1500.00
LOER	96.00	6.14	38.40	6.14	240.00	>1500.00

. The test item, M-100SC-OR2-C applied at rates ranging from 0.39 to 1500.00 mL/ha had a varied impact on vegetative vigour of the test plant species. The impact depended on the rate and species.
2. The test item caused no mortality of cabbag, soybean, and oats at all test item rates. After the application of the test item at the rates equal to 600.00 and 1500.00 mL/ha, 100% of tomato and lettuce mortality was observed. The most sensitive species was tomato with LR50 of 204.78 mL of the test item/ha.
3. Based on NOER, ER10, ER25 and ER50 values determined from the shoot length it was proved that the test item inhibited the process of growth of tomato, soybean, lettuce, and onion. The most sensitive

plant was lettuce with ER50 equal to 63.37 mL of the test item/ha. No oats growth inhibition was observed.
4. Based on NOER, ER10, ER25 and ER50 values determined from the dry shoot weight it was proved that the test item did not inhibit the process of growth of oats. The most sensitive plant was lettuce with ER50 of 38.44 mL of the test item/ha.
5. Based on NOER, ER10, ER25 and ER50 values determined from the phytotoxic symptoms it was proved that the test item did not inhibit the process of growth of oats. The most sensitive plant was tomato with ER50 of 103.07mL of the test item/ha. Phototoxic symptoms, i.e., stunted growth, wilting, chlorosis, necrosis, and dead plants were observed.

Validity of the test:

- Seedling emergence in the control was at least 70%
- In none of the control replications of any plants species there were any signs of intoxications visible
- Mean survival of plants in control was 100% for every species (required at least 90%)
- Environmental conditions and soil were identical for all used in the experiment plants species

Conclusion:
The lowest ER_{50} was determined for lettuce (plant dry weight) and is equal to 38.44 mL of the test item/ ha.

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