FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: MT-565SG-OR2-C

Product name(s): HAKSAR TOP 565 SG

Chemical active substance(s):

MCPA, 550 g/kg
Tribenuron methyl, 15 g/kg

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT (Poland)

(authorization)

Applicant: CIECH Sarzyna S.A.

Submission date: 01/2021

MS Finalisation date: 06/12/2021

Version history

When	What
January 2021	First submission of product authorization.
January 2021	Submission to the Polish Ministry of Agriculture and Rural Development
February 2021	Submission to the evaluation unit
August 2021	zRMS finalised evaluation
December 2021	Final RR

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9 Ecotoxicology (KCP 10)

This application was submitted by CIECH Sarzyna S.A. for approval of the formulation MT-565SG-OR2-C / HAKSAR TOP 565 SG containing following active substances: MCPA, 550 g/kg and Tribenuron methyl, 15 g/kg for use as herbicide in cereals, miscanthus and grasses.

This dRR report Part B reviews only ecotoxicological data (Annex III) and additional information that has not previously been considered within the EU review process.

The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations, and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information is struck through and shaded for transparency.

9.1 Critical GAP and overall conclusions

Table 9.1 1: Table of critical GAPs

GAP , date: January 2021

PPP (product name/code):	MT-565SG-OR2-C/HAKSAR TOP 565 SG	Formulation type:	SG [a, b]
Active substance 1:	MCPA	Conc. of as 1:	550 [c]
Active substance 2:	Tribenuron-methyl	Conc. of as 2:	15 [c]
Safener:	N/A	Conc. of safener:	N/A [c]
Synergist:	N/A	Conc. of synergist:	N/A [c]
Applicant:	CIECH Sarzyna S.A.	Professional use:	☒
Zone(s):	central [d]	Non professional use:	☐
Verified by MS:	~~no~~yes		

Field of use: herbicide

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
					Application				**Application rate**					**Conclusion**						
Use-No. [e]	**Member state(s)**	**Crop and/ or situa-tion (crop destination / purpose of crop)**	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests con-trolled** (additionally: developmental stages of the pest or pest group)	Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. inter-val 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	**PHI** (days)	**Remarks:** e.g. g safen-er/synergist per ha [f]	Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	PL	Winter soft wheat (TRZAW),	F	Annual dicoty-ledonous weeds	Broadcast - foliar	Autumn BBCH	a) 1 b) 1	n.a.	a) 1,00 kg/ha; b) 1,00 kg/ha	a) MCPA 550 g as/ha; tribenuron methyl 15 g	200 / 400	n.a.								

		Winter rye (SECCW), Winter triticale (TTLWI), Winter barley (HORVW)				13 – 23				as/ha b) MCPA 550 g as/ha; tribenuron methyl 15 g as/ha									
2	PL	Winter soft wheat (TRZAW), Spring soft wheat (TRZAS), Winter rye (SECCW), Winter triticale (TTLWI) Winter barley (HORVW) Spring barley (HORVS) Oats (AVESA)	F	Annual dicoty-ledonous weeds	Broadcast - foliar	Spring BBCH 13 – 39	a) 1 b) 1	n.a.	a) 1,00 kg/ha; b) 1,00 kg/ha	a) MCPA 550 g as/ha; tribenuron methyl 15 g as/ha b) MCPA 550 g as/ha; tribenuron methyl 15 g as/ha	200 / 400	n.a.								
3	DE	Winter soft wheat (TRZAW), Winter rye (SECCW), Winter triticale (TTLWI), Winter barley (HORVW)	F	Annual dicoty-ledonous weeds	Broadcast - foliar	Autumn BBCH 13 – 23	a) 1 b) 1	n.a.	a) 1,00 kg/ha; b) 1,00 kg/ha	a) MCPA 550 g as/ha; tribenuron methyl 15 g as/ha b) MCPA 550 g as/ha; tribenuron methyl 15 g as/ha	200 / 400	n.a.	To be further submitted via Mutual Recogni-tion procedure.							
4	DE	Winter soft wheat	F	Annual dicoty-ledonous	Broadcast - foliar	Spring BBCH	a) 1 b) 1	n.a.	a) 1,00 kg/ha; b) 1,00 kg/ha	a) MCPA 550 g as/ha; tribenuron	200 / 400	n.a.	To be further submitted via							

		(TRZAW), Spring barley (HORVS), Winter barley (HORVW) Winter rye (SECCW), Winter triticale (TTLWI),		weeds		13 – 39				methyl 15 g as/ha b) MCPA 550 g as/ha; tribenuron methyl 15 g as/ha			Mutual Recognition procedure.							
5	HU	Winter soft wheat (TRZAW), Spring barley (HORVS)	F	Annual dicotyledonous weeds	Broadcast - foliar	Spring BBCH 13 – 39	a) 1 b) 1	n.a.	a) 1,00 kg/ha; b) 1,00 kg/ha	a) MCPA 550 g as/ha; tribenuron methyl 15 g as/ha b) MCPA 550 g as/ha; tribenuron methyl 15 g as/ha	200 / 400	n.a.	To be further submitted via Mutual Recognition procedure.							
6	RO	Winter soft wheat (TRZAW), Spring barley (HORVS)	F	Annual dicotyledonous weeds	Broadcast - foliar	Spring BBCH 13 – 39	a) 1 b) 1	n.a.	a) 1,00 kg/ha; b) 1,00 kg/ha	a) MCPA 550 g as/ha; tribenuron methyl 15 g as/ha b) MCPA 550 g as/ha; tribenuron methyl 15 g as/ha	200 / 400	n.a.	To be further submitted via Mutual Recognition procedure.							

Minor uses according to Article 51 (zonal uses)																		
7	PL	Durum wheat (TRZDU), Spelt wheat (TRZSP), einkorn wheat (TRZMO) emmer wheat (TRZDI)	F	Annual dicoty-ledonous weeds	Broadcast - foliar	Autumn BBCH 13 – 23	a) 1 b) 1	n.a.	a) 1,00 kg/ha; b) 1,00 kg/ha	a) MCPA 550 g as/ha; tribenuron methyl 15 g as/ha b) MCPA 550 g as/ha; tribenuron methyl 15 g as/ha	200 / 400	n.a.							
8	PL	Durum wheat (TRZDU), Spelt wheat (TRZSP), Spring rye (SECCS), Spring triticale (TTLWS), einkorn wheat (TRZMO), emmer wheat (TRZDI)	F	Annual dicoty-ledonous weeds	Broadcast - foliar	Spring BBCH 13 – 39	a) 1 b) 1	n.a.	a) 1,00 kg/ha; b) 1,00 kg/ha	a) MCPA 550 g as/ha; tribenuron methyl 15 g as/ha b) MCPA 550 g as/ha; tribenuron methyl 15 g as/ha	200 / 400	n.a.							
9	PL	Miscanthus sp. (MISSS)	F	Annual dicoty-ledonous weeds	Broadcast - foliar	BBCH 12 -14	a) 1 b) 1	n.a.	a) 1,00 kg/ha; b) 1,00 kg/ha	a) MCPA 550 g as/ha; tribenuron methyl 15 g as/ha b) MCPA 550 g as/ha; tribenuron methyl 15 g as/ha	200 / 400	n.a.							
10	PL	Grasses grown for seeds	F	Annual dicoty-ledonous weeds	Broadcast - foliar	Spring BBCH 13 – 39	a) 1 b) 1	n.a.	a) 1,00 kg/ha; b) 1,00 kg/ha	a) MCPA 550 g as/ha; tribenuron methyl 15 g as/ha b) MCPA 550 g as/ha; tribenuron methyl 15 g	200 / 400	n.a.							

										as/ha										
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

Review Comments:

GAP presented in the Table 9.1-1 of this document is revised with consideration of the outcome of the evaluation performed in area of ecotoxicology.

9.1.1 Overall conclusions

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

The risk assessment performed for birds and mammals indicate acceptable acute and long-term risk to birds and mammals exposed to MCPA and tribenuron methyl with its relevant plant metabolite following application of HAKSAR TOP 565 SG acc. to intended GAP.

Long-term risk for use of the product in grasses showed unacceptable risk at TIER 1. Higher risk assessment was performed.

For use in grasses the refinement based on TWA for MCPA and PD refinement for tribenuron methyl was provided. Taking to consideration refinement the long-term risk for mammals has been accepted.

The acceptability of the refinement should be considered at the MS level.

As the active substances have a log Pow value of < 3 it was not necessary to consider the risk to birds and mammals from secondary poisoning.

No risk to birds or mammals via drinking water was identified, as the ratio of the effective application rate to relevant endpoints was < 50 (threshold relevant to the Koc of MCPA and tribenuron-methyl).

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

Furthermore, for mixture toxicity acceptable risk could be demonstrated.
For mammals for the long-term mixture toxicity the weight of evidence was performed by the zRMS. It should be considered on MS level.

9.1.1.2 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 in spring and winter cereals (autumn and spring application), and on minor uses for HAKSAR TOP 565 SG, provided that ~~following~~ appropriate risk mitigation measures are taken into account.

~~- a vegetative buffer zone of 20 m to surface water bodies is required.~~

However, as for Poland the relevant scenarios are D3, D4 and R1 only, no unacceptable risk is indicated following the HAKSAR TOP 565 SG application. Thus for Poland, none mitigate on measures are required on the label.

Concerned Member States must decide on the applicability of indicated risk mitigation measures at the product authorization.

9.1.1.3 Effects on bees (KCP 10.3.1)

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 (SAN-

CO/10329/2002 rev.2 (final), October 17, 2002).

The acute risk assessments for the active substances as well as for the formulated product HAKSAR TOP 565 SG 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 spring and winter cereals (autumn and spring application), and on minor uses according to the proposed GAP.
Therefore, a low risk to bees is expected from the application of HAKSAR TOP 565 SG and no mitigation measures are required.
According to Commission regulation (EU) No 284/2013, point 10.3.1. (Effects on bees): The Applicant has provided chronic test on bees on honey bee development with formulated product.

Since acceptable oral and contact acute risk have been concluded for bees exposed to HAKSAR TOP 565 SG at the Tier 1 level and the assessment was performed according to SANCO cMS must decide on the consideration of data requirements of the EFSA Bee guidance (2013) on National level.

9.1.1.4 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).

Based on results obtained for MT-565SG-OR2-C in laboratory studies on *T. pyri* and *A. rhopalosiphi* the 'in-field' and “off-field” HQ values was below the trigger value of 2, indicating that HAKSAR TOP 565 SG poses an acceptable risk to non-target arthropods in both in-field and off-field areas without the need for risk mitigation measures.

9.1.1.5 The in-field and off-field risk from exposure to MCPA and tribenuron methyl applied as HAKSAR TOP 565 SG for the intended uses in spring and winter cereals (autumn and spring application), and on minor uses is indicated to be acceptable for non-target arthropods other than bees also based on Tier 2 for *T.pyri* data without the need for risk mitigation measures.

Review Comments:

The evaluation of the risk for non-target arthropods was performed in accordance with the recommendations of the guidance document ESCORT 2.
Risk assessment presented by the Applicant has been updated.
For the zonal evaluations in the Risk assessment for off-field exposure default factor VDF of 10 was used by zRMS. VDF of 5 should be considered at for National authorisations.

The HQ for recommended species: *Typhlodromus pyri* and *Aphidius rhopalosiphi* is below the ESCORT 2 trigger value of 2, indicating acceptable in-field and off-field risk to non-target arthropods already at tier I level. All calculated HQ values based on a single maximum application rate indicated acceptable risk to cereals and minor crops according to the GAP.

On this basis acceptable risk for in-field and off-field habitats may be concluded with no need for risk mitigation measures.

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

Acute and chronic risk to earthworms arising from the application of HAKSAR TOP 565 SG according to the intended GAP uses can be excluded as the trigger values of 10 for acute risk and 5 for long-term risk were exceeded by far.

Additionally, performed long-term risk assessment for collembola and predatory mites indicates that TER_{lt} is above the trigger value of 5, indicating acceptable risk to soil organisms (other than earthworms) from the proposed uses of HAKSAR TOP 565 SG.

The risk to soil microorganisms is acceptable since effects on the nitrogen transformations are acceptable at concentration which is higher than the maximum relevant PEC soil for the maximum application rate of HAKSAR TOP 565 SG and relevant metabolites of Tribenuron methyl.

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

The risk for non-target plants in the off-crop area is indicated to be acceptable when either 75% drift reduction or a 5 m buffer strip is applied as risk mitigation measures.

Concerned Member States must decide on the applicability of indicated risk mitigation measures at the product authorization.

9.1.1.8 For the proposed use of HAKSAR TOP 565 SG, based on the highest application rate the risk for non-target plants in the off-crop area is indicated to be acceptable when either 75% drift reduction or a 5 m buffer strip is applied as risk mitigation measures.

Review comments:

Risk assessment performed by the Applicant for non-target terrestrial plants was accepted.

Acceptable risk for non-target terrestrial plants could be concluded for HAKSAR TOP 565 SG when following risk mitigation measures are applied:

Winter and spring cereals, grasses:

Deterministic approach:
- 10 m and use of 50% drift reducing technology or,
- 5 m and use of 75% drift reducing technology

Probabilistic approach:
- 1 m and use of 75% drift reducing technology or,
- 5 m with no drift reducing technology to non-agricultural land

Concerned Member States must decide on the applicability of indicated risk mitigation measures at the product authorization.

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

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

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 9.1-1: Critical use pattern of HAKSAR TOP 565 SG grouped according to time of application

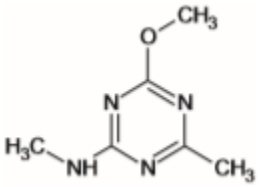
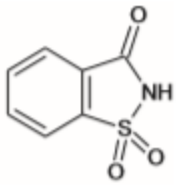
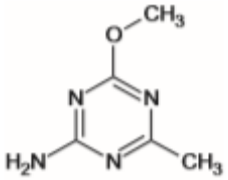
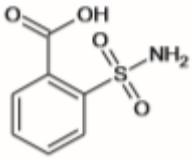
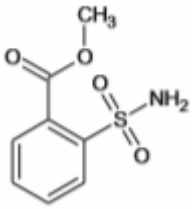
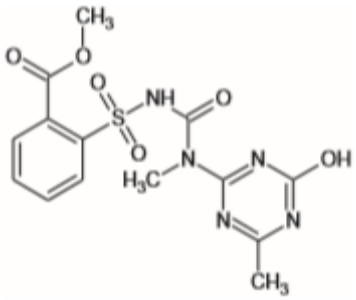
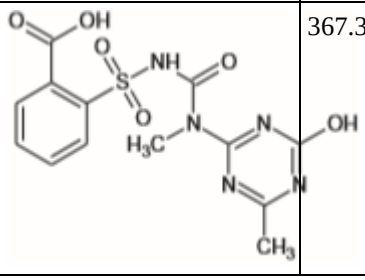
Grouping according to time of application			
Group	**Intended uses**	**relevant use parameters for grouping**	**Risk assessment**
1	Winter cereals – (autumn application) - post emergence, 1.00 kg/ha	-highest PECsw for MCPa and tribenuron methyl and its relevant metabolites -the highest exposure scenario -highest PEC soil for MCPA and tribenuron methyl and its relevant metabolites	-risk assessment for aquatic organisms, - risk assessment for bees, arthropods other than bees, non-target plants - risk assessment for soil organisms (covers the other intended uses)
2	Winter/Spring cereals – (spring application) - post emergence, 1.00 kg/ha	-the highest shortcut values -highest PECsw for MCPa and tribenuron methyl and its relevant metabolites	-risk assessment of the acute and long-term/reproductive risk for birds and mammals -risk assessment for aquatic organisms,
3	Grasses – spring appliaction, 1.00 kg/ha	-the highest shortcut values -highest PECsw for MCPa and tribenuron methyl and its relevant metabolites	-risk assessment of the acute and long-term/reproductive risk for birds and mammals -risk assessment for aquatic organisms,

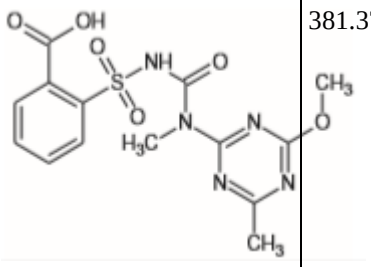
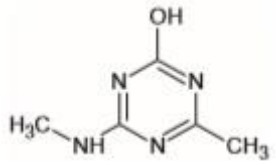
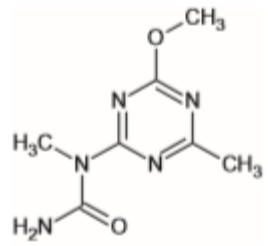
9.1.3 Consideration of metabolites

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 HAKSAR TOP 565 SG is indicated in the table.

Metabolites of MCPA potentially relevant for exposure assessment - No metabolites of MCPA were observed or identified.

Table 9.1-2 Metabolites of tribenuron methyl

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
IN-L5296		154.17	Soil :85.7% Water/sediment: up to 88.9% (total system, 56 d), max 42% in water (14 d), max 86% in sediment (56 d)	Yes, aquatics and soil organisms
IN-00581 saccharin		183.19	Soil :33.9% Water/sediment: up to 38.4% (total system, 14 d), max 32% in water (14 d), max 6.4% in sediment (14 d)	Yes, aquatics and soil organisms
IN-A4098		10.14	Soil :12.6% Water/sediment: 0.0001% , 0.0001 % in water, 0.0001% in sediment	Yes, aquatics and soil organisms
IN-D5119		201.20	Soil : 6.1% Water/sediment: up to 26.5% (total system, 56 d), max 19% in water (56 d), max 7.5% in sediment (56 d)	Yes, aquatics
IN-D5803		215.22	Soil :46.6% Water/sediment: 0.0001% , 0.0001 % in water, 0.0001% in sediment	Yes, aquatics
IN-GK521		381.37	Soil :32.1% Water/sediment: 0.0001% , 0.0001 % in water, 0.0001% in sediment	Yes, aquatics and soil organisms
IN-GN815		367.34	Soil : 6.8% Water/sediment: up to 13% (total system, 29 d), max 5.7% in water (42 d), max 9.2% in sediment (29 d)	Yes, aquatics

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
IN-R9803		381.37	Soil:9.1%	-
IN-R9805		140.15	Soil :7.6 % Water/sediment: up to 14.7% (total system, 71 d), max 9% in water (71 d), max 5.7% in sediment (71 d)	Yes, aquatics and soil organisms
M2		197.19	Soil :16.2 % Water/sediment: 0.0001% , 0.0001 % in water, 0.0001% in sediment	Yes, aquatics and soil organisms

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with MCPA and Tribenuron methyl. Full details of these studies are provided in the respective EU DAR and related documents.
Effects on birds of HAKSAR TOP 565 SG were not evaluated as part of the EU assessment of MCPA and Tribenuron methyl. No new studies for formulation are required.

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

Species	Substance	Exposure System	Results	Reference
Bobwhite quail	MCPA	Acute	LD_{50} = 270 mg/kg bw	SANCO/4062/2001 – final of 11.07.2008
Bobwhite quail	MCPA	Reproductive toxicity	NOEL = 93.2 mg/kg bw/day	SANCO/4062/2001 – final of 11.07.2008
Bobwhite quail	Tribenuron-methyl	Acute oral	LD_{50}>2250mg/kg bw per day	EFSA Journal 2017;15(7):4912
Bobwhite quail	Tribenuron-methyl	Reproduction, 23 weeks	NOEL = 22 ♂, 21 ♀mg/kg bw per day	EFSA Journal 2017;15(7):4912
Mallard duck	Tribenuron-methyl	Reproduction, 21 weeks	NOEL = 21 ♂, 23 ♀mg/kg bw per	EFSA Journal 2017;15(7):4912

Species	Substance	Exposure System	Results	Reference
			day (reproduction 21 weeks)	

9.2.1.1 Justification for new endpoints

No deviation from EU agreed endpoints.

9.2.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438). Risk assessments are presented for the intended use of HAKSAR TOP 565 SG in cereals and grassland (minor uses) according to the proposed GAP.

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 and First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of HAKSAR TOP 565 SG in cereals - MCPA

Intended use	**Cereals**				
Active substance/product	MCPA				
Application rate (g/ha)	1 × 550				
Acute toxicity (mg/kg bw)	270				
TER criterion	10				
Crop scenario Growth stage	**Generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening step	Small omnivorous bird	158.8	1	87.34	**3.09**
Cereals early (shoots)	Large herbivorous bird “goose”	30.5	1.0	16.78	16.09
Cereals BBCH 10 - 29	Small omnivorous bird “lark”	24.0	1.0	13.2	20.45
Cereals BBCH 30 - 39	Small omnivorous bird “lark”	12.0	1.0	6.6	40.91
Reprod. toxicity (mg/kg bw/d)	93.2				
TER criterion	5				
Crop scenario Growth stage	**Generic species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening step	Small omnivorous bird	64.8	0.53	18.88	**4.93**
Cereals	Large herbivorous bird	16.2	0.53	4.72	19.75

early (shoots) BBCH 10-29	"goose"				
Cereals BBCH 10 - 29	Small omnivorous bird "lark"	10.9	0.53	3.18	29.31
Cereals BBCH 30 - 39	Small omnivorous bird "lark"	5.4	0.53	1.57	59.36

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: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of HAKSAR TOP 565 SG in cereals – tribenuron methyl

Intended use	**cereals**				
Active substance/product	Tribenuron methyl				
Application rate (g/ha)	1 × 15				
Acute toxicity (mg/kg bw/d)	>2250				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals	Small omnivorous bird	158.8	1	2.38	>945
Reprod. toxicity (mg/kg bw/d)	21				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Winter and spring cereals	Small omnivorous bird	64.8	1*0.53	0.515	40.78

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 9.2-4: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of HAKSAR TOP 565 SG in grassland - MCPA

Intended use	**Grassland**				
Active substance/product	MCPA				
Application rate (g/ha)	1 × 550				
Acute toxicity (mg/kg bw/d)	270				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Grassland	Large herbivorous bird	30.5	1	16.8	16.1
Reprod. toxicity (mg/kg bw/d)	93.2				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**

Grassland	Large herbivorous bird	16.2	1*0.53	4.7	19.9

Table 9.2-5: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of HAKSAR TOP 565 SG in grassland – tribenuron methyl

Intended use	Grassland				
Active substance/product	Tribenuron methylu				
Application rate (g/ha)	1× 15				
Acute toxicity (mg/kg bw/d)	>2250				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Grassland	Large herbivorous bird	30.5	1	0.46	4891
Reprod. toxicity (mg/kg bw/d)	21				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Grassland	Large herbivorous bird	16.2	1*0.53	0.13	162

Plant metabolites

The overall highest maximum observed occurrence of metabolites in crops was 46% TRR in hay for the triazine amine metabolites and 44.6% TRR in grain for the sulphonamide-related compounds (EFSA Journal 2017;15(7):4912).

Since no experimental avian toxicity data were available for any of the metabolites of tribenuron-methyl, the risk assessment was performed for a "worst case hypothetical plant metabolite" which was assumed to be 10 times more toxic than the active substance and occur at 46% TRR. The resulting DDDs and TERs for acute and long term exposure are presented in the tables below.

Table 9.2-6: Screening assessment of the acute and long-term/reproductive risk for birds due to metabolites of Tribenuron-methyl after the use of HAKSAR TOP 565 SG in cereals

Intended use	**cereals**				
Metabolite/product	Worst case metabolite / HAKSAR TOP 565 SG				
Application rate (g/ha)	1 x 15 x Max. obs. =1 x 15 x 46%=6.9				
Acute toxicity (mg/kg bw/d)	>225**				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
cereals	Small omnivorous bird	158.8	1	1.09	206
Reprod. toxicity (mg/kg bw/d)	2.1**				
TER criterion	5				
Crop scenario	**Indicator/generic focal species**	**SV_m**	**MAF_m ×**	**DDD_m**	**TER_{lt}**

Growth stage			**TWA**	**(mg/kg bw/d)**	
cereals	Small omnivorous bird	64.8	1*0.53	0.23	9.1

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.

**assumption that metabolites 10 times more toxic than active substance

Mixture toxicity for birds - acute

Toxicity studies for birds with formulated products are typically not available. For the assessment of acute effects, a surrogate LD_{50} was calculated. A model often used to estimate the toxicity of mixtures is the assumption of dose/concentration additivity of toxicity (Loewe and Muischnek, 1926).

The following formula was used to derive a surrogate LD_{50} for the mixture of active substances with known toxicity assuming dose additivity:

$$LD_{50}(mix) = \left(\sum_i \frac{X_{(a.s._i)}}{LD_{50_{(a.s._i)}}} \right)^{-1}$$

With:

$X_{(a.s._i)}$ – fraction of active substance [i] in the mixture,

$LD_{50_{(a.s._i)}}$ – acute toxicity value for active substance [i].

Active substance	$\boldsymbol{LD_{50_{(a.s._i)}}}$ **(mg a.s./kg b.w./day)**	**Content in the product [g/kg]**	$\boldsymbol{X_{(a.s._i)}}$	$\boldsymbol{\frac{X_{(a.s._i)}}{LD_{50_{(a.s._i)}}}}$	$\boldsymbol{LD_{50}(mix)}$ **(mg a.s./kg b.w./day)**
MCPA	270	550	0.97	0.0036	278
Tribenuron methyl	2250	15	0.03	0.000013	

According to the EFSA/2009/1438 an endpoint for a mixture of active substances calculated assuming dose additivity should be conceived as an endpoint of a single virtual compound. Therefore the exposure calculation for the risk assessment is based as well on this assumption. Content in the formulation and application rate per hectare should thus be expressed in terms of that virtual compound. Therefore, the overall application rate for active substances combined of 0.565 kg virtual compound/ha is considered for the acute risk assessment.

Table 9.2-5: Screening assessment of the acute risk for birds due to the use of / HAKSAR TOP 565 SG in cereals

Intended use	**cereals** ~~**winter oilseed rape**~~				
Active substance/product	MCPA+Tribenuron methyl				
Application rate (g/ha)	1 × 565				
Acute toxicity (mg/kg bw)	278				
TER criterion	10				
Crop scenario	**Indicator focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Bare soil	Small granivorous bird	24.7	1	13.96	19.84
Cereals	Small omnivorous bird	158.8	1	89.72	**3.09**

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 9.2-6: First-tier assessment of the acute risk for birds due to the use of HAKSAR TOP 565 SG in cereals

Intended use	**cereals ~~winter and spring~~**				
Active substance/product	MCPA+Tribenuron methyl				
Application rate (g/ha)	1 × 565				
Acute toxicity (mg/kg bw)	278				
TER criterion	10				
Crop scenario Growth stage	**Generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals early (shoots) BBCH 10-29	Large herbivorous bird "goose"	30.5	1.0	17.23	16.13
Cereals BBCH 10 - 29	Small omnivorous bird "lark"	24.0	1.0	13.56	20.50
Cereals BBCH 30 - 39	Small omnivorous bird "lark"	12.0	1.0	6.78	41.00

Mixture toxicity for birds – long-term

According to Appendix B of EFSA (2009) recommends not using the predictive mixture toxicity calculation for chronic effects.

A combined risk assessment was performed using the following equation:

TER_{LT}, combi = trigger / ((trigger/lowest TERsubst 1) + (trigger/lowest TERsubst 2))

The combined risk assessment was conducted based on the first-tier assessment for MCPA and screening assessment for Tribenuron methyl:

TER_{LT}, combi = 5 / ((5/19.75) + (5/40.78)) = 13.51

The combined long-term risk assessment is acceptable.

9.2.2.2 Higher-tier risk assessment

No higher tier risk assessment was performed as the first-tier assessment for MCPA and screening assessment for Tribenuron methyl clearly indicate that there is no unacceptable risk for birds when using HAKSAR TOP 565 SG acc. to GAP.

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 HAKSAR TOP 565 SG is not a product for spray applications / not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not have to be considered.

Puddle scenario

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

With a K(f)oc <500 L/kg , Tribenuron-methyl belongs to the group of less sorptive substances.

Effective application rate (g/ha)	=	15		
Acute toxicity (mg/kg bw)	=	2250	quotient	= 0.0066
Reprod. toxicity (mg/kg bw/d)	=	21	quotient	= 0.71

With a K(f)oc <500 L/kg, MCPA belongs to the group of less sorptive substances.

Effective application rate (g/ha)	=	550		
Acute toxicity (mg/kg bw)	=	270	quotient	= 2.04
Reprod. toxicity (mg/kg bw/d)	=	93.2	quotient	= 5.90

No specific TER calculation for puddle scenario is necessary.

9.2.2.4 Effects of secondary poisoning

The log P_{ow} of MCPA and Tribenuron methyl do not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

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 risk for birds arising from acute and long-term exposure to HAKSAR TOP 565 SG is acceptable.

Review comments:

The acute and long-term risk assessment for birds performed by the Applicant is agreed by the zRMS. It was performed in line with recommendations of the EFSA (2009) with assumption of EU agreed endpoints. No formulation study was required.

The TER_A and TER_{LT} values for tribenuron-methyl and MCPA were calculated for the highest proposed application rate of 15 g Tribenuron methyl/ha and 550 g MCPA/ha.
In addition, a risk assessment for worst case hypothetical plant metabolite of tribenuron methyl was conducted and its risk assessment is acceptable.
Based on screening and TIER 1 step the acceptable acute and reproductive risk to birds was concluded for application of the formulation in winter and spring cereals and grasses according to the intended uses.

For mixture toxicity acceptable risk could be demonstrated.

HAKSAR TOP 565 SG presents no unacceptable risk to birds resulting from exposure via drinking water. Since the log Pow value of MCPA and tribenuron-methyl and its relevant soil and aquatic metabolites are all below the trigger of 3, the evaluation of the risk of secondary poisoning is not triggered.

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

9.3.1 Toxicity data

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

Effects on mammals of HAKSAR TOP 565 SG were not evaluated as part of the EU assessment of MCPA and Tribenuron methyl.

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

Species	Substance	Exposure System	Results	Reference
rat	MCPA	Acute	LD_{50} = 962 mg/kg bw	SANCO/4062/2001 – final of 11.07.2008
rat	MCPA	Reproductive toxicity	NOAEL = 37.8 mg/kg bw/d	SANCO/4062/2001 – final of 11.07.2008
Rat	Tribenuron-methyl	Acute oral	LD_{50}>5000	EFSA Journal 2017;15(7):4912
Rat	Tribenuron-methyl	Reproductive toxicity Two-generation study	NOAEL = 2	EFSA Journal 2017;15(7):4912
Rat	Triazine amine (IN-L5296)	Acute oral	LD_{50}=394	EFSA Journal 2017;15(7):4912
Rat	Triazine amine (IN-L5296)	4-week, repeated dose gavage	NOAEL= 8	EFSA Journal 2017;15(7):4912
Rat	Sulfonamide urea (IN-B5685)	Acute oral	LD_{50}>11000	EFSA Journal 2017;15(7):4912
Rat	Sulfonamide urea (IN-B5685)	10 days, repeated dose gavage	NOAEL ≥ 220 [a]	EFSA Journal 2017;15(7):4912
Rat	Sulfonamide (IN-D5803)	Acute oral	LD_{50}>7500[b]	EFSA Journal 2017;15(7):4912

a The study was considered of limited reliability but supportive
b Data from EFSA Conclusions for metsulfuron-methyl (EFSA, 2015a) and ethmetsulfuron (EFSA, 2014a)

9.3.1.1 Justification for new endpoints

No deviation from the EU agreed endpoints

9.3.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Mammals and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438). Risk assessments are presented for the intended use of HAKSAR TOP 565 SG in cereals and grassland (minor uses) according to the proposed GAP.

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 and First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of HAKSAR TOP 565 SG in winter and spring cereals

Intended use	cereals				
Active substance/product	MCPA				
Application rate (g/ha)	1 × 550				
Acute toxicity (mg/kg bw)	962				
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 herbivorous mammal	118.4	1.0	65.12	14.77
~~Cereals BBCH ≥ 20~~	~~Small insectivorous mammal "shrew"~~	~~5.4~~	~~1.0~~	~~2.97~~	~~323.9~~
~~Cereals early (shoots)~~	~~Large herbivorous mammal "lagomorph"~~	~~42.1~~	~~1.0~~	~~23.16~~	~~41.5~~
~~Cereals BBCH 10 - 29~~	~~Small omnivorous mammal "mouse"~~	~~17.2~~	~~1.0~~	~~9.46~~	~~101.7~~
~~Cereals BBCH 30 - 39~~	~~Small omnivorous mammal "mouse"~~	~~8.6~~	~~1.0~~	~~4.73~~	~~203.4~~
Reprod. toxicity (mg/kg bw/d)	37.8				
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 herbivorous mammal	48.3	0.53	14.07	**2.69**
Cereals BBCH ≥ 20	Small insectivorous mammal "shrew"	1.9	0.53	0.55	68.7
Cereals early (shoots)	Large herbivorous mammal "lagomorph"	22.3	0.53	6.5	5.8
Cereals BBCH 10 - 29	Small omnivorous mammal "mouse"	7.8	0.53	2.27	16.7

Cereals BBCH 30 - 39	Small omnivorous mammal "mouse"	3.9	0.53	1.14	33.2

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 9.3-3: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of HAKSAR TOP 565 SG in winter and spring cereals

Intended use	cereals				
Active substance/product	Tribenuron-methyl				
Application rate (g/ha)	1 × 15				
Acute toxicity (mg/kg bw)	>5000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
cereals	Small herbivorous mammal	118.4	1	1.77	2824
Reprod. toxicity (mg/kg bw/d)	2				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
cereals	Small herbivorous mammal	48.3	1*0.53	0.38	5.26

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 9.3-4: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of HAKSAR TOP 565 SG in winter and spring cereals

Intended use	winter and spring cereals				
Active substance/product	Tribenuron methyl				
Application rate (g/ha)	1 × 15				
Acute toxicity (mg/kg bw)	>5000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals BBCH 10 - 29	Small omnivorous mammal "mouse"	17.2	1.0	0.26	19230
Cereals BBCH 30 - 39	Small omnivorous mammal "mouse"	8.6	1.0	0.13	38462
Reprod. toxicity (mg/kg bw/d)	2				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals BBCH 10 - 29	Small omnivorous mammal "mouse"	7.8	0.53	0.06	33.3
Cereals BBCH 30 - 39	Small omnivorous mammal "mouse"	3.9	0.53	0.03	66.7

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 9.3-4 ~~5~~: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of HAKSAR TOP 565 SG in grassland - MCPA

Intended use	**Grassland**				
Active substance/product	MCPA				
Application rate (g/ha)	1 × 550				
Acute toxicity (mg/kg bw/d)	962				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Grassland	Small herbivorous mammal	136.4	1	75.02	12.82
Reprod. toxicity (mg/kg bw/d)	37.5				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Grassland	Small herbivorous mammal	72.3	1*0.53	21.08	**1.8**

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 9.3-5 ~~6~~: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of HAKSAR TOP 565 SG in grassland – tribenuron methyl

Intended use	**Grassland**				
Active substance/product	Tribenuron methyl ~~solo~~				
Application rate (g/ha)	1 × 15				
Acute toxicity (mg/kg bw/d)	>5000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Grassland	Small herbivorous mammal	136.4	1	2.05	2439.02
Reprod. toxicity (mg/kg bw/d)	2				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Grassland	Small herbivorous mammal	72.3	1*0.53	0.57	**3.5**

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 9.3-6a ~~7~~: Tier 1 assessment of the long-term/reproductive risk for mammals due to the use of HAKSAR TOP 565 SG in grassland - MCPA

Intended use	Grassland				
Active substance/product	MCPA				
Application rate (g/ha)	1 × 550				
Reprod. toxicity (mg/kg bw/d)	37.8				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
~~Grassland 'Growing shoots'~~	~~Large herbivorous bird "goose"~~	~~16.2~~	~~1*0.53~~	~~4.7~~	~~8.0~~
All season	Large herbivorous mammal	17.3	0.53	5	7.56
Late	Small insectivorous mammal "shrew"	1.9	0.53	0.55	68.7
All season	Small herbivorous mammal	72.3	0.53	21.1	**1.8**
Late season	New sown grass seeds	6.6	0.53	2	19

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.

Since unacceptable risk was indicated for vole further **higher tier risk assessment is needed.**

Table 9.3-7a ~~7~~: Tier 1 assessment of the long-term/reproductive risk for mammals due to the use of HAKSAR TOP 565 SG in grassland – tribenuron solo-

Intended use	Grassland				
Active substance/product	Tribenuron methyl ~~solo~~				
Application rate (g/ha)	1 × 15				
Reprod. toxicity (mg/kg bw/d)	2				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
~~Grassland 'Growing shoots'~~	~~Large herbivorous bird "goose"~~	~~16.2~~	~~1*0.53~~	~~0.13~~	~~15.38~~
All season	Large herbivorous mammal	17.3	0.53	0.13	15
Late	Small insectivorous mammal "shrew"	1.9	0.53	0.01	200
All season	Small herbivorous mammal	72.3	0.53	0.57	**3.5**
Late season	New sown grass seeds	6.6	0.53	0.05	40

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.

Acute risk assessment for IN-B5685, IN-GN815 (covering: IN-37739 and IN-QHP91) metabolites is not required since for active substance the wide margin of safety was confirmed (TER_a for the tribenuron methyl is 2439.02)
Nevertheless, a long-term screening assessment has been conducted for sulfonamide urea (IN-B5685) for which actual measured NOAEL value is available. The risk assessment was performed with the same approach as is taken in RAR for tribenuron-methyl metabolites (2017). The assumption was made that metabolite is 10 times more toxic than the active substance and occur at 45% and 17% in plant parts.

Table 9.3-8 Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of HAKSAR TOP 565 SG for sulfonamide urea (IN-B5685):

Intended use		Cereals, grassland			
Metabolite		IN-B5685			
Application rate (g/ha)		0.007 (application rate x max. obs. % of metabolite*)			
Reprod. toxicity (mg/kg bw/d)		220**			
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 cereals	Small herbivorous mammal	48.3	1*0.53	0.18	1222.2
Screening step grassland	Small herbivorous mammal	72.3	1*0.53	0.27	814

* Maximum occurrence of plant metabolite is 45%
**Endpoint is from the 10 days, repeated dose gavage study performed for mammals (EFSA Journal 2017;15(7):4912)

The following plant metabolites are not considered to be covered by any other risk assessment performed above:

– α-hydroxy-triazine amine (IN-37739); 15% occurrence in wheat
– 4-methoxy-6-(methylamino)-1,3,5-triazine-2-methanol (IN-QHP91); 12% occurrence in wheat
– O-demethyl-tribenuron free acid (IN-GN815); 17% occurrence in wheat

To cover these metabolites, the zRMS performed an additional assessment based on assumption that metabolite IN-GN815 is 10 times more toxic than the active substance and occur at 17% in plant parts.

Table 9.3-9: Long-term screening for IN-GN815

Intended use		Cereals, grassland			
Metabolite		IN-GN815			
Application rate (g/ha)		2.55 (application rate x max. obs. % of metabolite*)			
Reprod. toxicity (mg/kg bw/d)		0.2**			
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 cereals	Small herbivorous mammal	22.3	1*0.53	0.03	6.66
Screening step grassland	Small herbivorous mammal	72.3	1*0.53	0.09	**2.22**

* Maximum occurrence of plant metabolite is 17%
** metabolite 10 times more toxic than active substance

For metabolite IN-B5685 and IN-GN815 risk for mammals is acceptable.

Since TERlt was lower than 5, for other metabolites in grasses unacceptable long-term risk to mammals was indicated. Further assessment is needed.

Table 9.3-10: Long-term Tier 1 assessment for mammals exposed to IN-GN815

Intended use	grassland				
Metabolite	IN-GN815				
Application rate (g/ha)	2.55 (application rate x max. obs. % of metabolite*)				
Reprod. toxicity (mg/kg bw)	0.2**				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV_{90}	MAF_{90}	DDD90 (mg/kg bw/d)	TERlt
First Tier Risk Assessment					
Grassland- worst case All season	Small herbivorous mammal Vole	72.3	1*0.53	0.09	**2.22**

* Maximum occurrence of plant metabolite is 17%
** metabolite 10 times more toxic than active substance

Risk assessment provided for IN-GN815 in grasses covers risk assessment for IN-37739 and IN-QHP91 metabolites. Based on performed assessment, long-term risk is still unacceptable for proposed uses of MT-565SG-OR2-C / HAKSAR TOP 565 SG in grasses for vole. Further assessment should be performed.

Mixture toxicity for mammals - acute

For the assessment of acute effects, a surrogate LD_{50} was calculated. A model often used to estimate the toxicity of mixtures is the assumption of dose/concentration additivity of toxicity (Loewe and Muischnek, 1926).

The following formula was used to derive a surrogate LD_{50} for the mixture of active substances with known toxicity assuming dose additivity:

$$LD_{50}(mix) = \left(\sum_i \frac{X_{(a.s._i)}}{LD_{50(a.s._i)}} \right)^{-1}$$

With:

$X_{(a.s._i)}$ – fraction of active substance [i] in the mixture,

$LD_{50(a.s._i)}$ – acute toxicity value for active substance [i].

Active substance	$LD_{50(a.s._i)}$ (mg a.s./kg b.w./day)	Content in the product [g/kg]	$X_{(a.s._i)}$	$\frac{X_{(a.s._i)}}{LD_{50(a.s._i)}}$	$LD_{50}(mix)$ (mg a.s./kg b.w./day)
MCPA	>5000	550	0.97	0.000194	4444
Tribenuron methyl	962	15	0.03	0.000031	

According to the EFSA/2009/1438 an endpoint for a mixture of active substances calculated assuming dose additivity should be conceived as an endpoint of a single virtual compound. Therefore the exposure calculation for the risk assessment is based as well on this assumption. Content in the formulation and application rate per hectare should thus be expressed in terms of that virtual compound. Therefore, the overall application rate for active substances combined of 0.565 kg virtual compound/ha is considered for the acute risk assessment

Table 9.3-4: Screening assessment of the acute risk for mammals due to the use of HAKSAR TOP 565 SG in cereals ~~winter oilseed rape~~

Intended use	**winter and spring cereals**				
Active substance/product	MCPA & Tribenuron methyl				
Application rate (g/ha)	1 × 565				
Acute toxicity (mg/kg bw)	4444				
TER criterion	10				
Crop scenario	**Indicator focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Bare soil	Small granivorous mammal	14.4	1	8.1	548.6
Cereals	Small herbivorous mammal	118.4	1	66.9	66.4

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.

Mixture toxicity for mammals – long-term

According to Appendix B of EFSA (2009) recommends not using the predictive mixture toxicity calculation for chronic effects.

Therefore a combined risk assessment was performed using the following equation:

TER_{LT}, combi = trigger / ((trigger/lowest TERsubst 1) + (trigger/lowest TERsubst 2))

The combined risk assessment was conducted based on the first-tier assessment for MCPA and tribenuron methyl (For combined risk assessment Tier 1 refinement for tribenuron methyl was conducted):

TER_{LT}, combi = 5 / ((5/5.8) + (5/33.3)) = 5, cereals

TER_{LT}, combi = 5 / ((5/1.8 + (5/3.5)) = **1.2** grassess

Assessment after refinement : TER_{LT}, combi = 5 / ((5/5.94 + (5/5)) = **2.8** grasses

Overall conclusion

Based on higher tier assessment step, the calculated TERmix values for the long-term does achieve the acceptability criteria for for the f intended uses in cereals: MCPA 550 g as/ha; tribenuron methyl 15 g, equivalent to 1,00 kg/ha

The $TER_{LTcombi}$ value for the worst case exposure scenario (voles) is below the trigger value of 5. Thus, it can be concluded that the reproductive risk for mammals for the combined exposure to the two active substances in the application of Haksar is still unacceptable. zRMS performed weight of evidence below:

Weight of evidence: The combined long-term risk for mammals in grasses

Because HAKSAR TOP 565 SG contains more than one active substance, the effect of combination of these active substances has been considered. It should be noted that long-term effects of simultaneous exposure of several active substances is not a recommendation of EFSA B&M guideline. This is important for the national authorization procedures for product containing more than one active substance. It should be underline that the calculation is highly conservative approach and can overestimate the exposure of mixture toxicity.

Target source of the endpoint in the study:

MCPA and tribenuron methyl worse endpoints were used for calculations. It is necessary to consider that the target of the endpoints for both substances is different as follows:

For MCPA endpoint is derived from reproductive toxicity study and is NOAEL = 37.8 mg/kg bw/d

For Tribenuron methyl endpoint is derived from rreproductive toxicity two-generation study NOAEL= 2 mg/kg bw/d.

For the comparison for the studies (here we deal with two studies with the same species) they should be conducted according to similar guideline/protocol. This means it is very hard to compare two datasets from two different types of the studies.

Mode of action of active substances

The mode of action of MCPA is as an **auxin**, which are growth hormones that naturally exist in plants.

The mode of action of tribenuron methyl is the inhibition of acetolactate synthase.

It should be noted that in the aquatic risk assessment the calculated MDR values are between 0.2 and 5 for each organism expect *Lemna gibba,* indicating that the formulation does not cause (unexpected) an increased toxicity compared to the active substances for these organisms. No synergisms or additional toxicity occurs due to the co-formulants.

The apparent antagonism for *Lemna gibba* (toxicity of the formulation lower than expected) can be explained by the fact that endpoints for individual active substances are higher than values. When MDR <0.2 the predicted value should be used in the risk assessment of the formulation product. However, an assemssment to determine the presence of a toxic driver was conducted. Overall, it appears the MCPA is the toxic driver for daphnids (contributes 99 % of the formulation toxicity). For algae and aquatic plants tribenuron methyl occur to be the toxicity driver. Generally the toxicity is primarily influenced by the active substances. Thus, additive effects of mixture toxicity is not expected.

DT_{50} of active substance

For MCPA mean DT_{50} of 2.32 days should be taken to consideration. Than Mammals would not be exposed by both substances as long-term exposure.

Number of the application

Only one application is done during the season thus cumulative effects would not occur.

~~5 / ((5/5.8) + (5/33.3)) = 5,~~

In conclusion taking to consideration weight of evidence the combined long-term risk assessment is ~~un~~acceptable. Concerned MS should considered this on the MS level.

~~Although, due to different chemical nature of MCPA and Tribenuron methyl, and thus, their unsimilar mechanisms (mode of action), a combined effects should be not considered.~~

9.3.2.2 Higher-tier risk assessment

~~The risk assessment conducted at Tier 1 indicates an acceptable chronic risk to large herbivorous mammal "lagomorph" exposed to HAKSAR TOP 565 SG~~

~~Therefore, additional risk assessment is not required.~~

The TERlt for small herbivore vole fall below the trigger of 5 for MCPA, therefore further risk assessment was required.

Long- term refinement for MCPA

Default foliar 10 days used in Tier 1 and Tier 2 risk assessment would be refined on the basis of residue decline performed in MCPA Addendum Vol.3 (2004).

Below zRMS presented the detailed results directly from MCPA Addendum:

Residues in Feed

Data from 26 residue studies in cereals and pasture were used for the calculation. Treatment in the Southern European zone was at a lower rate than the maximum GAP of 1.8 kg/ha, but since this represents normal agronomic practice (maximum label rates in the range 800 – 1200g/ha in cereals and up to 1600g/ha in pasture) it is more realistic to use the initial residue figures from these data unaltered. Using the Southern data has the benefit of greatly increasing the number of data points used and therefore increases the confidence in the results.

	Crop	*Individual day 0 residues (mg/kg)*		*Arithmetic Mean Day 0 Residue*
		1999	*2000*	*(mg/kg)*
Northern	*Cereals*	*53, 89, 31, 66*	*69, 113, 46, 57*	***67.2***
Europe	*Pasture*	*80, 98*	*128, 85, 59, 10*	
Southern	*Cereals**	*20, 37, 25, 24*	*61, 19, 45, 19*	
Europe	*Pasture***	*138, 31*	*135, 208*	

** Trials treated at 1.2 kg/ha*
*** Trials treated at 1.6 kg/ha*
Northern European crops treated at 1.8 kg/ha

The mean initial residue value of 67.2 ppm is appropriate for the estimation of long term risk to mammals. The decline of residues is discussed below

Estimated Residue Decline

As the object is to review the long term risk to mammals the fact that the residue declines must be taken into account. The estimated worst case scenario is the application to long grass/pasture (where the crop cover means that little will be lost to bare ground). The estimate below is based on grassland data from 4 trials:

	Residue at day					***Est DT50****
	0	***7***	***14***	***28***	***56***	***days***
N Eurpe Grassland 1	*80*	*6.7*	*5.6*	*1.4*	*0.2*	***2.32***
N Eurpe Grassland 2	*98*	*4.9*	*1.1*	*0.76*	*0.38*	
S Eurpe Grassland 1	*138*	*19*	*11*	*7.5*	*3*	
S Eurpe Grassland 2	*31*	*7.4*	*6.6*	*3*	*0.66*	

**Based on 1st order kinetics between days 0 and 56 days*

N Europe Grassland trial IR 18196/R9117
S Europe Grassland Trial IR18200/R9119

The above data does not conform strictly to 1st or 2nd order kinetics (probably because it is influenced by the multiple factors of metabolism, growth dilution and wash off). Initial decay is rapid, but decay is slower later. However, an exponential plot of the decay (see Appendix 1) gives a mean ***DT_{50} of 2.32 days****.*
Detailed calculation of half-life time is presented in table and figure below.

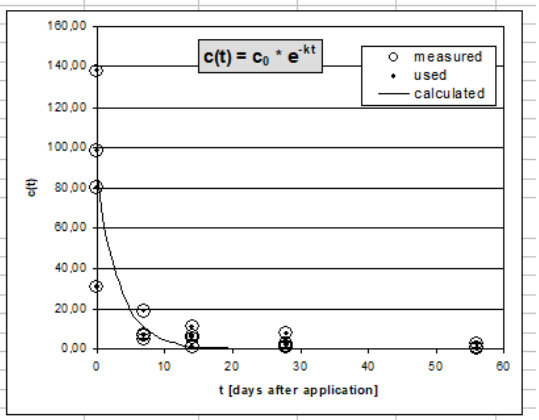
Appendix 1

Calculation of the half-life time of a substance from experimental data

assuming single-exponential first-order kinetics

Substance:	MCPA	**Operator:**	A L Bond
Study type:	Residue Decline	**Date:**	05.11.2002
Study no.:	Wild Mammal Risk Assessment		
Test system:	Pasture		
initial concentr.:	86,75	(mean of the 4 residue curves)	
degradation rate:	0,2990	d^{-1}	

data point	for optimisation	sampling time (DAA)	measured values	calculated values	squared difference
1	1	0	80,000	86,750	45,563
2	1	7	6,700	10,696	15,972
3	1	14	5,600	1,319	18,328
4	1	28	1,400	0,020	1,904
5	1	56	0,200	0,000	0,040
6	1	0	98,000	86,750	126,563
7	1	7	4,900	10,696	33,599
8	1	14	1,100	1,319	0,048
9	1	28	0,760	0,020	0,548
10	1	56	0,380	0,000	0,144
11	1	0	138,000	86,750	2626,563
12	1	7	19,000	10,696	68,949
13	1	14	11,000	1,319	93,724
14	1	28	7,500	0,020	55,950
15	1	56	3,000	0,000	9,000
16	1	0	31,000	86,750	3108,063
17	1	7	7,400	10,696	10,867
18	1	14	6,600	1,319	27,890
19	1	28	3,000	0,020	8,880
20	1	56	0,660	0,000	0,436
			sum of squared differences:		6253,027



DT-50:	2,32	days
DT-90:	7,70	days
B:	0,8299	

In conclusion taking to consideration calculated DT_{50}=2.32 new fTWA value for MCPA is 0.16 and it would be used in the higher tier refinement.

Table 9.3-6b: Tier 1 assessment of the long-term/reproductive risk for mammals due to the use of HAKSAR TOP 565 SG in grassland – MCPA

Intended use	Grassland
Active substance/product	MCPA
Application rate (g/ha)	1 × 550
Reprod. toxicity (mg/kg bw/d)	37.8
TER criterion	5

Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
All season	Small herbivorous mammal	72.3	0.16	6.36	5.94

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.

The risk assessment with the refined TWA is acceptable. Concerned member states must decide on the applicability of the refinement.

Higher-tier risk assessment

Refinements are required for the grassland application scenario (small herbivorous mammal 'vole'), Where for Tribenuron methyl TER_{LT} = 3.5 and for metabolite IN-GN815 TERlt=2.2.

Proportions of feed item in the diet (PD) according to Rinke (1991)

Studies by Rinke[1] and Lüthi et all[2] are commonly used for purposes of the refinement of the risk at National and Zonal level.

In the study by Rinke[1] were investigates vole feeding preferences (mono versus dicot) via stomach content analysis. No exact percentages of each per animal were determined, instead, animals were categorized into 5 potential categories of dicot consumption (20% intervals). Overall, despite the fact that more monocots were available in the surrounding areas (70%), voles showed a preference for dicots, with the majority of voles (all seasons, sexes, ages) showing >80% dicot material in stomach contents. For the chronic risk assessment, in spring and summer, the diet can be set on 25% monocots and 75% dicots.
Additionally, in Lüthi et all[2] also an extensive study on the diet of the common vole in monocot and dicot dominated fields was performed. The study is very detailed (considering that it is public literature) and a large number of samples/voles were considered.

A PD of 25% non-grass herbs and 75% grass and cereals is considered relevant for purposes of evaluation of the risk to vole in grasslands following application of Toscana Top 75 WG.

It should be noted that this approach has been already taken by the zRMS in the course of evaluation of several plant protection products at the national level. As the studies by Rinke (1991) and Lüthi et al. (2010) are publicly available, there are no restrictions regarding use of their results in the risk assessment.

As stated above, for the representative small herbivore as relevant for Tier 1 assessments, the vole conservatively is assumed solely to feed on grasses, the feed item reported to contain the highest residue levels (default mean RUD = 54.2 mg/kg) together with the lowest assimilation efficiency and low food energy.
Studies have demonstrated that common voles (*Microtus arvalis*) prefer to consume dicotyledons rather than monocotyledonous grasses. A study of the diet of the common vole in grassland in Germany examined the diet of 363 individuals caught by snap-trapping through analysis of stomach contents (Rinke (1990[1]; 1991[2]). The results showed that dicotyledons, such as Taraxacum officinale and Trifolium pratense, were preferred and were eaten at a higher frequency than would be expected from their relative occurrence in the grassland habitat in question. Overall, it was reported that dicotyledons comprised a mean volume percentage of 63.5% of stomach contents of common vole. Therefore, the risk assessment can be refined by considering a common vole consuming a diet comprising 60% dicotyledons (non-grass herbs) and 40% grasses.

Feed item	Food energy	Assimilation efficiency	Moisture content	Food energy	Daily energy expenditure	Fresh Food intake rate	Body weight	FIR/bw

[1] Rinke, T. 1990. Zur Nahrungsökologie von Microtus arvalis (Pallas, 1778) auf Dauergrünland. I. Allgemeine Nahrungspräferenzen. Zeitschrift für Säugetierkunde 55: 106-114
[2] Rinke, T. (1991). Percentage of volume versus number of species: availability and intake of grasses and forbs in Microtus arvalis. Folia Zoologica 40 (2): 143 – 151.

			FEi	AEi	MCi	FEi fresh	FEi, total fresh	DEE	FIRi, total fresh	bw	
Category	[%] (fraction)	PDi (fraction)	[kJ/g dry]	[%]	[%]	[kJ/g fresh]		[kJ/d]	[g fresh weight/d]	[g]	
Small herbivorous scenario – common vole (Approach 2)											
Monocotyledons	40	0.40	17.6	47	76.4	0.78	1.75	65.09	38.29	25	1.49
Dicotyledonous	60	0.60	17.8	76	88.1	0.97					
Sum	100										

Calculations of new short cut value based on data from EFSA B&M guidance (i.e. bw and RUDs).

Indicator/generic focal species	**Typ of food**	**FIR/bw**	**RUD_{mean}**	**PD**	**SV_m**
Small herbivorous mammal "vole"	Monocotyledons	1.49	54.2	0.25	20.2
	Dicotyledonos	1.49	28.7	0.75	32.1
SUM				1.0	52.3

The risk assessment based on the refined parameters is presented in table below.

Intended use	grassland
Active substance	Tribenuron methyl
Application rate [g a.s./ha]	1 × 15 g a.s./ha
Reprod. toxicity [mg/kg bw/d]	2
TER criterion	5

Focal species	Food category, % in diet	RUDm × DF [mg/kg food]	FIR/bw	MAFm × TWA	PT	DDDm [mg/kg bw/d]	TERlt
"herbivorous" Microtus arvalis (Common vole)	Monocotyledons 25%	54.2 × 1	1.49	1 × 0.53	1	∑=0.4	5
	Dicotyledonos 75%	28.7 × 1		1 × 0.53		∑=0.5	

As presented above zRMS performed the risk assessment based on the refined diet for vole.

The refinement of the risk demonstrated acceptable risk for small herbivores exposed to tribenuron-methyl after application of Haksar Top 565 SG (at rate 15.00 g a.s/ha).

Intended use	grassland
Metabolite	metabolite IN-GN815
Application rate [g a.s./ha]	2.55 metabolite/ha
Reprod. toxicity [mg/kg bw/d]	2
TER criterion	5

Focal species	Food category, % in diet	RUDm × DF [mg/kg food]	FIR/bw	MAFm × TWA	PT	DDDm [mg/kg bw/d]	TERlt

"herbivorous" Microtus arvalis (Common vole)	Monocotyledons 25%	54.2 × 1	1.49	1 × 0.53	1	∑=0.3	6.7
	Dicotyledonos 75%	28.7 × 1		1 × 0.53			

Risk assessment for metabolites is acceptable.

9.3.2.3 Drinking water exposure

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

Puddle scenario

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

With a K(f)oc <500 L/kg , Tribenuron-methyl belongs to the group of less sorptive substances.

Effective application rate (g/ha)	=	15		
Acute toxicity (mg/kg bw)	=	5000	quotient =	0.003
Reprod. toxicity (mg/kg bw/d)	=	2	quotient =	7.5

With a K(f)oc <500 L/kg, MCPA belongs to the group of less sorptive substances.

Effective application rate (g/ha)	=	550		
Acute toxicity (mg/kg bw)	=	962	quotient =	2.04
Reprod. toxicity (mg/kg bw/d)	=	37.8	quotient =	5.90

IN-L5296 is a metabolite which the highest content in comparison to other metabolites in water/sediment 88.9%. This metabolite also has the lowest acute toxicity (IN-L5296 LD_{50} = 394 mg/kg bw per day). That toxicity is lower than the toxicity of the active substance thus this metabolite need to be considered in the risk assessment. The other metabolites are considered covered under the risk envelope by the risk assessment conducted with that acute toxicity endpoint.

Effective application rate (g/ha)	=	15 x 88.9 % = 13.33 (application rate x max. obs. % of metabolite		
Acute toxicity (mg/kg bw)	=	394	quotient =	0.03
Reprod. toxicity (mg/kg bw/d)	=	8	quotient =	1.66

9.3.2.4 Effects of secondary poisoning

The log P_{ow} of MCPA and Tribenuron methyl do not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

Risk assessment for fish-eating mammals via secondary poisoning

Not required.

Risk assessment for earthworm-eating mammals via secondary poisoning

Not required.

9.3.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.3.4 Overall conclusions

The risk for mammals arising from acute and long-term exposure to HAKSAR TOP 565 SG is acceptable.

Review comments:

The acute and long-term risk assessment for mammals performed by the Applicant was not fully agreed by the zRMS. However it was performed in line with recommendations of the EFSA (2009) with assumption of EU agreed endpoints. No formulation study was required.

The TER_A and TER_{LT} values for tribenuron-methyl and MCPA were calculated for the highest pro-posed application rate of 15 g Tribenuron methyl/ha and 550 g MCPA/ha. Acceptable risk for both substances for mammals is indicated in cereals. For mixture toxicity also acceptable risk could be demonstrated in cereals.
It was noted that the Applicant did not present a risk assessment to plant metabolites of tribenuron methyl. zRMS presented risk assessment for relevant metabolites for use in cereals risk is acceptable.

For both substances and plant metabolite of tribenuron and mixture toxicity long-term risk for vole in grasses is unacceptable at TIER 1. Further refinement was needed.

zRMS used DT_{50} refinement for MCPA and refined proportions of feed item in the diet (PD) for tribenuron methyl (according to Rinke (1991)). After refinement risk assessment for both substances and relevant metabolites has been accepted for the use of HAKSAR TOP 565 SG in grasses.

Mixture toxicity for the use in grasses was acceptable on the basis on the weight of evidence. Concerned MS should consider these on the National level.

HAKSAR TOP 565 SG presents acceptable risk resulting from exposure via drinking water. Since the log Pow value of MCPA and tribenuron-methyl and its relevant soil and aquatic metabolites are all below the trigger of 3, the evaluation of the risk of secondary poisoning is not triggered.

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

No data/information available.

Review comments:

This issue is not assessed at the product level.

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 MCPA, Tribenuron-methyl and its metabolites. Full details of these studies are provided in the respective EU RAR and related documents.

Effects on aquatic organisms of HAKSAR TOP 565 SG were not evaluated as part of the EU assessment of MCPA and Tribenuron-methyl. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Table 9.5-1: Endpoints and effect values relevant for the risk assessment for aquatic organisms – MCPA, tribenuron-methyl and its metabolites

Species	Substance	Exposure System	Results	Reference
Fish				
Oncorhynchus mykiss	MCPA	96 h, f	LC_{50} = 50* mg a.s./L	SANCO/4062/2001-final of 11.07.2008
Pimephales promelas	MCPA	28 d, f	NOEC = 15 mg a.s./L	SANCO/4062/2001-final of 11.07.2008
Oncorhynchus mykiss Rainbow trout	Tribenuronmethyl	96h (static)	LC_{50} = 738 mg a.s./L $_{mm}$	EFSA Journal 2017;15(7):4912
Oncorhynchus mykiss Rainbow trout	IN-00581 = saccharin	96h (static)	LC_{50}>124 mg /L $_{mm}$	EFSA Journal 2017;15(7):4912
Oncorhynchus mykiss Rainbow trout	IN-A4098 = Ndemethyl triazine amine (AE F059411, CG A150829)	96h (static)	LC_{50}=200 mg /L $_{mm}$	EFSA Journal 2017;15(7):4912
Oncorhynchus mykiss Rainbow trout	IN-D5119 Acid sulfonamide	96h (static)	LC_{50}>115 mg /L $_{mm}$	EFSA Journal 2017;15(7):4912
Oncorhynchus mykiss Rainbow trout	IN-L5296 Methyl triazine amine	96h (static)	LC_{50} =172 mg /L $_{mm}$	EFSA Journal 2017;15(7):4912
Cyprinodon variegatus Sheepshead minnow	Tribenuron methyl	28d (flowthrough) Early lifestage (ELS)	NOEC =11.9 mg /L $_{mm}$	EFSA Journal 2017;15(7):4912
Aquatic invertebrates				
Daphnia magna	MCPA	48 h, f	EC_{50} >190 mg a.s./L	SANCO/4062/2001-final of 11.07.2008
Daphnia magna	MCPA	21 d, f	NOEC = 50* mg a.s./L	SANCO/4062/2001-final of 11.07.2008

Species	Substance	Exposure System	Results	Reference
Daphnia magna Water flea	Tribenuronmethyl	48h (static)	EC_{50}>894 mg a.s./L $_{mm}$	EFSA Journal 2017;15(7):4912
Daphnia magna Water flea	IN-00581 = saccharin	48h (static)	EC_{50}>118 mg/ L $_{mm}$	EFSA Journal 2017;15(7):4912
Daphnia magna Water flea	IN-A4098 = Ndemethyl triazine amine (AE F059411, CG A150829)	48h (static)	EC_{50}>99 mg/ L $_{mm}$	EFSA Journal 2017;15(7):4912
Daphnia magna Water flea	IN-D5119 Acid sulfonamide	48h (static)	EC_{50}>120 mg/ L (nom)	EFSA Journal 2017;15(7):4912
Daphnia magna Water flea	IN-L5296 Methyl triazine amine	48h (static)	EC_{50}>1020 mg/ L $_{mm}$	EFSA Journal 2017;15(7):4912
Daphnia magna Water flea	Tribenuronmethyl	21d (staticrenewal)	EC_{10}=52 mg/ L $_{mm}$	EFSA Journal 2017;15(7):4912
Daphnia magna Water flea	IN-A4098 = Ndemethyl triazine amine (AE F059411, CG A150829)	21d (static)	NOEC=97 mg/ L $_{mm}$	EFSA Journal 2017;15(7):4912
Daphnia magna Water flea	IN-L5296 Methyl triazine amine	21d (static)	NOEC=49 mg/ L $_{mm}$	EFSA Journal 2017;15(7):4912
Algae				
Selenastrum capricornutum	MCPA	120 h	E_rC_{50} > 392 mg a.s./L	SANCO/4062/2001-final of 11.07.2008
Navicula pelliculosa	MCPA	120 h	E_rC_{50} = 117 mg a.s./L	SANCO/4062/2001-final of 11.07.2008
Pseudokirchneriella subcapitata Green algae	Tribenuronmethyl	72h (static)	ErC_{50}=0.068 mg/ L $_{mm}$	EFSA Journal 2017;15(7):4912
Pseudokirchneriella subcapitata Green algae	IN-00581 = saccharin	72h (static)	ErC_{50}>10 mg/ L (nom)	EFSA Journal 2017;15(7):4912
Scenedesmus subspicatus Green algae	IN-A4098 = Ndemethyl triazine amine (AE F059411, CG A150829)	72h (static)	EbC_{50}>90 mg/ L (nom)	EFSA Journal 2017;15(7):4912
Pseudokirchneriella subcapitata Green algae	IN-R9805 Odemethyl triazine amine	72h (static)	ErC_{50}=142mg/ L (nom)	EFSA Journal 2017;15(7):4912
Pseudokirchneriella subcapitata Green algae	IN-GN815	72h (static)	ErC_{50}>120mg/ L (nom)	EFSA Journal 2017;15(7):4912
Pseudokirchneriella subcapitata Green algae	IN-GK521	72h (static)	ErC_{50}=12.9mg/ L (nom)	EFSA Journal 2017;15(7):4912
Pseudokirchneriella subcapitata Green algae	IN-D5803 Sulfonamide, methylsaccharin	72h (static)	ErC_{50}>19.7mg/ L (geometric mean of measured initial and	EFSA Journal 2017;15(7):4912

Species	Substance	Exposure System	Results	Reference
			measured at highest test concentration after 72h)	
Pseudokirchneriella subcapitata Green algae	M2 Triazine urea	72h (static)	ErC_{50}>100mg/ L (nom)	EFSA Journal 2017;15(7):4912
Pseudokirchneriella subcapitata Green algae	IN-D5119 Acid sulfonamide	72h (static)	ErC_{50}>10mg/ L (nom)	EFSA Journal 2017;15(7):4912
Pseudokirchneriella subcapitata Green algae	IN-L5296 Methyl triazine amine	72h (static)	ErC_{50}>10mg/ L (nom)	EFSA Journal 2017;15(7):4912
Anabaena flosaquae Cyanobacteria	Tribenuronmethyl	72h (static)	ErC_{50}>100mg/ L (nom)	EFSA Journal 2017;15(7):4912
Higher plants				
Lemna gibba	MCPA	14 d	IC_{50} = 0.152 mg a.s./L	SANCO/4062/2001-final of 11.07.2008
Lemna gibba Duck weed	Tribenuronmethyl	7d (static)	ErC_{50}= 0.0047mg/ L (nom)	EFSA Journal 2017;15(7):4912
Lemna gibba Duck weed	Tribenuronmethyl	16d low temperature (12°C), (static)	ErC_{50}= 0.0062mg/ L (nom)	EFSA Journal 2017;15(7):4912
Lemna gibba Duck weed	IN-00581 = saccharin	7d (static)	$ErCr_{50}$>11 mg/ L_{mm}	EFSA Journal 2017;15(7):4912
Lemna gibba Duck weed	IN-A4098 = Ndemethyl triazine amine (AE F059411, CG A150829)	7d (static)	ErC_{50}>100mg/ L (nom)	EFSA Journal 2017;15(7):4912
Lemna gibba Duck weed	IN-D5119 Acid sulfonamide	7d (static)	ErC_{50}>11mg/ L (nom)	EFSA Journal 2017;15(7):4912
Lemna gibba Duck weed	IN-L5296 Methyl triazine amine	14d (static)	ErC_{50}>10mg/ L (nom)	EFSA Journal 2017;15(7):4912
Lemna gibba Duck weed	IN-R9805 Odemethyl triazine amine	7d (static)	ErC_{50}>100mg/ L (nom)	EFSA Journal 2017;15(7):4912
Lemna gibba Duck weed	IN-GN815	7d (static)	ErC_{50}>120mg/ L (nom)	EFSA Journal 2017;15(7):4912
Lemna gibba Duck weed	IN-GK521	7d (static)	ErC_{50}=0.29mg/ L (nom)	EFSA Journal 2017;15(7):4912
Lemna gibba Duck weed	IN-D5803 Sulfonamide, methylsaccharin	7d (static)	ErC_{20}=10mg/ L (nominal degradation product saccharine)	EFSA Journal 2017;15(7):4912
Lemna gibba Duck weed	M2 Triazine urea	7d (static)	ErC_{50}>100mg/ L (nom)	EFSA Journal 2017;15(7):4912

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
* as for these aquatic end points is MCPA DMA

Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – HAKSAR TOP 565 SG

Species	Substance	Exposure System	Results [mg/L]	Reference
Daphnia magna	HAKSAR TOP 565 SG	48 h, s	EC_{50} > 100 mg formulation/L $_{nom}$ corresponding to 56.5mg/L of MCPA and 1.46 mg/L of Tribenuron-methyl	Tina Turek, Msc W/269/17 12.2017
Daphnia magna	HAKSAR TOP 565 SG	21 d, ss	EC50= >10 mg formulation/L nom ~~NOEC ≥ 10 mg formulation/L $_{nom}$~~	Paweł Bąk, Msc W/36/18 08.2018
Pseudokirchneriella subcapitata	HAKSAR TOP 565 SG	72 h	E_rC_{50} = 5.51 mg formulation/L $_{nom}$ E_rC_{50} = 3.112 mg MCPA/L $_{nom}$ E_rC_{50} = 0.08 mg tribenuron-methyl /L $_{nom}$ EbC_{50} = 0.94mg/L $_{nom}$ EbC_{50} = 0.531 mg MCPA/L $_{nom}$ EbC_{50} = 0.014 mg tribenuron-methyl /L $_{nom}$	Tina Turek, Msc W/270/17 01.2018
Navicula pelliculosa	HAKSAR TOP 565 SG	72 h	E_rC_{50} >100 mg forulation/L $_{nom}$ E_rC_{50} > 56.5 mg MCPA/L $_{nom}$ E_rC_{50} > 1.46 mg tribenuron/L $_{nom}$ EbC_{50} >100 mg forulation/L $_{nom}$ EbC_{50} > 56.5 mg MCPA/L $_{nom}$ EbC_{50} > 1.46 mg tribenuron-methyl /L $_{nom}$	Tina Turek, Msc W/271/17 12.2017
Lemna gibba	HAKSAR TOP 565 SG	7 d, s	E_rC_{50} = 1.482 mg formulation/L $_{nom}$ E_rC_{50} = 0.837 mg MCPA/L $_{nom}$ E_rC_{50} = 0.0215 mg tribenuron-methyl /L $_{nom}$ EbC_{50} =0.478 mgformulation/L $_{nom}$ EbC_{50} = 0.270 mg MCPA/L $_{nom}$ EbC_{50} = 0.0069 mg tribenuron-methyl /L $_{nom}$ (on basis of frond number)	Tina Turek, Msc W/272/17 02.2018
Myriophyllum spicatum	HAKSAR TOP 565 SG	14 d, s	ErC_{50} = 15.919 mg formulation/L $_{nom}$ E_rC_{50} = 8.269 mg MCPA/L E_rC_{50} = 0.179 mg tribenuron-methyl /L EbC_{50} = 5.367 mg formulation/L $_{nom}$ EbC_{50} = 2.610 mg MCPA/L EbC_{50} = 0.05968 mg tribenuron-methyl /L (on basis of total shoot length)	Tina Turek, Msc W/181/17 07.2018
Higher-tier studies (micro- or mesocosm studies)				
No study submitted, not required				

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 deviation from the EU agreed endpoints

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 tables below.

9.5.2.1 MCPA risk assemssment

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 MCPA for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of HAKSAR TOP 565 SG in winter cereals - autumn application

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**		**Aquatic plants**
Test species		***Oncorhynchus mykiss***	***Pimephales promelas***	***Daphnia magna***	***Daphnia magna***	***Scenedesmus quadricauda***	***Navicula pelliculosa***	***Lemna gibba***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	IC_{50}	E_rC_{50}	IC_{50}
(µg/L)		50 000	15 000	> 190 000	50 000	>392 000	117 000	152
AF		100	10	100	10	10	10	10
RAC (µg/L)		500	1 500	>1 900	5 000	>39 200	11 700	15.2
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**							
Step 1								
	175.4423	0.35	0.12	0.09	0.04	0.00	0.01	**11.54**
Step 2								
N-Europe	78.6745	-	-	-	-	-	-	**6.82**
Step 3								
D3/ditch	3.480	-	-	-	-	-	-	0.23
D4/pond	5.220	-	-	-	-	-	-	0.34
D4/stream	7.976	-	-	-	-	-	-	0.52
D5/ pond	3.669	-	-	-	-	-	-	0.24

Group		Fish acute	Fish pro-longed	Inverteb. acute	Inverteb. prolonged	Algae		Aquatic plants
D5/stream	5.475	-	-	-	-	-	-	0.36
R1/pond	0.1202	-	-	-	-	-	-	0.01
R1/stream	3.426	-	-	-	-	-	-	0.23
R3/stream	34.92	-	-	-	-	-	-	**2.30**
R4/stream	11.64	-	-	-	-	-	-	0.77

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

Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for MCPA for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of HAKSAR TOP 565 SG in spring, winter cereals and grass – spring application

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**		**Aquatic plants**
Test species		***Oncorhynchus mykiss***	***Pimephales promelas***	***Daphnia magna***	***Daphnia magna***	***Scenedesmus quadricauda***	***Navicula pelliculosa***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} 50 000	NOEC 15 000	EC_{50} > 190 000	NOEC 50 000	IC_{50} >392 000	E_rC_{50} 117 000	IC_{50} 152
AF		100	10	100	10	10	10	10
RAC (µg/L)		500	1 500	>1 900	5 000	>39 200	11 700	15.2
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**							
Step 1								
	175.4423	0.35	0.12	0.09	0.04	0.00	0.01	**11.54**
Step 2								
N-Europe	33.8255	-	-	-	-	-	-	**2.23**
Step 3								
spring cereals, spring application (550 g as/ha)								
D3/ditch	3.489	-	-	-	-	-	-	0.22
D4/pond	0.2110	-	-	-	-	-	-	0.01
D4/stream	2.874	-	-	-	-	-	-	0.19
D5/ pond	0.1685	-	-	-	-	-	-	0.01
D5/stream	3.101	-	-	-	-	-	-	0.20
R4/stream	26.36	-	-	-	-	-	-	**1.73**
Winter cereals, spring application (550 g as/ha)								
D3/ditch	3.485	-	-	-	-	-	-	0.23
D4/pond	0.1844	-	-	-	-	-	-	0.01
D4/stream	2.915	-	-	-	-	-	-	0.19
D5/pond	0.1681	-	-	-	-	-	-	0.01
D5/stream	2.823	-	-	-	-	-	-	0.19
R1/pond	0.1411	-	-	-	-	-	-	0.01
R1/stream	7.249	-	-	-	-	-	-	0.48
R3/stream	8.434	-	-	-	-	-	-	0.56
R4/stream	26.23	-	-	-	-	-	-	**1.73**
Grass, spring application (550 g as/ha)								
Step 1								

Group		Fish acute	Fish pro-longed	Inverteb. acute	Inverteb. prolonged	Algae		Aquatic plants
	175.4423	0.35	0.12	0.09	0.04	0.00	0.01	**11.54**
Step 2								
N-Europe	15.8860	-	-	-	-	-	-	**1.05**
Step 3								
D3/ditch	3.491	-	-	-	-	-	-	0.23
D4/pond	0.1203	-	-	-	-	-	-	0.01
D4/stream	2.666	-	-	-	-	-	-	0.18

For the intended uses cereals, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for aquatic plants as characterised by an IC_{50} for *Lemna gibba* of 152 µg a.s./L in connection with an assessment factor of 10 in several FOCUS Steps 1-3 scenarios. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies.

At Step 4 run-off mitigation via vegetated filter strip efficiency was calculated for 1m buffer using the VFSmod model. Additionaly the run-off reduction was considered with a vegetative buffer of 10-12 m (by reducing mass of pesticide in aqueous phase by 60% and mass of eroded sediment by 85%) and 18-20 m (by reducing mass of pesticide in aqueous phase by 70% and mass of eroded sediment by 95%). To see more details please refer to Section 8 (Environmental Fate), Chapter 8.9.2.1.

Table 9.5-4: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for MCPA based on FOCUS Step 4 calculations and toxicity data for aquatic plants with mitigation of spray drift and run-off for the use of HAKSAR TOP 565 SG in winter and spring cereals (spring application) and winter cereals (autumn application).

Intended use **Active substance** **Application rate (g/ha)**		Spring/Winter cereals MCPA 1 × 550		
Nozzle reduction	**No-spray buffer (m)**	**1**	**10**	**20**
	Vegetated filter strip (m) - VFSmod	**1**	**-**	**-**
	Vegetative buffer (m)	**-**	**10-12**	**18-20**
	spring cereals, spring application			
None	**R4/stream**	6.131	11.90	-
	winter cereals, spring application			
None	**R4/stream**	6.166	11.84	-
	winter cereals, autumn application			
None	**R3/stream**	12.57	15.70	8.194
RAC (µg/L) 15.2		**PEC/RAC ratio**		

	spring cereals, spring application			
None	**R4/stream**	0.40	0.78	-
	winter cereals, spring application			
None	**R4/stream**	0.41	0.78	-
	winter cereals, autumn application			
	R3/stream	0.83	**1.03**	0.54

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

9.5.2.2 Tribenuron-methyl risk assessment

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 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Tribenuron-methyl for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of HAKSAR TOP 565 SG in spring and winter cereals (pH<7) – spring application

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**		**Aquatic plants**
Test species		***Oncorhynchus mykiss***	***Cyprinodon variagatus***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchinella subcapitata***	***Anabaena flosaque***	***Lemna gibba***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	ErC_{50}	E_rC_{50}	ErC_{50}
(µg/L)		738000	11900	>894000	52000	68	>100000	4.7
AF		100	10	100	10	10	10	10
RAC (µg/L)		7380	1190	8940	5200	6.8	10000	0.47
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**							
Step 1								
	4.89	0.0007	0.004	0.0005	0.0009	0.72	0.0005	**10.40**
Step 2								
N-Europe	**0.68**	-	-	-	-	-	-	**1.45**
Step 3								
spring cereals, spring application (15 g as/ha)								
D3/ditch	0.0951	-	-	-	-	-	-	0.20
D4/pond	0.003287	-	-	-	-	-	-	0.01
D4/stream	0.07778	-	-	-	-	-	-	0.17

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. pro-longed	Algae		Aquatic plants
D5/pond	0.003297	-	-	-	-	-	-	0.01
D5/stream	0.08299	-	-	-	-	-	-	0.18
R4/stream	0.4781	-	-	-	-	-	-	**1.02**
winter cereals, spring application (15 g as/ha)								
D3/ditch	0.09496	-	-	-	-	-	-	0.20
D4/pond	0.003286	-	-	-	-	-	-	0.01
D4/stream	0.07930	-	-	-	-	-	-	0.17
D5/pond	0.003281	-	-	-	-	-	-	0.01
D5/stream	0.07587	-	-	-	-	-	-	0.16
R1/pond	0.003280	-	-	-	-	-	-	0.01
R1/stream	0.1513	-	-	-	-	-	-	0.32
R3/stream	0.09678	-	-	-	-	-	-	0.21
R4/stream	0.4726	-	-	-	-	-	-	**1.01**

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

Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Tribenuron-methyl for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of HAKSAR TOP 565 SG in winter cereals (pH<7) –autumn application

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**		**Aquatic plants**
Test species		***Oncorhynchus mykiss***	***Cyprinodon variagatus***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchinella subcapitata***	***Anabaena flosaque***	***Lemna gibba***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	ErC_{50}	E_rC_{50}	ErC_{50}
(µg/L)		738000	11900	>894000	52000	68	>100000	4.7
AF		100	10	100	10	10	10	10
RAC (µg/L)		7380	1190	8940	5200	6.8	10000	0.47
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**							
Step 1								
	4.89	0.0007	0.004	0.0005	0.0009	0.72	0.0005	**10.40**
Step 2								
N-Europe	**1.52**	-	-	-	-	-	-	**3.23**
Step 3								
winter cereals, autumn application (15 g as/ha)								
D3/ditch	0.09475	-	-	-	-	-	-	0.20
D4/pond	0.02370	-	-	-	-	-	-	0.05
D4/stream	0.08223	-	-	-	-	-	-	0.17
D5/pond	0.03949	-	-	-	-	-	-	0.08
D5/stream	0.08871	-	-	-	-	-	-	0.19

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae		Aquatic plants
R1/pond	0.003280	-	-	-	-	-	-	0.01
R1/stream	0.09029	-	-	-	-	-	-	0.19
R3/stream	0.9270	-	-	-	-	-	-	**1.97**
R4/stream	0.2127	-	-	-	-	-	-	0.45

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

Table 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Tribenuron-methyl for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of HAKSAR TOP 565 SG in spring and winter cereals (pH >7) – spring application

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae		Aquatic plants
Test species		***Oncorhynchus mykiss***	***Pimephales promelas***	***Daphnia magna***	***Daphnia magna***	***Scenedesmus quadricauda***	***Navicula pelliculosa***	***Lemna gibba***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	ErC_{50}	E_rC_{50}	ErC_{50}
(µg/L)		738000	11900	>894000	52000	68	>100000	4.7
AF		100	10	100	10	10	10	10
RAC (µg/L)		7380	1190	8940	5200	6.8	10000	0.47
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**							
Step 1								
	5.08	0.0007	0.004	0.0006	0.001	0.75	0.0005	**10.81**
Step 2								
N-Europe	0.96	-	-	-	-	-	-	**2.04**
Step 3								

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. pro-longed	Algae		Aquatic plants
spring cereals, spring application (15 g as/ha)								
D3/ditch	0.1145	-	-	-	-	-	-	0.24
D4/pond	0.02886	-	-	-	-	-	-	0.06
D4/stream	0.08899	-	-	-	-	-	-	0.19
D5/pond	0.005828	-	-	-	-	-	-	0.00
D5/stream	0.08454	-	-	-	-	-	-	0.18
R4/stream	0.7697	-	-	-	-	-	-	**1.64**
winter cereals, spring application (15 g as/ha)								
D3/ditch	0.1099	-	-	-	-	-	-	0.23
D4/pond	0.02840	-	-	-	-	-	-	0.06
D4/stream	0.08939	-	-	-	-	-	-	0.19
D5/pond	0.006148	-	-	-	-	-	-	0.01
D5/stream	0.07754	-	-	-	-	-	-	0.16
R1/pond	0.003280	-	-	-	-	-	-	0.01
R1/stream	0.2349	-	-	-	-	-	-	0.50
R3/stream	0.1129	-	-	-	-	-	-	0.24
R4/stream	0.7669	-	-	-	-	-	-	**1.63**

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

Table 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Tribenuron-methyl for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of HAKSAR TOP 565 SG in winter cereals (pH>7) –autumn application

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. pro-longed**	**Algae**		**Aquatic plants**
Test species		***Oncorhynchus mykiss***	***Cyprinodon variagatus***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchinella subcapitata***	***Anabaena flosaque***	***Lemna gibba***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	ErC_{50}	E_rC_{50}	ErC_{50}
(µg/L)		738000	11900	>894000	52000	68	>100000	4.7
AF		100	10	100	10	10	10	10
RAC (µg/L)		7380	1190	8940	5200	6.8	10000	0.47
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**							
Step 1								
	5.08	0.0007	0.004	0.0006	0.001	0.75	0.0005	**10.81**
Step 2								
N-Europe	2.21	-	-	-	-	-	-	**4.7**
Step 3								
winter cereals, autumn application (15 g as/ha)								
D3/ditch	0.1621	-	-	-	-	-	-	0.34
D4/pond	0.3806	-	-	-	-	-	-	0.81
D4/stream	0.3119	-	-	-	-	-	-	0.67
D5/pond	0.3940	-	-	-	-	-	-	0.84
D5/stream	0.2668	-	-	-	-	-	-	0.57

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae		Aquatic plants
R1/pond	0.003280	-	-	-	-	-	-	0.01
R1/stream	0.09197	-	-	-	-	-	-	0.20
R3/stream	1.181	-	-	-	-	-	-	**2.51**
R4/stream	0.1606	-	-	-	-	-	-	0.34

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

Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Tribenuron-methyl for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of HAKSAR TOP 565 SG in grassland (pH<7, pH>7) – spring application, 15 g a. s./ha

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae		Aquatic plants
Test species		***Oncorhynchus mykiss***	***Cyprinodon variagatus***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchinella subcapitata***	***Anabaena flosaque***	***Lemna gibba***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	ErC_{50}	E_rC_{50}	ErC_{50}
(µg/L)		738000	11900	>894000	52000	68	>100000	4.7
AF		100	10	100	10	10	10	10
RAC (µg/L)		7380	1190	8940	5200	6.8	10000	0.47
FOCUS Scenario	PEC_{gl-max} (µg/L) pH <7/pH >7							
Step 1								
	4.89 /5.08	0.00 / 0.00	0.00 / 0.00	0.00 / 0.00	0.00 /	0.90 / 0.93	0.00 / 0.00	**13.009 / 13.51**
Step 2								
N-Europe	0.34/0.45	-	-	-	-	-	-	0.91 / **1.2**

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. pro-longed	Algae		Aquatic plants
Step 3								
grass, spring application (15 g as/ha)								
D3/ditch	0.0952/0.1077	-	-	-	-	-	-	0.20 / 0.23
D4/pond	0.0033/0.0051	-	-	-	-	-	-	0.01 / 0.01
D4/stream	0.0727/0.0757	-	-	-	-	-	-	0.15 / 0.16

Table 9.5-9: The risk assessment for aquatic species (most sensitive species of each group) for Tribenuron-methyl metabolites (winter and spring cereals).

Use pattern	Organism	Test substance	Toxicity endpoint mg/L	FOCUS Step 1 PEC_{sw} µg/L	RAC	PEC/RAC	PEC/RAC <1
Crop: Winter	Fish *Oncorhynchus mykiss*	IN-00581	96 h LC50 >124	1.69	1240	0.0014	YES
		IN-A4098	96 h LC50 = 0.93	0.21	9.3	0.023	
		IN - D5119	96 h LC50 >115	0.84	1150	0.0007	
		IN-L5296	96 h LC50 = 172	3.11	1720	0.0018	
	Invertebrate *Daphnia magna*	IN-0581	48 h EC_{50} >118	1.69	1180	0.0014	YES
		IN-A4098	48 h EC_{50} >99	0.21	990	0.0002	
		IN-D5119	48 h EC_{50} >120	0.84	1200	0.0007	
		M2	48 h EC_{50} =23	0.37	230	0.002	
		IN-L5296	48 h EC_{50} >1020	3.11	10200	0.0003	
	Algae *Pseudokirchneriella subcapitata*	IN-00581	72 h E_rC_{50} >10	1.69	1000	0.0017	YES
		IN-A4098	72 h E_bC_{50} >90	0.21	9000	0.000022	
		IN-R9805	72 h E_rC_{50} = 142	0.35	14200	0.000025	
		IN-GN815	72 h E_rC_{50} >120	0.92	12000	0.000077	
		IN-GK521	72 h E_rC_{50} = 12.9	1.51	1290	0.001	
		IN-D5803	72 h E_rC_{50} >19.7	1.24	1970	0.00063	
		M2	72 h E_rC_{50} >100	0.37	10000	0.00004	
		IN-D5119	72 h E_rC_{50} >10	0.84	1000	0.0008	
		IN-L5296	72 h E_rC_{50} >10	3.11	1000	0.003	
	Aquatic plants *Lemna sp.*	IN-00581	7 d E_rC_{50} > 11	1.69	1100	0.0015	YES
		IN-A4098	7 d E_rC_{50} > 100	0.21	10000	0.00002	
		IN-R9805	7 d E_rC_{50} > 100	0.35	10000	0.000035	
		IN-GN815	7 d E_rC_{50} > 120	0.92	12000	0.00008	
		IN-GK521	7 d E_rC_{50} = 0.29	1.51	29	0.052	
		IN-D5803	7 d E_rC_{20} =10	1.24	1000	0.0012	
		M2	7 d E_rC_{50} > 100	0.37	10000	0.00004	
		IN-D5119	7 d E_rC_{50} > 11	0.84	1100	0.0008	
		IN-L5296	7 d E_rC_{50} > 10	3.11	1000	0.003	

For the intended uses cereals, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for aquatic plants as characterised by ErC50 for *Lemna gibba* of 0.47 µg a.s./L in connection with an assessment factor of 10) in several FOCUS Steps 1-3 scenarios. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies. The calculated PEC/RAC ratios indicate the risk for trubenuron methyl of pH>7 covers the risk of tribenuron methyl of pH < 7.

At Step 4 run off mitigation of the pesticide was calculated for 1and 3 m via vegetated filter strip efficiency using the VFSmod model. Additionally, the run off mitigation was considered with a vegetative buffer of 10-12 m and 18-20 m. To see more details please refer to Section 8 (Environmental Fate), Chapter 8.9.2.2.

Table 9.5-10: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for tribenuron-methyl based on FOCUS Step 4 calculations and toxicity data for aquatic plants with mitigation of spray drift and run-off for the use of HAKSAR TOP 565 SG in winter and spring cerals (spring application) and winter cereals (autumn application).

Intended use **Active substance** **Application rate (g/ha)**		Spring/Winter cereals Tribenuron methyl pH >7 1 × 15			
Nozzle reduction	**No-spray buffer (m)**	**1**	**3**	**10**	**20**
	Vegetated filter strip (m) – VFSmod	**1**	**3**	**-**	**-**
	Vegetative buffer (m)	**-**	**-**	**10 - 12**	**18 - 20**
	spring cereals, spring application				
None	**R4/stream**	0.2212	0.03589	0.3474	0.1814
None	winter cereals, spring application				
	R4/stream	0.2221	0.03589	0.3462	0.1807
	winter cereals, autumn application				
None	**R3/stream**	0.4886	0.3831	0.5312	0.2774
RAC (µg/L) **0.47**		**PEC/RAC ratio**			
	spring cereals, spring application				
None	**R4/stream**	0.47	-	0.74	-
None	winter cereals, spring application				
	R4/stream	0.47	-	0.74	-
	winter cereals, autumn application				
None	**R3/stream**	**1.04**	0.82	**1.13**	0.59

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

9.5.2.3 HAKSAR TOP 565 SG Risk Assessment

In addition, the risk of formulation was assessed according to the endpoints derived with formulation studies. For this purpose the PEC values of HAKSAR TOP 565 SG in surface water have been assessed with the Drift calculator in FOCUS (see section B8). The PEC_{SW} for the formulation was calculated for highest single application rate (1.00 kg product/ha). The maximum PEC_{SW} values were used to determine the risk factors for aquatic organisms. Regulatory acceptable concentration (RAC) was calculated on basis of relevant endpoints from studies performed on formulation (Ref. KCP 10.2.1).

Table 9.5 - 11 Assessment of the risk for aquatic organisms due to the use HAKSAR TOP 565 SG in winter and spring cereals.

Test substance	Organism/ Study Reference	Toxicity endpoint [µg product/L]	AF	PEC_{sw} (Drift calculator) µg/L	RAC µg/L	PEC/RAC	PEC/RAC <1 YES/NO
	Daphnia magna/ KCP 10.2.1/01	EC_{50} – 48h >100000	100	**6.4246**	1000	0.006	YES
	Pseudokirchneriella subcapitata/ KCP 10.2.1/02	ErC_{50}-72 h = 5510	10		551	0.12	YES
	Navicula pelliculosa/ KCP 10.2.1/03	ErC_{50}-72 h >100000	10		1000	0.006	YES
	Lemna gibba/ KCP 10.2.1/04	ErC_{50}-7d = 1482	10		148.2	0.04	YES
	Myriophyllum spicatum/ KCP 10.2.1/05	ErC50-14d = 15919	10		1591.9	0.004	YES
	Daphnia magna/ KCP 10.2.2	NOEC-21d ≥ 10000	10		1000	0.006	YES

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

The calculated PEC/RAC ratios considering the formulation endpoint for the most sensitive organism and the formulation PEC_{SW} resulting from drift event, indicate an acceptable risk for the most sensitive group of aquatic organisms (*Lemna gibba*).

Additionally, because HAKSAR TOP 565 SG contains more than one active substance, the effect of combination of these active substances has been considered. MCPA and tribenuron methyl worse endpoints were used for calculations. According to the EFSA Scientific Opinion (EFSA, 2013) measured and calculated mixture toxicity should be compared to determine synergistic, additive or antagonistic effects of the formulation. In the following the concentration addition (CA) model is based on the following equation, for deriving theoretical LC_{50}/EC_{50}.

$$ECx_{mix-CA} = \left(\sum_{i=1}^{n} \frac{p_i}{ECx_i} \right)^{-1}$$

where:

n: number of mixture components
i: index from 1…n mixture components
p_i: the i^{th} component as a relative fraction of the mixture composition (note: Σp_i must be 1)
ECx_i: concentration of component i provoking x % effect (pragmatically, $NOEC_i$ may be inserted, too).

To determine the respective formulation effect, EFSA, 2013 proposed to calculate MDR (model deviation ratio), which divides the calculated mixture toxicity by the measured mixture toxicity. If MDR is between 0.2 and 5, the observed and calculated mixture toxicities are considered in agreement.

Table 9.5 - 12 Summary of results obtained in the studies with formulated product HAKSAR TOP 565 SG and comparison of calculated and measured mixture toxicity.

Compound	Content in the formulation (%)	Fraction in mixture (%)	LC_{50} (mg a.s/L)	Fraction /LD50	Tox per fraction	Contribution to predicted toxicity (%)
Daphnia						
MCPA	55	97.35	190	0.00512	195.1818	99.4237185
Tribenuron methyl	1.5	2.65	894	0.0000297	33674	0.57628147
Total	56.5	100.00	-	0.00515	-	100
HAKSAR TOP 565 SG	-	-	>100 mg product/L (measured) 56,5 mg a.s./L (measured)	-	ECx (mix-CA) 194.16 (predicted)	MDR = 3.44
Pseudokirchinella						
MCPA	55	97.35	117	0.00832	120.1909	2.08658782
Tribenuron methyl	1.5	2.65	0.068	0.39042	2.561333	97.9134122
Total	56.5	100.00	-	0.39874	-	100
HAKSAR TOP 565 SG	-	-	5.51 mg product/L (measured) 3.11 mg a.s./L (measured)	-	ECx (mix-CA) 2.51 (predicted)	MDR = 0.81
Lemna gibba						
MCPA	55	97.35	0.152	6.40429	0.1561	10.183179
Tribenuron methyl	1.5	2.65	0.0047	5.64865	0.0177	89.816821
Total	56.5	100.00	-	12.0529	-	100
HAKSAR TOP 565 SG	-	-	1.482 mg product/L (measured) 0.84 mg a.s./L (measured)	-	$ECx_{(mix-CA)}$ 0.08 (predicted)	MDR = 0.10

The calculated MDR values are between 0.2 and 5 for each organism expect *Lemna gibba*, indicating that the formulation does not cause (unexpected) an increased toxicity compared to the active substances for these organisms. No synergisms or additional toxicity occurs due to the co-formulants. The apparent antagonism for *Lemna gibba* (toxicity of the formulation lower than expected) can be explained by the fact that endpoints for individual active substances are higher than values. When MDR <0.2 the predicted value should be used in the risk assessment of the formulation product. However, an assemssment to determine the presence of a toxic driver was conducted. Overall, it appears the MCPA is the toxic driver for daphnids (contributes 99 % of the formulation toxicity). For algae and aquatic plants tribenuron methyl occur to be the toxicity driver. Generally the toxicity is primarily influenced by the active substances.

9.5.3 Overall conclusions

The use in spring cereals is covered by risk assessment for winter cereals. Moreover, autumn application covers the risk assesment of spring application.
Based on PEC/RAC calculations, no unacceptable risk is indicated for aquatic organisms considering all envisaged GAP uses in spring and winter cereals (autumn and spring application), and on minor for HAKSAR TOP 565 SG, provided that following risk mitigation measures are taken into account:

- a vegetative buffer strip of 20 m to surface water bodies is required.

According to "Working Document of the Central Zone in the Authorization of Plant Protection Products - Part B section 8 - Environmental fate and behavior, Version 1 rev. 1 – June 2018" it should be checked with individual MS wheather VFSmod approach is acceptable or not. Therefore, a vegetative filter strip of 3 m (VFSmod) should be considered if required.

However, for Poland the relevant scenarios are D3, D4 and R1. Thus, no unacceptable risk is indicated following the HAKSAR TOP 565 SG application. None mitigation measures are required.

Review Comments:

The evaluation of the risk for aquatic was performed in accordance with 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(EFSA Journal 2013;11(7):3290).

For the active substance MCPA
For the active substance calculated PEC/RAC ratios for winter and spring cereals and spring grasslands did indicate an acceptable risk in almost all FOCUS Steps 1-3 scenarios with the exception of Scenario: R3stream (autumn application) and R4stream (spring application)

Lowest RAC for MCPA = 15.2 µg a.s/L would be taken to consideration.

To refine the risk to aquatic organisms of **MCPA** for scenario R3 stream and R4stream appropriate risk mitigation measures are required:
At Step 4 the run-off mitigation via vegetated filter strip was calculated for 1 m buffer using the VFS mode. Additionally, the run-off reduction was considered with a vegetative buffer strip of 10 m and 20 m.

SWAN model. Max. PECsw, (µg/L)

Crop	Application rate g a.s./ha	MCPA 10 m VBS* + 10 m NSS	MCPA 20 m VBS* + 20 m NSS
Winter cereals autumn application	550.0	15.70 R3 stream	8.194 R3 stream
Winter cereals spring application	550.0	11.84 R4 stream	6.180 R4 stream
Spring cereals	550.0	11.90 R4 stream	6.210 R4 stream

***vegetated buffer strip**

Following mitigation measures are required when run-off reduction in STEP 4 is considered with a vegetative buffer zone of 10 m and 20 m.

Winter cereals autumn application - 10 m VBS + 10 m NSS

Winter cereals spring application - 10 m VBS + 10 m NSS

Spring cereals - 10 m VBS + 10 m NSS

For grass no mitigation measure was proposed.

VFSmod. Max. PECsw, (µg/L)

Crop	Application rate g a.s./ha	MCPA 1 m VFS + 1 m NSS
Winter cereals autumn application	550.0	12.57 R3 stream
Winter cereals spring application	550.0	6.166 R4 stream
Spring cereals	550.0	6.131 R4 stream

Following mitigation measures are required when VFSmod is considered

All cereals according to critical GAP: 1 m VFS + 1 m NSS

For grass no mitigation measure was proposed.

For the active substance **tribenuron-methyl** calculated PEC/RAC ratios for winter and spring cereals and spring grasslands did indicate an acceptable risk in almost all FOCUS Steps 1-3 scenarios with the exception of Scenario: R3stream and R4stream as follows:

spring and winter cereals (pH<7) – spring application
R4/stream - spring cereals, spring application (15 g as/ha)
R4/stream - winter cereals, spring application (15 g as/ha)

winter cereals (pH<7) –autumn application
R3/stream - winter cereals, autumn application (15 g as/ha)

spring and winter cereals (pH >7) – spring application
R4/stream - spring cereals, spring application (15 g as/ha)
R4/stream - winter cereals, spring application (15 g as/ha)

winter cereals (pH>7) –autumn application
R3/stream – winter cereals, autumn application (15 g as/ha)

For tribenuron-methyl in grasslands (pH<7, pH>7) – spring application, 15 g a. s./ha PEC/RAC ratios indicate an acceptable risk already at FOCUS Steps 1-3. For grass no mitigation measure was proposed.

For all tribenuron-methyl metabolites, calculated PEC/RAC ratios for winter and spring cereals and grasses indicate an acceptable risk already at FOCUS Steps 1.

To refine the risk to aquatic organisms of **tribenuron methyl** for scenario R3 stream and R4stream appropriate risk mitigation measures are required:

Lowest RAC for tribenuron methyl = 0.47 µg a.s/L would be taken to consideration.

The mitigation measures for spring and winter cereals were proposed. At Step 4 the run-off mitigation via vegetated filter strip was calculated for 1 and 3 m buffer using the VFS model. Additionally, the run-off reduction was considered with a vegetative buffer strip of 10 m and 20 m.

SWAN model. Max. PECsw, (µg/L)

Crop	**Application rate g a.s./ha**	**Tribenuron-methyl pH < 7 10 m VBS* + 10 m NSS**	**PEC/RAC**	**Tribenuron-methyl pH > 7 10 m VBS*+ 10 m NSS**	**PEC/RAC**
Winter cereals autumn application	15.00	0.4170 R3 stream	0.88	0.5312 R3 stream	**1.13**
Winter cereals spring application	15.00	0.2133 R4 stream	0.45	0.3462 R4 stream	0.72
Spring cereals	15.00	0.2158 R4 stream	0.45	0.3474 R4 stream	0.72

***vegetated buffer strip**

Crop	**Application rate g a.s./ha**	**Tribenuron-methyl pH < 7 20 m VBS* + 20 m NSS**	**PEC/RAC**	**Tribenuron-methyl pH > 7 20 m VBS*+ 20 m NSS**	**PEC/RAC**
Winter cereals autumn application	15.00	0.2178 R3 stream	-	0.2774 R3 stream	0.48
Winter cereals spring application	15.00	0.1114 R4 stream	-	0.1807 R4 stream	-
Spring cereals	15.00	0.1126 R4 stream	-	0.1814 R4 stream	-

***vegetated buffer strip**

Following mitigation measures are required when run-off reduction in STEP 4 is considered with a vegetative buffer zone of 10 m and 20 m.

All application in cereals according to critical GAP with pH < 7 and >7

- 10 m VBS + 10 m NSS

With the exception of:

Winter cereals autumn application pH > 7

- 20 m VBS + 20 m NSS

For grass no mitigation measure was proposed.

VFSmod. Max. PECsw, (µg/L)

Crop	Application rate g a.s./ha	Tribenuron-methyl pH < 7 1 m VFS + 1 m NSS	PEC/RAC	Tribenuron-methyl pH > 7 1 m VFS + 1 m NSS	PEC/RAC
Winter cereals autumn application	15.00	0.3439 R3 stream	0.73	0.4886 R3 stream	1.0
Winter cereals spring application	15.00	0.08931 R4 stream	0.18	0.2221 R4 stream	0.47
Spring cereals	15.00	0.1165 R4 stream	0.24	0.2212 R4 stream	0.47

Following mitigation measures are required when VFSmode is considered

All cereals according to critical GAP: 1 m VFS + 1 m NSS

For grass no mitigation measure was proposed.

For the formulated product, no potential risks are identified for aquatic organisms following application of to HAKSAR TOP 565 SG winter and spring cereals and grasslands when appropriate mitigation measures are used.

The submitted PECsw assessment for formulation was accepted. The PECsw were calculated for single application and for the highest application rate recommended for use in winter/spring cereals and grass. Drift calculator for formulation PECsw assessment was used. Assessment for the formulation is presented in Table 9.5 – 11.
For the formulation no mitigation measures are required.

For scenarios relevant for Poland: D3, D4, R1 no mitigation measures are required.

Concerned Member States must decide on the consideration of risk mitigation measures

9.6 Effects on bees (KCP 10.3.1)

9.6.1 Toxicity data

Studies on the toxicity to bees have been carried out with the active substances MCPA and tribenuron methyl. Full details of these studies are provided in the respective EU DAR and related documents as well as in Appendix 2 of this document (new studies).

Effects on bees of HAKSAR TOP 565 SG were not evaluated as part of the EU assessment of MCPA and tribenuron methyl. 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 deviate from the results of the EU review processes, a justification is given below. Risk assessments in agreement with current guidance are only presented for acute effects on honeybees. Chronic and larval development toxicity data are presented in

the list of endpoints.

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

Species	Substance	Exposure System	Results	Reference
Apis mellifera	MCPA	Oral	LD_{50} > 200 µg/bee	SANCO/4062/2001-final of 11.07.2008
		Contact	LD_{50} > 200 µg/bee	
Apis mellifera	Tribenuron methyl technical	Oral	LD_{50} >9.1µg a.s./bee	EFSA Journal 2017;15(7):4912
		Contact	LD_{50} >98.4µg a.s./bee	
Apis mellifera	Tribenuron methyl 75WG	Contact	LD_{50}>100 µg a.s./bee	EFSA Journal 2017;15(7):4912
		Oral	LD_{50} >186 µg a.s./bee	
Apis mellifera	Tribenuron methyl 50SG	Contact	LD_{50}>100 µg a.s./bee	EFSA Journal 2017;15(7):4912
		Oral	LD_{50} >77.1 µg a.s./bee	
Apis mellifera	Tribenuron methyl 50SG + DPX-KG691 surfactant	Contact	LD_{50}>200 µg a.s./bee	EFSA Journal 2017;15(7):4912
		Oral	LD_{50} =33.7 µg a.s./bee	
Apis mellifera	Tribenuron methyl 750 g/kg WG	Contact	LD_{50}>100 µg a.s./bee	EFSA Journal 2017;15(7):4912
		Oral	LD_{50} >108.8 µg a.s./bee	
Apis mellifera	Tribenuron-methyl technical	10 days Chronic	Oral LD_{50} > 74.3 µg a.s./bee/day Oral $NOEL_{mortality}$=74.3 µg a.s./bee/day	EFSA Journal 2017;15(7):4912
Apis mellifera	Tribenuron-methyl 75 WG	10 days Chronic	Oral LD_{50} > 147.5 µg a.s./bee/day Oral $NOEL_{hpg}$ =147.5 µg a.s./bee/day Oral $NOEL_{mortality}$=124.7 µg a.s./bee/day	EFSA Journal 2017;15(7):4912
Apis mellifera	HAKSAR TOP 565 SG (Formulation MT-565 SG-OR2-C)	Larval toxicity test, repeated exposure	LC_{50} = 316.262 mg of test item/kg of food NOEC = 81.25 mg of test item/kg of food	W. Londzin, 0016/01202/E, 2020
Apis mellifera	HAKSAR TOP 565 SG (Formulation MT-565 SG-OR2-C)	10 days Chronic	LC_{50} = 4460 mg/kg 10 d LDD_{50} = 100.62 µg /bee/day	P.Parma B/26/18, 2019
Apis mellifera	HAKSAR TOP 565 SG (Formulation MT-565 SG-OR2-C)	Oral	LD_{50} = 95.3 µg formulation/bee	N. Lemańska B/09/17, 2018
Apis mellifera	HAKSAR TOP 565 SG (Formulation MT-	Contact	LD_{50} > 200 µg formulation/bee	N. Lemańska B/10/17, 2018

Species	Substance	Exposure System	Results	Reference
	565 SG-OR2-C)			
Higher-tier studies (tunnel test, field studies)				
Not available				

9.6.1.1 Justification for new endpoints

No deviation from the EU agreed endpoints.

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).
Risk assessments are presented for the intended use of HAKSAR TOP 565 SG in spring and winter cereals (autumn and spring application), and on minor uses according to the proposed GAP.

The risk assessment is presented for the maximum single application rate of 1 kg product/ha (i.e. 550 g MCPA/ha and 15 g tribenuron methyl/ha), covering all the remaining application patterns with also proposed minor uses.

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 HAKSAR TOP 565 SG in cereals and in minor uses

Intended use **Active substance** **Application rate (g as/ha)**	All intendent uses MCPA 1 × 550		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	>200	550	< 2.75
Contact toxicity	>200		< 2.75
Intended use **Active substance** **Application rate (g a.s./ha)**	All intendent uses Tribenuron methyl 1 × 15		
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	> 9.1	15	< 1.64
Contact toxicity	> 98.4		< 0.15
Product **Application rate (g product/ha)**	HAKSAR TOP 565 SG 1× 1000		

Test design	**LD_{50} (lab.) (µg product/bee)**	**Single application rate (g product/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	>95.3	1000	< 10.49
Contact toxicity	>200		5

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

As outlined in the table above, both the Hazard Quotients for oral (QHO) and contact exposure (QHC) are well below the trigger of 50 for the active substances and the product. Therefore, a low risk to bees is expected from the application of HAKSAR TOP 565 SG for the intended uses.

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

Not relevant.

Review Comments:

Since acceptable acute risk have been concluded for bees exposed to HAKSAR TOP 565 SG at the Tier 1 level, a higher-tier risk assessment is not required for the proposed uses of HAKSAR TOP 565 SG.

9.6.3 Effects on bumble bees

No data/information available.

Review Comments:
According to SANCO/10329/2002 rev 2 final, the risk assessment for bumblebees is not required.

9.6.4 Effects on solitary bees

No data/information available.

Review Comments:
According to SANCO/10329/2002 rev 2 final, the risk assessment for bumblebees is not required.

9.6.5 Overall conclusions

The acute risk assessments for the active substances as well as for the formulated product HAKSAR TOP 565 SG 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 spring and winter cereals (autumn and spring application), and on minor uses. Therefore, a low risk to bees is expected from the application of HAKSAR TOP 565 SG according to the proposed GAP.

Review Comments:

The evaluation has been performed in line with SANCO/10329/2002 rev 2 final.
The risk assessment performed for active substances: MCPA and tribenuron-methyl and the formulated

product is agreed by the zRMS.

Risk assessments are based on the maximum single application rate of 1 kg product/ha (corresponding to 550 g MCPA/ha and 15 g tribenuron methyl/ha) in cereals and minor crops. All calculated hazard quotients are lower than 50, indicating that the acute oral and contact risk to bees is acceptable following the use according to the proposed use pattern of HAKSAR TOP 565 SG.

The Applicant provided chronic test on bees and evaluation of effects on honey bee development with formulated product. The chronic studies for HAKSAR TOP 565 SG were evaluated and accepted by zRMS but not taken to consideration in risk assessment since the evaluation was done according to SANCO/10329/2002 rev 2 final.

Concerned Member States must decide on the consideration of data requirements on national 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 active substances MCPA and tribenuron methyl. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target arthropods of HAKSAR TOP 565 SG were not evaluated as part of the EU assessment of MCPA and tribenuron methyl. 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
Typhlodromus pyri (protonymphs)	MCPA	Laboratory test glass plates (2D)	LR_{50} > 2000	SANCO/4062/2001-final of 11.07.2008
Aphidius rhopalosiphi (adults)	MCPA	Extended laboratory test barley plants (3D)	LR_{50} > 2100	SANCO/4062/2001-final of 11.07.2008
Aphidius rhopalosiphi (adults)	Tribenuron methyl 75 WG	Laboratory test glass plates (2D)	48-h LR_{50} > 30	EFSA Journal 2017;15(7):4912
	Tribenuron methyl 50SG	Laboratory test glass plates (2D)	48-h LR_{50} > 300	EFSA Journal 2017;15(7):4912
	Tribenuron methyl 50SG + DPX-KG691 surfactant	Laboratory test glass plates (2D)	48-h LR_{50} > 29.6	EFSA Journal 2017;15(7):4912
	Tribenuron-Methyl 750 g/kg WG	Laboratory test glass plates (2D)	48-h LR_{50} > 150	EFSA Journal 2017;15(7):4912
Typhlodromus pyri	Tribenuron methyl 75 WG	Laboratory test glass plates (2D)	7-d LR_{50} >30	EFSA Journal 2017;15(7):4912

Species	Substance	Exposure System	Results	Reference
(protonymphs)	Tribenuron methyl 50SG	Laboratory test glass plates (2D)	7-d LR_{50} > 300	EFSA Journal 2017;15(7):4912
	Tribenuron methyl 50SG + DPX-KG691 surfactant	Laboratory test glass plates (2D)	7-d LR_{50} > 29.6	EFSA Journal 2017;15(7):4912
	Tribenuron-Methyl 750 g/kg WG	Laboratory test glass plates (2D)	7-d LR_{50} > 150	EFSA Journal 2017;15(7):4912
Typhlodromus pyri (protonymphs)	HAKSAR TOP 565 SG	Laboratory test glass plates (2D)	LR_{50} > 1 kg/ha ER_{50} <0.25 kg/ha	Aneta Glanas, B/12/17, 2018
Aphidius rhopalosiphi (adults)	HAKSAR TOP 565 SG	Laboratory test glass plates (2D)	LR_{50} > 1 kg product /ha ER_{50} > 1 kg product/ha	Aneta Glanas, B/11/17, 2018
Typhlodromus pyri (protonymphs)	HAKSAR TOP 565 SG	TIER 2, 2D Extended laboratory test discs cut out of bean leaves	LR>1 kg product /ha ER_{50} =0.448 kg product/ha NOERreproduction < 0.125 kg product/ha.	Paweł Parma B/39/18, 2018

Review comments:

Table above has been updated by zRMS of the study B/39/18 by Parma (2018) with formulation, which was nod indicated by the Applicant.
Studies on toxicity of HAKSAR TOP 565 SG to arthropods other than bees were evaluated by zRMS and accepted for risk assessment purposes. For details please see, Appendix 2.

9.7.1.1 Justification for new endpoints

No deviation from EU agreed endpoints.
Endpoints are provided for the actual formulated product HAKSAR TOP 565 SG for the standard test species *Aphidius rhopalosiphi* and *Typhlodromus pyri*.

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 spring and winter cereals (autumn and spring application), and on minor uses - 1.00 kg product/ ha (i.e. 550 g MCPA/ha and 15 g tribenuron methyl/ha), covering all the remaining application patterns with also proposed minor uses. The calculation was performed for all uses presented in GAP.

Table 9.7-2: First- ~~and higher~~ tier assessment of the in-field risk for non-target arthropods due to the use of HAKSAR TOP 565 SG in cereals and in minor uses

Intended use	All intendent uses		
Active substance/product	HAKSAR TOP 565 SG		
Application rate (g product/ha)	1 × 1000		
MAF	1		
Test species Tier I	**LR_{50} (lab.) (g product/ha)**	**$PER_{in\text{-}field}$ (g product/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	>1000	1000	1
Aphidius rhopalosiphi	>1000		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.

Based on results obtained for HAKSAR TOP 565 SG in laboratory studies on *A. rhopalosiphi* and *T.pyri* the "in-field" hazard quotient is below the trigger value of 2 indicating an acceptable "in-field" risk to *A. rhopalosiphi*, following application of HAKSAR TOP 565 SG according to the proposed GAP

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 spring and winter cereals (autumn and spring application), and on minor uses (respectively 1.00 kg product/ha and 2.77%).
The worst PERoff field value is equal 2.77 ~~5.54~~ g formulation/ha for all of the intended uses.

Table 9.7-3: First- ~~and higher tier~~ assessment of the off-field risk for non-target arthropods due to the use of HAKSAR TOP 565 SG in cereals and in minor uses

Intended use	All intended uses				
Active substance/product	HAKSAR TOP 565 SG				
Application rate (g product /ha)	1 × 1000				
MAF	1				
vdf	10 (Tier 1) ~~5~~ [a)]				
Test species Tier I	**LR_{50} (lab.) (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	>1000	0.0277	2.77 ~~5.54~~	10	0.027 ~~0.0554~~
Aphidius rhopalosiphi	>1000				0.027 ~~0.0554~~

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.

~~a) In accordance with EFSA (2019). Technical report on the outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2019:EN-1673. 117 pp. doi:10.2903/sp.efsa.2019.EN-1673.~~

Based on results obtained for HAKSAR TOP 565 SG in laboratory studies on *T. pyri* and *A. rhopalosiphi*

the “off-field” hazard quotients are below the trigger value of 2 indicating an acceptable “off-field” risk to non-target arthropods following application of HAKSAR TOP 565 SG according to the proposed GAP

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 MCPA and tribenuron methyl applied as HAKSAR TOP 565 SG for the intended uses in spring and winter cereals (autumn and spring application), and on minor uses is indicated to be acceptable for non-target arthropods other than bees also based on Tier 2 for *T.pyri* data without the need for risk mitigation measures.

Review Comments:

The evaluation of the risk for non-target arthropods was performed in accordance with the recommendations of the guidance document ESCORT 2.
Risk assessment presented by the Applicant has been updated.
For the zonal evaluations in the Risk assessment for off-field exposure default factor VDF of 10 was used by zRMS. VDF of 5 should be considered at for National authorisations.

The HQ for recommended species: *Typhlodromus pyri* and *Aphidius rhopalosiphi* is below the ESCORT 2 trigger value of 2, indicating acceptable in-field and off-field risk to non-target arthropods already at tier I level. All calculated HQ values based on a single maximum application rate indicated acceptable risk to cereals and minor crops according to the GAP.

On this basis acceptable risk for in-field and off-field habitats may be concluded with no 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 MCPA and Tribenuron methyl and its metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of HAKSAR TOP 565 SG were not evaluated as part of the EU assessment of MCPA or Tribenuron methyl. 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	End point	Results	Reference
Earthworms					
HAKSAR TOP 565 SG					
Eisenia andrei	MCPA + TRIBENURON METYL 565 SG	Test item incorporated into the soil / 10% peat Chronic, 56 days	NOEC EC_{10}	180 mg formulation/kg dry soil **102.653 mg formulation/kg dry soil**	Aneta Gierbuszewska, G/158/17
Tribenuron-methyl, relevant degradation products and related formulated products from EU review					
Eisenia fetida	Tribenuron-methyl technical	Test item incorpo-rated into the soil / 5% peat Chronic, 56 days	NOEC [a]	**3.2 mg a.s./kg dry soil**	EFSA Journal 2017;15(7):4912
Eisenia fetida	Tribenuron methyl 50SG + DPX-KG691 surfactant	Test item incorpo-rated into the soil / 5% peat Chronic, 56 days	NOEC EC10 repro	61.75 mg a.s./kg dry soil 70.5 mg a.s./kg dry soil	EFSA Journal 2017;15(7):4912
Eisenia fetida	IN-00581 (saccharin)	Test item incorpo-rated into the soil / 10% peat Chronic, 56 days	NOEC [a]	0.05 mg/kg dry soil	EFSA Journal 2017;15(7):4912
Eisenia fetida	IN-00581 (saccharin)	Test item incorpo-rated into the soil / 5% peat Chronic, 56 days	NOEC [a]	**100 mg/kg dry soil**	EFSA Journal 2017;15(7):4912
Eisenia fetida	IN-00581 (saccharin)	This metabolite was also evaluated for metsulfuron-methyl, ethametsulfuron-methyl and propoxycarbazone-sodium (EFSA, 2014a, 2015a, 2016b). The lowest reported NOECs were lower than 100 mg/kg dry soil, it is noted that this endpoints were set at the highest concentration tested. There was only a NOEC = 0.04 mg/kg dry soil not set at the highest concentration tested, however, concerns were raised during the peer review on this endpoint and it was not used in the risk assessment.			EFSA Journal 2017;15(7):4912
Eisenia fetida	IN-A4098 (N-demethyl-triazine amine)	Test item incorpo-rated into the soil / 10% peat Chronic, 56 days	NOEC [a]	0.2 mg/kg dry soil	EFSA Journal 2017;15(7):4912
Eisenia fetida	IN-A4098	Test item	NOEC [a]	**8.0 mg/kg dry soil**	EFSA Journal

Species	Substance	Exposure System	End point	Results	Reference
	(N-demethyl-triazine amine)	incorpo-rated into the soil / 10% peat Chronic, 56 days			2017;15(7):4912
Eisenia fetida	IN-A4098 (N-demethyl-triazine amine)	This metabolite was also evaluated for chlorsulfuron, iodosulfuron-methyl-sodium, thifensulfuron-methyl, metsulfuron-methyl, prosulfuron and triasulfuron (EFSA, 2008; 2014b; 2015a,b,c; 2016a). The lowest reported NOECs were 0.2 and 0.202 mg/lg dry soil The use of these endpoints in the risk assessment would not change the outcome, therefore, the risk assessment was not updated.			EFSA Journal 2017;15(7):4912
Eisenia fetida	IN-D5119 (acid sulfonamide) ***	Test item incorpo-rated into the soil / 10% peat Chronic, 56 days	NOEC [a]	1000 mg/kg dry soil	EFSA Journal 2017;15(7):4912
Eisenia fetida	IN-D5119 (acid sulfonamide) ***	This metabolite was also evaluated for ethametsulfuron-methyl (EFSA, 2014a). The reported NOEC was 1000 mg/kg dry soil.			EFSA Journal 2017;15(7):4912
Eisenia fetida	IN-D5803 (sulfonamide) ***	Test item incorpo-rated into the soil / 5% peat Chronic, 56 days	NOEC [a]	**100 mg/kg dry soil**	EFSA Journal 2017;15(7):4912
Eisenia fetida	IN-D5803 (sulfonamide) ***	This metabolite was also evaluated for metsulfuron-methyl and ethametsulfuron-methyl (EFSA, 2014a, 2015a). The lowest reported NOEC was 100 mg/kg dry soil.			EFSA Journal 2017;15(7):4912
Eisenia fetida	IN-GK521 (O-demethyl-tribenuron methyl)	Test item incorpo-rated into the soil / 10% peat Chronic, 56 days	NOEC [a]	**100 mg/kg dry soil**	EFSA Journal 2017;15(7):4912
Eisenia fetida	IN-GK521 (O-demethyl-tribenuron methyl)	Test item incorpo-rated into the soil / 5% peat Chronic, 56 days	NOEC [a]	1.52 mg/kg dry soil	EFSA Journal 2017;15(7):4912
Eisenia fetida	IN-GN815 (O-demethyl-tribenuron free acid) ***	Test item incorpo-rated into the soil / 10% peat Chronic, 56 days	NOEC [a]	100 mg/kg dry soil	EFSA Journal 2017;15(7):4912
Eisenia fetida	IN-GN815	Test item	NOEC [a]	3.2 mg/kg dry soil	EFSA Journal

Species	Substance	Exposure System	End point	Results	Reference
	(O-demethyl-tribenuron free acid) ***	incorpo-rated into the soil / 5% peat Chronic, 56 days			2017;15(7):4912
Eisenia fetida	IN-L5296 (triazine amine)	Test item incorpo-rated into the soil / 10% peat Chronic, 56 days	NOEC [a]	**0.2 mg/kg dry soil**	EFSA Journal 2017;15(7):4912
Eisenia fetida	IN-R9803 (tribenuron free acid) ***	Test item incorpo-rated into the soil / 10% peat Chronic, 56 days	NOEC [a]	**6.0 mg/kg dry soil**	EFSA Journal 2017;15(7):4912
Eisenia fetida	IN-R9805 (O-demethyl-triazine amine)	Test item incorpo-rated into the soil / 5% peat Chronic, 56 days	NOEC [a]	100 mg/kg dry soil	EFSA Journal 2017;15(7):4912
Eisenia fetida	IN-R9805 (O-demethyl-triazine amine)	Test item incorpo-rated into the soil / 10% peat Chronic, 56 days	NOEC [a]	**1000 mg/kg dry soil**	EFSA Journal 2017;15(7):4912
Eisenia fetida	M2 (triazine urea)	Test item incorpo-rated into the soil / 5% peat Chronic, 56 days	NOEC [a]	**5.0 mg/kg dry soil**	EFSA Journal 2017;15(7):4912
MCPA products from EU review					
Eisenia fetida	MCPA	14 d, acute	LC_{50}	**325 mg a.s./kg dw**	SANCO/4062/2001-final of 11.07.2008
Other soil macroorganisms					
MCPA + TRIBENURON METYL 565 SG					
Folsomia candida	MCPA + TRIBENURON METYL 565 SG	Mixed into substrate 28 d, chronic 5 % peat content	NOEC EC_{10}	32.0 mg formulation/kg dry soil **14.5 mg formulation/kg dry soil**	Agnieszka Stalmach, G/135/18
Hypoaspis aculeifer	MCPA + TRIBENURON METYL 565 SG	Mixed into substrate 14 d, chronic 5 % peat content	NOEC	≥1000 mg formulation/kg dry soil	Agnieszka Stalmach, G/136/18

Species	Substance	Exposure System	End point	Results	Reference
Tribenuron-methyl, relevant degradation products and related formulated products from EU review					
Folsomia candida	Tribenuron methyl 50SG + DPX-KG691 surfactant	Test item incorpo-rated into the soil / 5% peat Chronic, 28 days	NOEC EC10 EC20	62.5 mg prod./kg dry soil = **30.8 mg a.s./kg dry soil** 53.2 mg a.s./kg dry soil 113.6 mg a.s./kg dry soil	EFSA Journal 2017;15(7):4912
Folsomia candida	IN-00581 (saccharin)	Test item incorpo-rated into the soil / 10% peat Chronic, 28 days	NOEC [a]	**100 mg/kg dry soil**	EFSA Journal 2017;15(7):4912
Folsomia candida	IN-00581 (saccharin)	Test item incorpo-rated into the soil / 10% peat Chronic, 28 days	NOEC [a]	**100 mg/kg dry soil**	EFSA Journal 2017;15(7):4912
Folsomia candida	IN-00581 (saccharin)	Test item incorpo-rated into the soil / 10% peat Chronic, 28 days	NOEC [a]	**100 mg/kg dry soil**	EFSA Journal 2017;15(7):4912
Folsomia candida	IN-00581 (saccharin)	This metabolite was also evaluated for metsulfuron-methyl, ethametsulfuron-methyl and propoxycarbazone-sodium (EFSA, 2014a, 2015a, 2016b). The reported NOEC values were 9.0 and 100 mg/kg dry soil. The NOEC of 9.0 mg/kg dry soil was based on absence of statistically significant effects up to and including the highest tested concentration of 9.0 mg/kg soil. Hence, it is not considered as adverse data.			EFSA Journal 2017;15(7):4912
Folsomia candida	IN-A4098 (N-demethyl-triazine amine)	Test item incorpo-rated into the soil / 10% peat Chronic, 28 days	NOEC [a]	0.225 mg/kg dry soil	EFSA Journal 2017;15(7):4912
Folsomia candida	IN-A4098 (N-demethyl-triazine amine)	Test item incorpo-rated into the soil / 5% peat Chronic, 28 days	NOEC [a]	**37.1 mg/kg dry soil**	EFSA Journal 2017;15(7):4912
Folsomia candida	IN-A4098 (N-demethyl-triazine amine)	This metabolite was also evaluated for chlorsulfuron, iodosulfuron-methyl-sodium, thifensulfuron-methyl, metsulfuron-methyl, prosulfuron and triasulfuron (EFSA,			EFSA Journal 2017;15(7):4912

Species	Substance	Exposure System	End point	Results	Reference
		2008, 2014b, 2015a,b,c, 2016a). The lowest reported NOEC was 0.045 mg/kg dry soil from the RAR for thifensulfuron-methyl. Based on the information provided by EFSA it was not possible to conclude if this NOEC should be considered as adverse data, since possible effects at higher concentrations were not reported.			
Folsomia candida	IN-D5119 (acid sulfonamide) ***	Test item incorpo-rated into the soil / 10% peat Chronic, 28 days	NOEC [a]	100 mg/kg dry soil	EFSA Journal 2017;15(7):4912
Folsomia candida	IN-D5119 (acid sulfonamide) ***	This metabolite was also evaluated for ethametsulfuron-methyl (EFSA, 2014a). The reported NOEC was 100 mg/kg dry soil.			EFSA Journal 2017;15(7):4912
Folsomia candida	IN-D5803 (sulfonamide) ***	Test item incorpo-rated into the soil / 5% peat Chronic, 28 days	NOEC [a]	**100 mg/kg dry soil**	EFSA Journal 2017;15(7):4912
Folsomia candida	IN-D5803 (sulfonamide) ***	This metabolite was also evaluated for ethametsulfuron-methyl (EFSA, 2014a). The reported NOEC was 100 mg/kg dry soil.			EFSA Journal 2017;15(7):4912
Folsomia candida	IN-GK521 (O-demethyl-tribenuron methyl)	Test item incorpo-rated into the soil / 5% peat Chronic, 28 days	NOEC [a]	**100 mg/kg dry soil**	EFSA Journal 2017;15(7):4912
Folsomia candida	IN-GN815 (O-demethyl-tribenuron free acid) ***	Test item incorpo-rated into the soil / 5% peat Chronic, 28 days	NOEC [a]	100 mg/kg dry soil	EFSA Journal 2017;15(7):4912
Folsomia candida	IN-L5296 (triazine amine)	Test item incorpo-rated into the soil / 10% peat Chronic, 28 days	NOEC [EC10 EC20]	**0.116 mg/kg dry soil** [0.47 mg/kg dry soil 1.01 mg/kg dry soil]	EFSA Journal 2017;15(7):4912
Folsomia candida	IN-R9803 (tribenuron free acid) ***	Test item incorpo-rated into the soil / 5% peat Chronic, 28 days	NOEC [a]	**100 mg/kg dry soil**	EFSA Journal 2017;15(7):4912

Species	Substance	Exposure System	End point	Results	Reference
Folsomia candida	IN-R9805 (O-demethyl-triazine amine)	Test item incorpo-rated into the soil / 5% peat Chronic, 28 days	NOEC [a]	**100 mg/kg dry soil**	EFSA Journal 2017;15(7):4912
Folsomia candida	M2 (triazine urea)	No data available. Estimated as 10 times more toxic than the active substance	NOEC	**3 mg/kg dry soil**	EFSA Journal 2017;15(7):4912
Hypoaspis aculeifer	Tribenuron methyl 50SG + DPX-KG691 surfactant	Test item incorpo-rated into the soil / 5% peat Chronic, 28 days	NOEC	**493 mg a.s./kg dry soil ***	EFSA Journal 2017;15(7):4912
Hypoaspis aculeifer	IN-00581 (saccharin)	Test item incorpo-rated into the soil / 5% peat Chronic, 28 days	NOEC [a]	**100 mg/kg dry soil**	EFSA Journal 2017;15(7):4912
Hypoaspis aculeifer	IN-00581 (saccharin)	Test item incorpo-rated into the soil / 5% peat Chronic, 28 days	NOEC	1000 mg/kg dry soil**	EFSA Journal 2017;15(7):4912
Hypoaspis aculeifer	IN-00581 (saccharin)	This metabolite was also evaluated for metsulfuron-methyl, ethametsulfuron-methyl and propoxycarbazone-sodium (EFSA, 2014a, 2015a, 2016b). The reported NOEC values were 10 and 100 mg/kg dry soil. The NOEC of 10 mg/kg dry soil was based on absence of statistically significant effects up to and including the highest tested concentration of 10 mg/kg soil. Hence, it is not considered as adverse data.			EFSA Journal 2017;15(7):4912
Hypoaspis aculeifer	IN-A4098 (N-demethyl-triazine amine)	Test item incorpo-rated into the soil / 5% peat Chronic, 28 days	NOEC [a]	**100 mg/kg dry soil**	EFSA Journal 2017;15(7):4912
Hypoaspis aculeifer	IN-A4098 (N-demethyl-triazine amine)	Test item incorpo-rated into the soil / 5% peat Chronic, 28 days	NOEC EC10 EC20	**100 mg/kg dry soil** 111 mg/kg dry soil 248 mg/kg dry soil	EFSA Journal 2017;15(7):4912
Hypoaspis aculeifer	IN-A4098 (N-demethyl-	This metabolite was also evaluated for iodosulfuron-methyl-sodium, thifensulfuron-methyl, metsulfuron-			EFSA Journal 2017;15(7):4912

Species	Substance	Exposure System	End point	Results	Reference
	triazine amine)	methyl, prosulfuron and triasulfuron (EFSA, 2014b, 2015a,b,c, 2016a). NOEC values of 98.7 and 100 mg/kg dry soil were reported.			
Hypoaspis aculeifer	IN-D5119 (acid sulfonamide) ***	Test item incorpo-rated into the soil / 5% peat Chronic, 28 days	NOEC [a]	100 mg/kg dry soil	EFSA Journal 2017;15(7):4912
Hypoaspis aculeifer	IN-D5803 (sulfonamide) ***	Test item incorpo-rated into the soil / 5% peat Chronic, 28 days	NOEC [a]	**100 mg/kg dry soil**	EFSA Journal 2017;15(7):4912
Hypoaspis aculeifer	IN-GK521 (O-demethyl-tribenuron methyl)	Test item incorpo-rated into the soil / 5% peat Chronic, 28 days	NOEC	**25 mg/kg dry soil**	EFSA Journal 2017;15(7):4912
Hypoaspis aculeifer	IN-GN815 (O-demethyl-tribenuron free acid) ***	Test item incorpo-rated into the soil / 5% peat Chronic, 28 days	NOEC [a]	100 mg/kg dry soil	EFSA Journal 2017;15(7):4912
Hypoaspis aculeifer	IN-L5296 (triazine amine)	Test item incorpo-rated into the soil / 5% peat Chronic, 28 days	NOEC [a]	100 mg/kg dry soil	EFSA Journal 2017;15(7):4912
Hypoaspis aculeifer	IN-L5296 (triazine amine)	Test item incorpo-rated into the soil / 5% peat Chronic, 28 days	NOEC EC10 EC20	562 mg/kg dry soil **403 mg/kg dry soil** 667 mg/kg dry soil	EFSA Journal 2017;15(7):4912
Hypoaspis aculeifer	IN-R9803 (tribenuron free acid) ***	Test item incorpo-rated into the soil / 5% peat Chronic, 28 days	NOEC [a]	**100 mg/kg dry soil**	EFSA Journal 2017;15(7):4912
Hypoaspis aculeifer	IN-R9805 (O-demethyl-triazine amine)	Test item incorpo-rated into the soil / 5% peat Chronic, 28 days	NOEC	**50 mg/kg dry soil**	EFSA Journal 2017;15(7):4912
Hypoaspis	M2 (triazine	-	-	No data available	EFSA Journal

Species	Substance	Exposure System	End point	Results	Reference
aculeifer	urea)				2017;15(7):4912
MCPA					
Folsomia candida	-	-	-	-	-
Hypoaspis aculeifer	-	-	-	-	-
	Field studies				
	Not available				
	Litter bag test				
	Not available				

* In the study from Schöbinger 2013, a 26% increase in reproduction was observed. It is uncertain whether such effect could be considered as adverse at population level. In addition it is noted that the standard deviation in the control was 18.2% and a higher mortality in the control than in the treatment was observed (13% against 0%). This adds uncertainties on the biological significance of this finding. In light of the above the endpoint was set at 493 mg a.s./kg soil.
** An increase in the reproduction was observed at this concentration. It is uncertain whether such effect could be considered as adverse at population level
*** Endpoints for metabolite IN-D5119, IN-GN815, IN-D5803, IN-9803 are presented, however these metabolites were not included in the risk assessment presented below. IN-D5119, IN-GN815 are not included in the residue definition for soil Moreover according to experts' consultation (PPR TC 139, March 2017) it was agreed not to consider metabolite IN-R9803 and IN-D5803in the risk assessment.

a All values = the highest tested concentrations.

9.8.1.1 Justification for new endpoints

No deviation from the 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 tribenuron methyl and MCPA but needs to be considered for some relevant metabolites of tribenuron methyl (IN-00581, IN-A4098, IN-L5296, IN-R9805). Initial PEC_{soil} is considered for the formulated product.
To achieve a concise risk assessment, the risk envelope approach is applied. The assessment presented below covers application in on spring cereals (spring spraying) and winter cereals (autumn and spring spraying) and minor crops presented in the GAP table (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 HAKSAR TOP 565 SG in cereals.

Intended use			
Acute effects on earthworms			
Product/active substance	**LC_{50} (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_a (criterion TER ≥ 10)**
MCPA	325	0.7333	443
Chronic effects on earthworms			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
MCPA + TRIBENURON METYL 565 SG	102.653	1.333	77
Tribenuron -methyl	3.2	0.020	160
IN-00581	100	0.0035*	28571
IN-A4098	8.0	0.0010*	8000
IN-L5296	0.2	0.0092*	22
IN-GK521	100	0.0062	16129
IN-R9805	1000	0.0007*	1428571
M2	5.0	0.0016	3125
MCPA	No data available	0.7333	443
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			
MCPA + TRIBENURON METYL 565 SG	14.5	1.333	10.88
Tribenuron -methyl	30.8	0.020	1540
IN-00581	100	0.0035*	28571
IN-A4098	37.1	0.0010*	37100
IN-L5296	0.116	0.0092*	13
IN-GK521	100	0.0062	16129
IN-R9805	100	0.0007*	142857
M2	3	0.0016	1875
MCPA	No data available	0.7333	-
Hypoaspis aculeifer			
MCPA + TRIBENURON METYL 565 SG	≥1000	1.333	750
Tribenuron -methyl	493	0.020	24650
IN-00581	100	0.0035*	28571
IN-A4098	100	0.0010*	100000
IN-L5296	403	0.0092*	43804

IN-GK521	25	0.0062	4032
IN-R9805	50	0.0007*	71429
M2	No data available	0.0016	-
MCPA	No data available	0.7333	-

*PEC accumulation was used

9.8.2.2 Higher-tier risk assessment

Not relevant.

Review comments:

A higher tier assessment is not required based on the low risk indicated in the chronic assessment on earthworms, collembolan, and soil mite.

9.8.3 Overall conclusions

Acute and chronic risk to earthworms arising from the application of HAKSAR TOP 565 SG according to the intended GAP uses can be excluded as the trigger values of 10 for acute risk and 5 for long-term risk were exceeded by far.
Additionally, performed long-term risk assessment for collembola and predatory mites indicates that TER_{lt} is above the trigger value of 5, indicating acceptable risk to soil organisms (other than earthworms) from the proposed uses of HAKSAR TOP 565 SG.

Review comments:

The risk assessment for earthworms exposed to MCPA, tribenuron-methyl, its relevant metabolites and formulation following application of HAKSAR TOP 565 SG according to critical GAP was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology" (SANCO/10329/2002) and was accepted by the zRMS. Applicant presented also assessment for *Folsomia* and *Hypoaspis.*

According to experts' consultation (PPR TC 139, March 2017) it was agreed not to consider metabolite IN-R9803 and IN-D5803in the risk assessment.

TERlt values calculated for all considered compounds and HAKSAR TOP 565 SG were above the triggers indicating acceptable long-term risk to earthworms and other non-target soil organisms No further evaluation is deemed necessary.

Overall, acceptable risk could be concluded for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of HAKSAR TOP 565 SG and other non-target soil organisms in cereals and minor crops according to GAP.

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 MCPA, Tribenuron methyl and its metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on soil microorganisms of HAKSAR TOP 565 SG were not evaluated as part of the EU assessment of tribenuron methyl. 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 from the EU review process are provided below.

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

Endpoint	Substance	Exposure System	No effects (< 25 % difference to control) up to	Reference
N-mineralisation	HAKSAR TOP 565 SG	56 days	13.50 mg formulation/kg of soil	Agnieszka Woźniak 2020, Study code 0016/0103/E
	IN-00581 (saccharine)	28 days	0.20 mg/kg dry soil	EFSA Journal 2017;15(7):4912
	IN-00581 (saccharine)	28 days	0.0511 mg/kg dry soil	EFSA Journal 2017;15(7):4912
	IN-00581 (saccharine)	42 days	0.204 mg/kg dry soil	EFSA Journal 2017;15(7):4912
	IN-00581 (saccharine)	This metabolite was also evaluated for metsulfuron-methyl, ethametsulfuron-methyl and propoxycarbazone-sodium (EFSA, 2014a, 2015a, 2016b). No adverse data were identified.		EFSA Journal 2017;15(7):4912
	IN-A4098 (N-demethyl-triazine amine)	28 days	0.0397 mg/kg dry soil	EFSA Journal 2017;15(7):4912
	IN-A4098 (N-demethyl-triazine amine)	This metabolite was also evaluated for chlorsulfuron, iodosulfuronmethyl-sodium, thifensulfuron-methyl, metsulfuron-methyl, prosulfuron and triasulfuron (EFSA, 2008, 2014b, 2015a,b,c, 2016a). No adverse data were identified.		EFSA Journal 2017;15(7):4912
	IN-D5119 (acid sulfonamide) *	28 days	0.0533 mg/kg dry soil	EFSA Journal 2017;15(7):4912
	IN-D5119 (acid sulfonamide) *	This metabolite was also evaluated for ethametsulfuron-methyl (EFSA, 2014a). No adverse data were identified.		EFSA Journal 2017;15(7):4912
	IN-D5803 (sulfonamide) *	42 days	0.0597 mg/kg dry soil	EFSA Journal 2017;15(7):4912
	IN-D5803 (sulfonamide) *	This metabolite was also evaluated for metsulfuron-methyl and ethametsulfuron-methyl (EFSA, 2014a, 2015a). No adverse data were identified		EFSA Journal 2017;15(7):4912
	IN-GK521 (O-demethyl tribenuron methyl)	28 days	1.62 mg/kg dry soil	EFSA Journal 2017;15(7):4912

Endpoint	Substance	Exposure System	No effects (< 25 % difference to control) up to	Reference
	IN-GK521 (O-demethyl tribenuron methyl)	28 days	0.2 mg/kg dry soil	EFSA Journal 2017;15(7):4912
	IN-L5296 (triazine amine)	No data	0.02 mg/kg dry soil, estimated as 10 times more toxic than the active substance	EFSA Journal 2017;15(7):4912
	IN-GN815 (O-demethyl-tribenuron free acid) *	28 days	1.41 mg/kg dry soil	EFSA Journal 2017;15(7):4912
	IN-GN815 (O-demethyl-tribenuron free acid) *	28 days	0.2 mg/kg dry soil	EFSA Journal 2017;15(7):4912
	IN-R9805 (O-demethyl-triazine amine)	28 days	200 mg/kg dry soil	EFSA Journal 2017;15(7):4912
	IN-R9803 (tribenuron free acid)*	28 days	1.17 mg/kg dry soil	EFSA Journal 2017;15(7):4912
	IN-R9803 (tribenuron free acid)*	28 days	0.4 mg/kg dry soil	EFSA Journal 2017;15(7):4912
	M2 (triazine urea)	28 days	0.2 mg/kg dry soil	EFSA Journal 2017;15(7):4912
	MCPA	28 d	26.7 mg/kg dry soil	SANCO/4062/2001-final of 11.07.2008

* Endpoints for metabolite IN-D5119, IN-GN815, IN-D5803, IN-9803 are presented, however these metabolites were not included in the risk assessment presented below. IN-D5119, IN-GN815 are not included in the residue definition for soil Moreover according to experts' consultation (PPR TC 139, March 2017) it was agreed not to consider metabolite IN-R9803 and IN-D5803in the risk assessment.

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 0).

To achieve a concise risk assessment, the risk envelope approach is applied. The assessment presented below covers application in on spring cereals (spring spraying) and winter cereals (autumn and spring spraying) and minor crop (see 9.1.2).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of HAKSAR TOP 565 SG in cereals covering minor crops

Intended use	cereals		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
HAKSAR TOP 565 SG	13.50	1.333	Yes
IN-00581 (saccharine)	0.20	0.0035*	yes
IN-00581 (saccharine)	0.204	0.0035*	yes
IN-A4098 (N-demethyl-triazine	0.0397	0.0010*	yes
IN-GK521 (O-demethyl tribenuron methyl)	1.62	0.0062	yes
IN-GK521 (O-demethyl tribenuron methyl)	0.2	0.0062	yes
IN-L5296 (triazine amine)	0.02	0.0092*	yes
IN-R9805 (O-demethyl-triazine amine)	200	0.0007*	yes
M2 (triazine urea)	0.2	0.0016	yes
MCPA	26.7	0.7333	yes

9.9.3 Overall conclusions

The risk to soil microorganisms is acceptable since effects on the nitrogen transformations are acceptable at concentration which is higher than the maximum relevant PEC soil for the maximum application rate of HAKSAR TOP 565 SG and relevant metabolites of Tribenuron methyl.

Review comments:

The risk assessment for soil micro-organisms exposed to MCPA, tribenuron-methyl, its relevant metabolites and the formulated product MT-565SG-OR2-C / HAKSAR TOP 565 SG following the proposed uses of was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology" (SANCO/10329/2002).
The risk assessment presented in Table 9.9-2 is agreed by the zRMS. 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).
Based on the obtained results, soil nitrate formation rates were below the 25% trigger value. Thus, it is concluded that HAKSAR TOP 565 SG had no significant impact on soil microorganisms when applied at test item concentrations up to 13.5 mg formulation/kg soil dry weight.

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

Effects on non-target terrestrial plants of HAKSAR TOP 565 SG were not evaluated as part of the EU assessment of MCPA or Tribenuron methyl. 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
Daucus carota$_d$ *Helianthus annuus*$_d$ *Brassica olerace var. capitata*$_d$ *Pisum sativum*$_d$ *Phaseolus vulgaris*$_d$ *Solanum lycopersicon*$_d$ *Allium cepa*$_m$ *Lolium perenne*$_m$ *Avena sativa*$_m$ *Triticum aestivum*$_m$	MCPA + TRIBENURON METYL 565 SG	21 d Seedling emergence	ER_{50} emergence = 82.1 g formulation/ha *(Daucus carota)* ER_{50} plant weight = 49.3 g formulation/ha Cabbage ~~96.5 g formulation/ha (Daucus carota)~~ ER_{50} plant height = 26.7 g formulation/ha *(Daucus carota)*	Aneta Gierbuszewska, G/160/17
Daucus carota$_d$ *Helianthus annuus*$_d$ *Brassica olerace var. capitata*$_d$ *Pisum sativum*$_d$ *Phaseolus vulgaris*$_d$ *Solanum lycopersicon*$_d$ *Allium cepa*$_m$ *Lolium perenne*$_m$ *Avena sativa*$_m$ *Triticum aestivum*$_m$	MCPA + TRIBENURON METYL 565 SG	21 d Vegetative vigour	[1)] ER_{50} mortality = 100.1 g formulation/ha *(Helianthus annuus)* ER_{50} plant weight = **9.3 g formulation /ha** *(Daucus carota)* ER_{50} plant height = 28.2 g formulation/ha sunflower ~~54.3 g formulation/ha (Allium cepa)~~	Weronika Dec, G/161/17

m: monocotyledonous; d: dicotyledonous

9.10.1.1 Justification for new endpoints

Initial risk assessments are presented based on the lowest ER_{50} estimate from the vegetative vigour study by Dec, G/161/17 (2018) for *Daucus carota* (plant weight) For further refinement, in addition to the deterministic approach, a probabilistic data evaluation based on the individual effect data for vegetative vigour by Dec, G/161/17 (2018) is performed.

A Species Sensitivity Distribution (SSD) assessment based on the available ER_{50} estimates was performed by calculating normal distribution of the data sets and plotting 'Fraction affected' against "log10 Toxicity data" using ETX 2.1 software[3]. In accordance with the terrestrial guidance document (SAN-

[3] ETX 2.1 – A Program to Calculate Hazardous Concentrations and Fraction Affected Based on Normally Distributed Toxicity Data, P.L.A. van Vlaardingen, T.P. Traas, A.M. Wintersen & T. Aldenberg, RIVM Report 601501028/2004

CO/10329/2002 rev.)[4], 'the risk for terrestrial plants is assumed to be acceptable', if 'the $ED[R]_{50}$ for less than 5% of the species is below the highest predicted exposure level' (i.e. TER trigger of 1 for acceptability of risk).

The following table present the individual median effect rates (ER_{50}) for the more sensitive parameter shoot dry weight for MCPA+TRIBENURON METYL 565 SG for vegetative vigour. For more details reference is made to the study summary in Appendix 2.

Table 9.10-2: Median effect rates from the vegetative vigour study – shoot dry weight (biomass)

Plant species	**Plant group**	**ER_{50} (95% CI)** [g formulation/ha]
Vegetative vigour		
Allium cepa	Monocots	29.6 (20.8 – 42.0)
Lolium perenne		456.7 (276.6 – 927.0)
Avena sativa		>1000 (641.1 – n.d.)
Triticum aestivum		>1000 (852.3 – >1000)
Daucus carota	Dicots	**9.3 (4.4 – 19.6)**
Helianthus annuus		18.4 (11.8 – 28.9)
Brassica olerace var. capitata		199.9 (130.6 – 320.7)
Pisum sativum		243.9 (112.7 – 616.9)
Phaseolus vulgaris		290.8 (144.9 – 827.7)
Solanum lycopersicon		31.2 (18.8 – 51.8)

Worst-case ER_{50} estimate is highlighted in **bold**

Thus, reliable ER_{50} values are available for all of the 10 tested plant species, mono- and dicotyledonous plants.

The results of the SSD and data distributions are presented in the following tables and graphs.

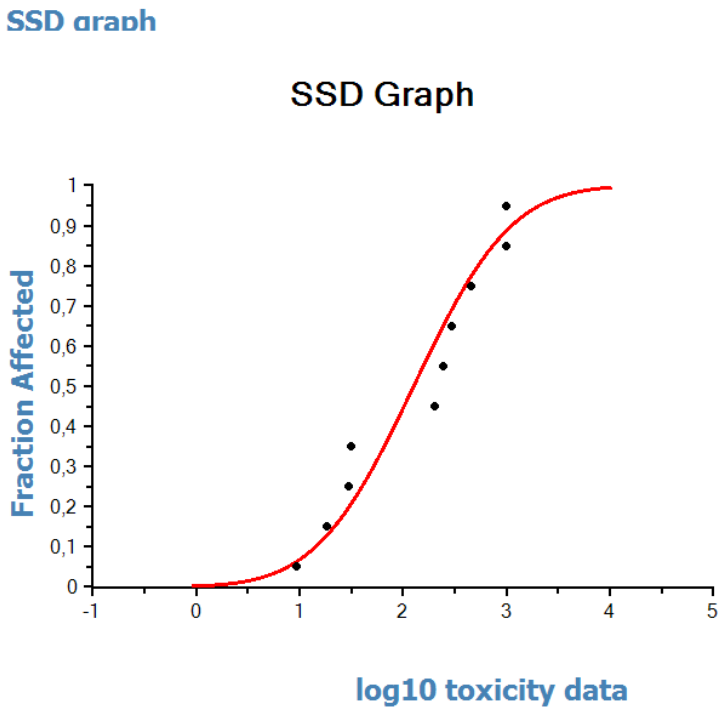
Table 9.10-3: SSD over ER_{50} from the relevant vegetative vigour data for HAKSAR TOP 565 SG

Parameter:	**ER_{50} shoot dry weight (n = 10)** [g formulation/ha]
Goodness of fit of toxicity data (normal distribution)	
Anderson-Darling test for normality	Accepted [a)]
Kolmogorov-Smirnov test for normality	Accepted [a)]
Cramer von Mises test for normality	Accepted [a)]
Median HC_5	**6.947**
95% confidence limits	0.88 – 22.29

[a)] Acceptable normal distribution at all significance level

Thus, the data fulfil the criterion for normal distribution even at the lowest significance level and in accordance with all tests for normality.

[4] European Commission. Health & Consumer Protection Directorat – General (2002). Draft Working Document. Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC. SANCO/10329/2002 rev.



Graph 1: SSD over ER_{50} from the relevant vegetative vigour data for MCPA+TRIBENURON METYL 565 SG

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.

To achieve a concise risk assessment, the risk envelope approach is applied. The assessment presented below covers application in on spring cereals (spring spraying) and winter cereals (autumn and spring spraying) and minor crops (see 9.1.2).

Table 9.10-4: Assessment of the risk for non-target plants due to the use of HAKSAR TOP 565 SG in cereals and minor crops

Intended use	Cereals, grasses
Active substance/product	HAKSAR TOP 565 SG
Application rate (g/ha)	1 × 1000 g/ha
MAF	1

Test species	**ER_{50} (g formulation /ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**TER criterion: TER ≥ 5 for deterministic and ≥ 1 for probabilistic assessment**
Daucus carota Deterministic approach	9.3	2.77%	27.7	**0.33**
HC_5 (n = 10) Probabilistic approach	6.947	2.77	27.7	**0.25**

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 9.10-5: Risk assessment for non-target terrestrial plants due to the use of HAKSAR TOP 565 SG in cereals and grasses considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Cereals, grasses			
Active substance/product		HAKSAR TOP 565 SG			
Application rate (g/ha)		1 × 1000 g/ha			
MAF		1			
Buffer strip (m)	**Drift rate (%)**	**$PER_{off\text{-}field}$ (g/ha)**	**$PER_{off\text{-}field}$ 50 % drift red. (g/ha)**	**$PER_{off\text{-}field}$ 75 % drift red. (g/ha)**	**$PER_{off\text{-}field}$ 90 % drift red. (g/ha)**
1	2.77	27.7	13.85	6.925	2.77
5	0.57	5.7	2.85	1.425	0.57
10	0.29	2.9	1.45	0.725	0.29
Toxicity value ER_{50} = 9.3 g/ha Deterministic approach		**TER criterion: TER ≥ 5**			
1		**0.33**	**0.67**	**1.34**	**3.36**
5		**1.63**	**3.26**	6.53	-
10		**3.2**	6.41	-	-
Toxicity value HC_5 (n = 10) = 6.947 g/ha Probabilistic approach		**TER criterion: TER ≥ 1**			
1		**0.25**	**0.50**	1.003	-
5		1.2	-	-	-

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

In conclusion, based on the deterministic approach an acceptable risk is indicated when applying a 10 m buffer strip with 50% drift reduction is applied.

Based on the probabilistic approach, assessment indicate an acceptable risk under consideration of the TER of 1 when either 75% drift reduction or a 5 m buffer strip is applied.

9.10.3 Overall conclusions

For the proposed use of HAKSAR TOP 565 SG, based on the highest application rate the risk for non-target plants in the off-crop area is indicated to be acceptable when either 75% drift reduction or a 5 m buffer strip is applied as risk mitigation measures.

Review comments:

Risk assessment performed by the Applicant for non-target terrestrial plants was accepted.

Acceptable risk for non-target terrestrial plants could be concluded for HAKSAR TOP 565 SG when following risk mitigation measures are applied:

Winter and spring cereals, grasses:

Deterministic approach:
- 10 m and use of 50% drift reducing technology or,
- 5 m and use of 75% drift reducing technology

Probabilistic approach:
- 1 m and use of 75% drift reducing technology or,
- 5 m with no drift reducing technology to non-agricultural land

Concerned Member States must decide on the applicability of indicated risk mitigation measures at the product authorization.

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

HAKSAR TOP 565 SG 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.

Ingredients classified as hazardous to the aquatic environment are:
- active substance MCPA in a concentration of 55 % classified as “Aquatic Acute 1” and “Aquatic Chronic 1”.
- active substance tribenuron methyl in a concentration of 1.5 % classified as “Aquatic Acute 1” and “Aquatic Chronic 1”.

Taking into account the results of toxicity tests performed on HAKSAR TOP 565 SG it should be concluded:

- In accordance with point (a) of Table 4.1.0 in CLP Regulation, HAKSAR TOP 565 SG is classified as Acute 1 (Based on the acute toxicity data EC_{50} for Lemna gibba = 0.478 mg formulation/L, which is ≤ 1 mg/l).
- In accordance with point b (i) of Table 4.1.0 in CLP Regulation, HAKSAR TOP 565 SG is classified as Chronic 1 (Based on the toxicity data NOEC for Lemna gibba = 0.061 mg formulation/L, which is ≤ 0.1 mg/l).

CLASSIFICATION	
Hazard class(es), categories:	Aquatic Acute 1 (H400) , Aquatic Chronic 1 (H410)
LABELLING	
Hazard pictograms:	 GHS09
Signal word:	Warning
Hazard statement(s):	H410 – Very toxic to aquatic life with long lasting effects.
Precautionary statement(s):	P273 – Avoid release to the environment. P391 – Collect spillage
Additional phrase(s):	EUH401 - To avoid risks to human health and the environment, comply with the instructions for use.

Standard phrases under Regulation (EU) No 547/2011

SP 1	Do not contaminate water with the product or its container (Do not clean application equipment near surface water/Avoid contamination via drains from farmyards and roads).
SPe3	To protect aquatic organisms an unsprayed buffer zone of 20 m to surface water bodies.
SPe3	To protect non-target plants respect an unsprayed buffer zone of 5 m or 1m with 75% drift reduction to non-agricultural land.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
KCP 10.2.1/01	Tina Turek	2017	MCPA + Tribenuron metyl 565 SG, *Daphnia magna*, Acute Immobilization Test W/269/17 Intitute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.2.1/02	Tina Turek	2018	MCPA + Tribenuron metyl 565 SG, *Pseudokirchinella subcapitata*, Growth inhibition Test, W/270/17 Intitute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.2.1/03	Tina Turek	2017	MCPA + Tribenuron metyl 565 SG, *Navicula pelliculosa*, Growth Inhibition Test, W/271/17, Intitute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.2.1/04	Tina Turek	2018	MCPA + Tribenuron metyl 565 SG, *Lemna gibba*, Growth Inhibition Test, W/272/17 Intitute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.2.1/05	Anna Świerkot	2018	MCPA + Tribenuron metyl 565 SG, Water-sediment *Myriophyllum spicatum*, Toxicity Test, W/181/17	N	CIECH Sarzyna S.A.

			Intitute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished		
KCP 10.2.2/01	Paweł Bąk	2018	MCPA + Tribenuron metyl 565 SG, Daphnia magna, Reproduction Test, W/36/18 Intitute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.3.1/01	Natalia Lemańska	2018	MCPA + Tribenuron metyl 565 SG Honeybees (*Apis mellifera L.*), Acute Oral Toxicity Test B/09/17 Intitute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.3.1/02	Natalia Lemańska	2018	MCPA + Tribenuron metyl 565 SG Honeybees (*Apis mellifera L.*), Acute Contact Toxicity Test B/10/17 Intitute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.3.1/03	Paweł Parma	2019	MCPA + Tribenuron metyl 565 SG Honeybees (*Apis mellifera L.*), Chronic Oral Toxicity Test B/26/18 Łukasiewicz Research Network-Intitute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.3.1/04	Wiesław Londzin	2020	Chronic Toxicity Test for Honey Bee Larvae according to OECD GD 239 Study code: 0016/0102/E Test item: MT-565 SG-OR2-C SORBOLAB Research Laboratory LLC GLP Unpublished	N	CIECH Sarzyna S.A.

KCP 10.3.2/01	Aneta Glanas	2018	A laboratory test for evaluating the effects of MCPA + Tribenuron metyl 565 SG on the parastic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez) B/11/17 Intitute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.3.2/02	Aneta Glanas	2018	A laboratory test for evaluating the effects of MCPA + Tribenuron metyl 565 SG on the predatory mite , *Typhlodromus pyri* (Sch.) B/12/17 Intitute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.3.2/03	Paweł Parma	2018	An extended laboratory test for evaluating the effects of MCPA + Tribenuron metyl 565 SG on the predatory mite, Typhlodromus pyri (Sch.) B/39/18 Intitute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.4.1	Aneta Gierbuszewska	2018	MCPA + TRIBENURON METYL 565 SG Earthworm Reproduction Test (Eisenia andrei) G/158/17 Intitute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.4.2.1/01	Agnieszka Stalmach	2018	MCPA + Tribenuron metyl 565 SG Collembolan *(Folsomia candida)* Reproduction Test G/136/18 Intitute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.4.2.1/02	Agnieszka Stalmach	2018	MCPA + Tribenuron metyl 565 SG, Predatory mite *(Hypoaspis (Geolaelaps) aculeifer)* G/135/18 Intitute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A.

KCP 10.5	Agnieszka Woźniak	2020	Study of impact on soil microorganisms - nitrogen transformation test according to Guideline OECD 216 0016/0103/E SORBOLAB Research Laboratory LLC GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.6.2/01	Aneta Gierbuszewska	2018	MCPA + TRIBENURON METYL 565 SG Terrestrial Plant Test: Seedling Emer-gence and Seedling Growth Test G/160/17 Intitute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.6.2/02	Weronika Dec	2018	MCPA + TRIBENURON METYL 565 SG, Terrestrial Plant Test: Vegetative Vigour Test G/161/17 Intitute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A.

Appendix 2 Detailed evaluation of the new studies

Review Comment:

In order to provide sufficient details, where appropriate, the study summaries have been adapted by the zRMS from the full study reports provided in the dossier. zRMS text is highlighted in grey. The comments on individual studies are provided in grey comment boxes.

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

Summarised in Section 6 (Mammalian Toxicology)

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

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

A 2.2 KCP 10.2 Effects on aquatic organisms

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

A.2.2.1.1 Study 1

Comments of zRMS:	The study was conducted to OECD guideline 202 and according to the principles of GLP. No deviations to the guideline were noted. In the definitive test the validity criteria were met: - The immobilization of Daphnia magna in the control was 0% (criterion: not more than 10%), - The dissolved oxygen concentrations in the test vessels were within the range of 8.6– 8.8 mg/L (criterion: not less than 3 mg/L). The analytical measurements demonstrated that the test item concentrations throughout the test was within 80-120% of nominal and for this reason endpoints are expressed as nominal concentrations. The study is reliable and suitable for the risk assessment.

Reference:	KCP 10.2.1_01
Report	MCPA + Tribenuron metyl 565 SG *Daphnia magna*, Acute Immobilization Test, Tina Turek, MSc, 2017, STUDY CODE: W/269/17, Institute of Industrial Organic Chemistry, Branch Pszczyna
Guideline(s):	Yes. According to the OECD Guideline No. 202 (2004)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

SUMMARY

Immobilization of *Daphnia magna* exposed to the test item, MCPA + Tribenuron metyl 565 SG, was investigated during a 48-hour static test. The test was performed with a single test item concentration 100 mg/L as a limit test plus the control. Four replicates of either test item concentration or the control with five *Daphnia magna* per replicate were used. *Daphnia magna* were observed for immobilisation after 24 and 48 h of exposure. No immobilization of *Daphnia magna* was observed during the period of exposure, neither in the control, nor in the test item concentration of 100 mg/L.

Materials and methods

Test item: MCPA + Tribenuron metyl 565 SG,
content: 14.6 g/kg tribenuron methyl and 565.0 g/kg MCPA as active ingredients
batch number: 4.5/16
production date: 14.11.2016
expiry date: 13.11.2018

Test organism:	*Daphnia magna* Straus (< 24 h old at exposure initiation); not first brood progeny; neonates collected from a laboratory culture cultivated at the Institute of Industrial Organic Chemistry, Branch Pszczyna.
Test design:	Static test (48 h of exposure); 4 replicates for the control and the test item concentration; 5 *Daphnia magna* in each replicate.
Nominal test item concentration:	100 mg/L plus the control.
Test conditions:	Temperature: 18.9 – 20.1°C; pH of the control: 7.91 – 7.92; dissolved oxygen concentration: 8.6 – 8.8 mg/L; daily cycle 16 h light : 8 h dark; no feeding; no aeration; medium: Elendt M7.
Chemical determinations:	The concentrations of MCPA and tribenuron methyl were determined with a validated chromatographic method with UV-Vis detection.
Endpoint value:	EC_{50}.

Immobilization of young *Daphnia magna* (< 24 h old) exposed to the test item, MT-565SG-OR2-C was investigated during a 48-hour static test. A single test item concentration of 100 mg/l and a control were used (limit test). Four replicates of test item concentration and the control with five *Daphnia magna* per replicate were used. *Daphnia magna* were observed for immobilization after 24 and 48 h of exposure.

Analytical measurements

The determined concentration of MCPA at exposure initiation was 107.6% of the nominal concentration, whereas the determined concentration of tribenuron methyl was 102.7% of the nominal concentration. The results confirm that the test item concentration was prepared correctly. The determined concentration of MCPA at exposure termination was 99.7% of the nominal concentration, whereas the determined concentration of tribenuron methyl was 104.1% of the nominal concentration. Therefore, the concentrations of MCPA and tribenuron methyl were stable during test conditions. The endpoint values were determined based on nominal test item concentration.

Table 8. Concentration and stability of MCPA, definitive test

Nominal test item concentration [mg/L]	Nominal concentration of MCPA in the test item [mg/L]	Average determined concentration of MCPA (n=3) in samples collected			
		at exposure initiation [mg/L]	% of nominal concentration	at exposure termination [mg/L]	% of nominal concentration
Control	---	< LoD	---	< LoD	---
100	56.500	60.78	107.6	56.33	99.7

LoQ = 0.001 mg/L
LoD = 0.0003 mg/L
--- no value

Table 9. Concentration and stability of tribenuron methyl, definitive test

Nominal test item concentration [mg/L]	Nominal concentration of tribenuron methyl in the test item [mg/L]	Average determined concentration of tribenuron methyl (n=3) in samples collected			
		at exposure initiation [mg/L]	% of nominal concentration	at exposure termination [mg/L]	% of nominal concentration
Control	---	< LoD	---	< LoD	---
100	1.460	1.50	102.7	1.52	104.1

LoQ = 0.001 mg/L
LoD = 0.0003 mg/L
--- no value

Results and discussions

In the control and in the test item concentration of 100 mg/l no immobilization was observed during exposure. The endpoints were determined on the basis of nominal test item concentration. In the test item concentration of 100 mg/l and in the control no immobilization of *Daphnia magna* was observed during exposure. Therefore, no statistical analysis was needed. The EC_{50} value after 48h of exposure is higher than 100 mg/l. The LOEC/48h value is higher than 100mg/l. The NOEC/48h value is higher than or equal to 100 mg/l.

Table 7. Immobilisation of *Daphnia magna*, definitive test

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

Time of exposure: 31.10.2017 – 02.11.2017

Conclusion

<u>The endpoint values based on nominal test item concentrations are given below.</u>
The EC_{50}/24 h value is higher than 100 mg/L (56.5mg/L of MCPA and 1.46 mg/L of Tribenuron-methyl - (limit test) .
The EC_{50}/48 h value is higher than 100 mg/L (56.5mg/L of MCPA and 1.46 mg/L of Tribenuron-methyl - (limit test) .

A.2.2.1.2 Study 2

Comments of zRMS:	The study was conducted to OECD guideline 201 and according to the principles of GLP. No deviations to the guideline were noted. In the definitive test the validity criteria were met: - The biomass in the control increased by a factor of 143.7within 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 1.8 % (criterion: it must not exceed 7%),

	- The mean coefficient of variation for the section-by-section growth rate in the control culture was 14.8% (criterion: it must not exceed 35%). The analytical measurements demonstrated that the test item concentrations throughout the test was within 80-120% of nominal and for this reason endpoints are expressed as nominal concentrations. The study is reliable and suitable for the risk assessment.

Reference:	KCP 10.2.1_02
Report	MCPA + Tribenuron metyl 565 SG *Pseudokirchinella subcapitata*, Growth inhibition Test, Tina Turek, MSc, 2018, STUDY CODE: W/270/17, Institute of Industrial Organic Chemistry, Branch Pszczyna
Guideline(s):	Yes. According to the OECD Guideline No. 201 (2006) ~~202 (2004)~~
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Aim of the study

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

Analytical measurements

The determined concentrations of MCPA in samples collected at exposure initiation were in the range of 84.0 – 111.5% of the nominal concentration. The determined concentrations of tribenuron methyl in samples collected at exposure initiation were in the range of 96.3 – 112.1% of the nominal concentration. The results confirm correct preparation of the test item concentrations.
The determined concentrations of MCPA in samples collected at exposure termination were in the range of 89.3 – 106.4% of the nominal concentration. The determined concentrations of tribenuron methyl in samples collected at exposure termination were in the range of 97.8 – 117.9% of the nominal concentration.
Therefore, concentrations of MCPA and tribenuron methyl were stable under the test conditions.

Table 12. Concentration and stability of MCPA, definitive test

Nominal test item concentration [mg/L]	Nominal concentration of MCPA in the test item[mg/L]	Average determined concentration of MCPA (n=3) in samples collected			
		exposure initiation [mg/L]	% of nominal concentration	exposure termination [mg/L]	% of nominal concentration
Control	---	< LoD	---	< LoD	---
0.14	0.079	0.0703	88.8	0.0706	89.3
0.41	0.232	0.223	96.2	0.243	104.7
1.23	0.695	0.584	84.0	0.711	102.3
3.7	2.091	2.043	97.7	2.007	96.0
11	6.215	6.932	111.5	6.615	106.4
33	18.645	20.527	110.1	19.733	105.8
100	56.500	54.505	96.5	56.218	99.5

LoQ = 0.001 mg/L
LoD = 0.0003 mg/L
--- no value

Table 13. Concentration and stability of tribenuron methyl, definitive test

Nominal test item concentration [mg/L]	Nominal concentration of tribenuron methyl in the test item [mg/L]	Average determined concentration of tribenuron methyl (n=3) in samples collected			
		exposure initiation [mg/L]	% of nominal concentration	exposure termination [mg/L]	% of nominal concentration
Control	---	< LoD	---	< LoD	---
0.14	0.0020	0.00214	104.6	0.00211	103.1
0.41	0.0060	0.00577	96.3	0.00608	101.5
1.23	0.0180	0.0181	100.8	0.0176	97.8
3.7	0.0540	0.0605	112.1	0.0637	117.9
11	0.161	0.168	104.9	0.170	105.8
33	0.482	0.505	104.8	0.556	115.4
100	1.460	1.432	98.1	1.587	108.7

LoQ = 0.001 mg/L
LoD = 0.0003 mg/L
--- no value

Materials and methods

Test item: MCPA + Tribenuron metyl 565 SG,
content: 14.6 g/kg tribenuron methyl and 565.0 g/kg MCPA as active ingredients
batch number: 4.5/16
production date: 14.11.2016
expiry date: 14.11.2018

Biological test system: Unicellular freshwater green algae Pseudokirchneriella subcapitata (Reinsch) Korshikov (syn. Selenastrum capricornutum Prinz, Raphidocelis subcapitata Korshikov), specifica-

tion SAG 61.81, cultured in the Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicology, Laboratory of Aquatic Toxicology, stock from the Culture Collection of Algae at Gottingen University, Germany.

Test design:	72 hours of exposure; three replicates per each test item concentration; six replicates per the control; a background for the control and each test item concentration; initial algal cell density: 1 x 10^4 cells/mL.
Chemical determinations:	The concentrations of MCPA and tribenuron methyl were determined with a validated chromatographic method with UV-Vis detection.
Statistics:	Probit method calculations and analyses by: Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Williams Multiple Sequential t-test Procedure, Multiple Sequentially-rejective Welsh-t-test After Bonferroni-Holm.
Endpoint values:	E_rC_{50}, E_yC_{50}, NOEC, LOEC.

The growth of the green algae *Pseudokirchneriella subcapitata* exposed to the test item MT-565SG-OR2-C was investigated during 72-hour test. The test was performed in glass flasks with a capacity of 250 ml. Each of them contained 100 ml of a given test item concentration and the control. The initial density of the algae was $1x10^4$ cells/ml. The following test item concentrations were used: 100, 33, 11, 3.7, 1.23, 0.41, 0.14 mg/l (in three replicates) plus the control (in six replicates).
Density of algae cells was determined in each replicate after 24, 48 and 72h of exposure. Morphology observations of the algae cells were performed at exposure termination.

Results and discussions

The endpoint values were determined on the basis of the nominal test item concentrations. The E_rC_x and the E_yC_x values were calculated with the probit method. The lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC) were determined on the basis of the results of statistical analyses. To conduct statistical analyses, the ToxRat Professional commercial software was used. Results are shown in Tables below.
In all test item concentrations no differences of algae cells were reported as compared to the algae cells in the control.

Table 1. Growth rate endpoint values based on the nominal test item concentrations, definitive test

Endpoint value [mg/L]	**Time of exposure:**		
	24 h	**48 h**	**72 h**
E_rC_{50}	n.d.	6.27 (5.42 – 7.25)	5.51 (4.95 – 6.12)
E_rC_{20}	8.60 (3.52 – 16.70)	0.95 (0.74 – 1.17)	1.00 (0.83 – 1.17)
E_rC_{10}	0.96 (0.13 – 2.57)	0.35 (0.25 – 0.47)	0.41 (0.32 – 0.50)
LOEC	1.23	1.23	1.23
NOEC	0.41	0.41	0.41

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

Table 2. Yield endpoint values based on the nominal test item concentrations, definitive test

Endpoint value [mg/L]	**Time of exposure:**		
	24 h	**48 h**	**72 h**

E_yC_{50}	38.99 (16.31 – 192.55)	1.00 (0.86 – 1.17)	0.94 (0.85 – 1.03)
E_yC_{20}	1.27 (0.15 – 3.42)	0.42 (0.32 – 0.52)	0.53 (0.44 – 0.61)
E_yC_{10}	0.21 (0.01 – 0.88)	0.27 (0.18 – 0.35)	0.40 (0.31 – 0.47)
LOEC	3.70	1.23	1.23
NOEC	1.23	0.41	0.41

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

Table 3. Growth rate endpoint values based on the nominal concentrations of MCPA in the test item, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	**48 h**	**72 h**
E_rC_{50}	n.d.	3.541 (3.065 – 4.096)	3.112 (2.800 – 3.460)
E_rC_{20}	4.861 (1.990 – 9.438)	0.535 (0.418 – 0.661)	0.563 (0.472 – 0.659)
E_rC_{10}	0.545 (0.076 – 1.450)	0.199 (0.142 – 0.265)	0.230 (0.181 – 0.284)
LOEC	0.695	0.695	0.695
NOEC	0.232	0.232	0.232

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

Table 4. Yield endpoint values based on the nominal concentrations of MCPA in the test item, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	**48 h**	**72 h**
E_yC_{50}	22.031 (9.218 – 108.805)	0.567 (0.485 – 0.663)	0.531 (0.483 – 0.581)
E_yC_{20}	0.716 (0.083 – 1.931)	0.240 (0.180 – 0.295)	0.301 (0.252 – 0.343)
E_yC_{10}	0.119 (0.003 – 0.497)	0.153 (0.103 – 0.200)	0.224 (0.176 – 0.265)
LOEC	2.091	0.695	0.695
NOEC	0.695	0.232	0.232

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

Table 5. Growth rate endpoint values based on the nominal concentrations of tribenuron methyl in the test item, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_rC_{50}	n.d.	0.092 (0.079 – 0.106)	0.080 (0.072 – 0.089)
E_rC_{20}	0.126 (0.051 – 0.244)	0.014 (0.011 – 0.017)	0.015 (0.012 – 0.017)
E_rC_{10}	0.014 (0.002 – 0.037)	0.005 (0.004 – 0.007)	0.006 (0.005 – 0.007)
LOEC	0.018	0.018	0.018
NOEC	0.006	0.006	0.006

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

Table 6. Yield endpoint values based on the nominal concentrations of tribenuron methyl in the test item, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_yC_{50}	0.570 (0.238 – 2.818)	0.015 (0.013 – 0.017)	0.014 (0.013 – 0.015)
E_yC_{20}	0.018 (0.002 – 0.050)	0.006 (0.005 – 0.008)	0.008 (0.007 – 0.009)
E_yC_{10}	0.003 (0.000 – 0.013)	0.004 (0.003 – 0.005)	0.006 (0.005 – 0.007)
LOEC	0.054	0.018	0.018
NOEC	0.018	0.006	0.006

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

The determined concentrations of MCPA in samples collected at exposure initiation were in the range of 84.0 – 111.5% of the nominal concentration.
The determined concentrations of tribenuron methyl in samples collected at exposure initiation were in the range of 96.3 – 112.1% of the nominal concentration. The results confirm correct preparation of the test item concentrations.
The determined concentrations of MCPA in samples collected at exposure termination were in the range of 89.3 – 106.4% of the nominal concentration.
The determined concentrations of tribenuron methyl in samples collected at exposure termination were in the range of 97.8 – 117.9% of the nominal concentration.

Therefore, concentrations of MCPA and tribenuron methyl were stable under the test conditions.

Conclusion

The endpoint values determined based on the nominal test item concentrations are given below:
The ErC_{50}/72 h value is 5.51 mg/L (95% confidence interval: 4.95 – 6.12);
The LOEC/72 h value for growth rate is 1.23 mg/L;

The NOEC/72 h value for growth rate is 0.41 mg/L;
The E_yC_{50}/72 h value is 0.94 mg/L (95% confidence interval: 0.85 – 1.03);
The LOEC/72 h value for yield is 1.23 mg/L;
The NOEC/72 h value for yield is 0.41 mg/L.

A.2.2.1.3 Study 3

Comments of zRMS:	The study was conducted to OECD guideline 201 and according to the principles of GLP. No deviations to the guideline were noted. In the definitive test the validity criteria were met: - the biomass in the control increased by a factor of 58.4 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 4.5% (criterion: it must not exceed 7%). - the mean coefficient of variation for the section-by-section growth rate in the control culture was 18.9% (criterion: it must not exceed 35%). The analytical measurements demonstrated that the test item concentrations throughout the test was within 80-120% of nominal and for this reason endpoints are expressed as nominal concentrations. The study is reliable and suitable for the risk assessment.

Reference:	KCP 10.2.1_03
Report	MCPA + Tribenuron metyl 565 SG *Navicula pelliculosa* SAG 1050-3, Growth Inhibition Test, Tina Turek, MSc, 2017, STUDY CODE: W/271/17, Institute of Industrial Organic Chemistry, Branch Pszczyna
Guideline(s):	Yes. According to the OECD Guideline No. 201 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:

MCPA + Tribenuron metyl 565 SG,
content: 14.6 g/kg tribenuron methyl and 565.0 g/kg MCPA as active ingredients
batch number: 4.5/16
production date: 14.11.2016
expiry date: 14.11.2018

Biological test system: The freshwater diatom Navicula pelliculosa (Bréb.) Hilse specification SAG 1050 – 3, cultivated at the Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicology, Laboratory of Aquatic Toxicology.
The diatoms were obtained from the Culture Collection of Algae at Göttingen University, Germany

Test design:	72 hours of exposure; six replicates of the test item concentration and the control; initial diatoms cell density: 1 x 10^4 cells/mL.
Nominal test item concentration:	100 mg/L plus the control.
Test conditions:	Temperature: 22.2 – 22.5°C; pH of the control: 7.39; mean light intensity: 6058 – 6243 lux; constant illumination and shaking; medium: AAP-Si.
Chemical determinations:	The concentrations of MCPA and tribenuron methyl were determined with a validated chromatographic method with UV-Vis detection.
Statistics:	Probit method calculations and analyses by: Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Two-sample t-test Procedure.
Endpoint values:	E_rC_{50}, E_yC_{50}.

The growth of the green algae *Navicula pelliculosa* exposed to the test item MT-565SG-OR2-C was investigated during 72-hour test. The test was performed in glass flasks with a capacity of 250 ml. Each of them contained 100 ml of a given test item concentration and the control. The initial density of the algae was $1x10^4$ cells/ml. The test was performed with a single test item concentration of 100 mg/L (in six replicates) as a limit test plus the control. Density of algae cells was determined in each replicate after 24, 48 and 72h of exposure. Morphology observations of the algae cells were performed at exposure termination.

Results and discussions

The endpoint values were determined on the basis of the nominal test item concentrations. The E_rC_x and the E_yC_x values were calculated with the probit method. The lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC) were determined on the basis of the results of statistical analyses. Results are shown in Tables below.
In all test item concentrations no differences of algae cells were reported as compared to the algae cells in the control.

Table 1. Growth rate endpoint values based on the nominal test item concentrations, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_rC_{50}	> 100	> 100	> 100

Table 2. Yield endpoint values based on the nominal test item concentrations, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_yC_{50}	> 100	> 100	> 100

Table 3. Growth rate endpoint values based on the nominal concentrations of MCPA in the test item, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_rC_{50}	> 56.5	> 56.5	> 56.5

Table 4. Yield endpoint values based on the nominal concentrations of MCPA in the test item, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_yC_{50}	> 56.5	> 56.5	> 56.5

Table 5. Growth rate endpoint values based on the nominal concentrations of tribenuron methyl in the test item, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_rC_{50}	> 1.46	> 1.46	> 1.46

Table 6. Yield endpoint values based on the nominal concentrations of tribenuron methyl in the test item, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_yC_{50}	> 1.46	> 1.46	> 1.46

Results of the chemical determinations

The determined concentration of MCPA at exposure initiation was 92.1% of the nominal concentration, whereas the determined concentration of tribenuron methyl was 91.1% of the nominal concentration.
The results confirm that the test item concentration was prepared correctly.
The determined concentration of MCPA at exposure termination was 87.9% of the nominal concentration, whereas the determined concentration of tribenuron methyl was 97.9% of the nominal concentration.
Therefore, concentrations of MCPA and tribenuron methyl were stable under the test conditions.

Table 11. Concentration and stability of MCPA, definitive test

Nominal test item concentration [mg/L]	Nominal concentration of MCPA in the test item[mg/L]	Average determined concentration of MCPA (n=3) in samples collected			
		exposure initiation [mg/L]	% of nominal concentration	exposure termination [mg/L]	% of nominal concentration
Control	---	< LoD	---	< LoD	---
100	56.500	52.06	92.1	49.65	87.9

LoQ = 0.001 mg/L
LoD = 0.0003 mg/L
--- no value

Table 12. Concentration and stability of tribenuron methyl, definitive test

Nominal test item concentration [mg/L]	Nominal concentration of tribenuron methyl in the test item [mg/L]	Average determined concentration of tribenuron methyl (n=3) in samples collected			
		exposure initiation [mg/L]	% of nominal concentration	exposure termination [mg/L]	% of nominal concentration
Control	---	< LoD	---	< LoD	---
100	1.460	1.33	91.1	1.43	97.9

LoQ = 0.001 mg/L
LoD = 0.0003 mg/L
--- no value

Conclusion

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

The ErC_{50}/72 h and the EyC_{50}/72 h values are higher than 100 mg/L.

A.2.2.1.4 Study 4

Comments of zRMS:	The study was conducted to OECD guideline 221 and according to the principles of GLP. No deviations to the guideline were noted. In the definitive test the validity criteria were met: - The doubling time of frond number in the control was 2.46 days, criterion: less than 2.5 days (the factor of frond number in the control between 0 and 7 day was 7.2). - The average specific growth rate in the control between day 0 and day 7 was 0.282 d^{-1} (minimum requirement: higher than 0.275 d-1). It should be noted that based on the nominal concentrations of tribenuron methyl in the test item for growth rate and yield the NOEC/7 d value is lower than 0.0009 mg/L, whereas the LOEC/7 d value lower than or equal to 0.0009 mg/L this is below LOQ value (values based on the frond number). Also for growth rate the NOEC/7 d value is 0.0009 mg of tribenuron methyl/L (Endpoint values based on the dry weight). The analytical measurements demonstrated that the test item concentrations throughout the test was within 80-120% of nominal and for this reason endpoints are expressed as nominal concentrations. The study is reliable and suitable for the risk assessment. The study is considered to be reliable and suitable for the risk assessment. All results refer to nominal concentrations.

Reference: KCP 10.2.1_04

Report **MCPA + Tribenuron metyl 565 SG** *Lemna gibba*, Growth Inhibition Test, Tina Turek, MSc, 2018, STUDY CODE: W/272/17, Institute of Industrial Organic Chemistry, Branch Pszczyna

Guideline(s):	Yes. According to the OECD Guideline No. 221 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:	MCPA + Tribenuron metyl 565 SG, content: 14.6 g/kg tribenuron methyl and 565.0 g/kg MCPA as active ingredients batch number: 4.5/16 production date: 14.11.2016 expiry date: 14.11.2018
Biological test system:	The freshwater aquatic plant, Lemna gibba L. CPCC 310 cultivated at the Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicology, Laboratory of Aquatic Toxicology; the plants were obtained from the Canadian Phycological Culture Centre (CPCC), Department of Biology, University of Waterloo, Ontario, Canada

The growth of *Lemna gibba* exposed to the test item MT-565SG-OR2-C was investigated during a 7-day static test. The test was performed in glass beakers containing 400 mL of each test item concentration and the control per replicate. The initial frond number in each test item concentration and in the control was nine. The following test item concentrations were used : 1000, 250, 62.5, 15.63, 3.91, 0.98, 0.24, 0.061 mg/L plus the control. The total number of fronds in each test vessel was counted twice during exposure (day 3 and 5) and at exposure termination.

Results and discussions

The observations of plant development, i.e. size of fronds, necrosis, chlorosis, colony break-up, gibbosity, changes in the appearance of roots were performed at the same time. In the control normal size, shape and colour of fronds, normal development of colonies and length of roots were observed. After 7 days of exposure, in all test item concentrations smaller young fronds was observed. Moreover, in the test item concentrations of 250, 62.5, 15.63, 3.91, 0.98 mg/L bending up of fronds and in the test item concentration of 1000 mg/L necrosis and breaking up of colonies were observed.

The endpoint values were determined on the basis of the nominal test item concentrations. The EC_x values were calculated using the probit method. The lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC) were determined on the basis of the results of statistical analyses. To make calculations and to conduct statistical analyses, the ToxRat Professional commercial software was used. The endpoint values are given in Tables 1, 2.

Table 1. Growth rate endpoint values based on the nominal test item concentrations [mg/L], definitive test.

Endpoint values [mg/l]	Frond number			Dry weight
	3 d	5 d	7 d	7 d
E_rC_{50}	0.742 (0.649 – 0.849)	1.177 (1.025 – 1.349)	1.482 (1.329 – 1.652)	41.132 (35.861 – 47.228)

E_rC_{20}	0.167 (0.133 – 0.203)	0.143 (0.113 – 0.117)	0.188 (0.156 – 0.222)	4.195 (3.337 – 5.141)
E_rC_{10}	0.076 (0.056 – 0.099)	< 0.061	0.064 (0.050 – 0.080)	1.272 (0.932 – 1.670)
LOEC	0.24	0.24	≤ 0.061	0.24
NOEC	0.061	0.061	< 0.061	0.061

Table 2. Yield endpoint values based on the nominal test item concentrations [mg/L], definitive test.

Endpoint values [mg/l]	**Frond number**			**Dry weight**
	3 d	**5 d**	**7 d**	**7 d**
EyC_{50}	0.444 (0.408 – 0.484)	0.456 (0.413 – 0.503)	0.478 (0.454 – 0.504)	6.453 (5.868 – 7.097)
EyC_{20}	0.210 (0.184 – 0.235)	0.153 (0.130 – 0.176)	0.123 (0.113 – 0.134)	0.526 (0.450 – 0.609)
EyC_{10}	0.142 (0.119 – 0.164)	0.087 (0.069 – 0.104)	0.061 (0.054 – 0.068)	0.142 (0.115 – 0.172)
LOEC	0.24	0.24	≤ 0.061	0.98
NOEC	0.061	0.061	< 0.061	0.24

Chemical measurements

The determined concentrations of MCPA in samples collected from all test item concentrations at exposure initiation were in the range of 80.5 – 108.1% of the nominal concentration.
The determined concentrations of tribenuron methyl in samples collected from the test item concentrations in the range of 0.24 – 1000 mg/L at exposure initiation were in the range of 84.1 – 98.6% of the nominal concentration. The results confirm correct preparation of the test item concentrations.
The determined concentrations of MCPA in samples collected from all test item concentrations at exposure termination were in the range of 86.3 – 110.2% of the initial concentration.
The determined concentrations of tribenuron methyl in samples collected in the test item concentrations in the range of 0.24 – 1000 mg/L at exposure termination were in the range of 82.9 – 107.3% of the initial concentration. Therefore, concentrations of MCPA and tribenuron methyl were stable under the test conditions.

Table 15. Concentration and stability of MCPA, definitive test

Nominal test item concentration [mg/L]	Nominal concentration of MCPA in the test item[mg/L]	Average determined concentration of MCPA (n=3) in samples collected			
		exposure initiation [mg/L]	% of nominal concentration	exposure termination [mg/L]	% of initial concentration
Control	---	< LoD	---	< LoD	---
0.061	0.034	0.0319	92.5	0.0341	106.9
0.24	0.136	0.109	80.5	0.120	110.1
0.98	0.554	0.518	93.6	0.447	86.3
3.91	2.209	2.221	100.6	2.114	95.2
15.63	8.831	7.453	84.4	7.494	100.6
62.5	35.313	31.796	90.0	35.039	110.2
250	141.25	141.64	100.3	128.36	90.6
1000	565	610.63	108.1	569.99	93.3

LoQ = 0.001 mg/L
LoD = 0.0003 mg/L
--- no value

Table 16. Concentration and stability of tribenuron methyl, definitive test

Nominal test item concentration [mg/L]	Nominal concentration of tribenuron methyl in the test item [mg/L]	Average determined concentration of tribenuron methyl (n=3) in samples collected			
		exposure initiation [mg/L]	% of nominal concentration	exposure termination [mg/L]	% of nominal concentration
Control	---	< LoD	---	< LoD	---
0.061	0.0009	< LoQ	---	< LoQ	---
0.24	0.0035	0.00346	98.6	0.00321	92.8
0.98	0.014	0.0135	94.1	0.012	88.9
3.91	0.057	0.0509	89.1	0.0546	107.3
15.63	0.228	0.192	84.1	0.193	100.5
62.5	0.913	0.841	92.1	0.816	97.0
250	3.65	3.362	92.1	2.786	82.9
1000	14.6	13.47	92.3	12.25	90.9

LoQ = 0.001 mg/L
LoD = 0.0003 mg/L
--- no value

The endpoint values were determined based on nominal test item concentrations. ~~and nominal~~

Conclusion

Endpoint values based on the frond number:
The ErC50/7 d value is 1.482 mg/L (95% confidence interval 1.329 – 1.652).
The EyC50/7 d value is 0.478 mg/L (95% confidence interval 0.454 – 0.504).

For growth rate and yield the NOEC/7 d value is lower than 0.061 mg/L, whereas the LOEC/7 d value lower than or equal to 0.061 mg/L.

Endpoint values based on the dry weight: The ErC50/7 d value is 41.132 mg/L (95% confidence interval 35.861 – 47.228).
For growth rate the NOEC/7 d value is 0.061 mg/L, whereas the LOEC/7 d value is 0.24 mg/L.
The EyC50/7 d value is 6.453 mg/L (95% confidence interval 5.868 – 7.097).
For yield the NOEC/7 d value is 0.24 mg/L, whereas the LOEC/7 d value is 0.98 mg/L.

The endpoint values based on the nominal concentrations of MCPA in the test item:

Endpoint values based on the frond number:
The ErC50/7 d value is 0.837 mg/L (95% confidence interval 0.751 – 0.933).
The EyC50/7 d value is 0.270 mg/L (95% confidence interval 0.256 – 0.285).
For growth rate and yield the NOEC/7 d value is lower than 0.034 mg/L, whereas the LOEC/7 d value lower than or equal to 0.034 mg/L.

Endpoint values based on the dry weight:
The ErC50/7 d value is 23.241 mg/L (95% confidence interval 20.263 – 26.685).
For growth rate the NOEC/7 d value is 0.034 mg/L, whereas the LOEC/7 d value is 0.136 mg/L.
The EyC50/7 d value is 3.647 mg/L (95% confidence interval 3.316 – 4.010).
For yield the NOEC/7 d value is 0.136 mg/L, whereas the LOEC/7 d value is 0.554 mg/L.

The endpoint values based on the nominal concentrations of tribenuron methyl in the test item:

Endpoint values based on the frond number:
The ErC50/7 d value is 0.0215 mg/L (95% confidence interval 0.0193 – 0.0240).
The EyC50/7 d value is 0.0069 mg/L (95% confidence interval 0.0066 – 0.0073).
For growth rate and yield the NOEC/7 d value is lower than 0.0009 mg/L, whereas the LOEC/7 d value lower than or equal to 0.0009 mg/L.
Endpoint values based on the dry weight:
The ErC50/7 d value is 0.5999 mg/L (95% confidence interval 0.5229 – 0.6889).
For growth rate the NOEC/7 d value is 0.0009 mg/L, whereas the LOEC/7 d value is 0.0035 mg/L.
The EyC50/7 d value is 0.0938 mg/L (95% confidence interval 0.0853 – 0.1031).
For yield the NOEC/7 d value is 0.0035 mg/L, whereas the LOEC/7 d value is 0.0140 mg/L.

A.2.2.1.5 Study 5

Comments of zRMS:	The study was conducted to OECD guideline 239 and according to the principles of GLP. Following deviations were noted: In the definitive test, the temperature during the exposure phase was in the range of 19.2 – 26.6ºC (22.9 ± 3.7°C i.e. not within the range of 20 ± 2°C). Therefore, range and fluctuations of the temperature were greater than stated in the OECD No. 239 (2014). However, the growth of plants in the control was sufficient and the validity criteria were met. Therefore, the impact of the temperature fluctuations on the generated results is assumed not significant (negligible). In the definitive test, the plants were dried at approx. 60°C according to OECD No. 239 (2014). The deviations did not have any impact on the results generated during the study. It should be noted that based on the nominal concentrations of tribenuron methyl in the test item for growth rate and yield the NOEC/7 d value is lower than 0.0009 mg/L, whereas the LOEC/7 d value lower than or equal to 0.0009 mg/L this is below LOQ value (values based on the frond number). Also for growth rate the

	NOEC/7 d value is 0.0009 mg of tribenuron methyl/L (Endpoint values based on the dry weight). Thus these values seems to be unreliable. In the definitive test the validity criteria were met: - the mean total shoot length in the control in comparison with the mean total shoot length at exposure initiation increased 2.1-fold. The criterion of at least doubling the total shoot length was met; - the mean fresh weight in the control in comparison with the mean fresh weight for representative group at exposure initiation increased 2.1-fold. The criterion of at least doubling the fresh weight was met; - the plants in the control were without visual symptoms of chlorosis and during the exposure phase no contamination with algae, fungi or bacteria on the plants, on the sediment surface or in the test medium was observed; - the mean coefficient of variation for yield based on fresh weight in replicates of the control in a period from exposure initiation to termination was 11.7%; did not exceed 35%. The analytical measurements demonstrated that the test item concentrations throughout the test was out of the range of 80-120% of nominal and for this reason endpoints are expressed as measured concentrations. The study is reliable and suitable for the risk assessment.

Reference:	KCP 10.2.1_06
Report	MCPA + Tribenuron metyl 565 SG Water-sediment *Myriophyllum spicatum*, Toxicity Test, Tina Turek ~~Anna Świerkot~~, MSc, 2018, STUDY CODE: W/181/17, Institute of Industrial Organic Chemistry, Branch Pszczyna
Guideline(s):	Yes. According to the OECD Guideline No. 239 (2014)
Deviations:	Yes ~~No~~
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:	MCPA + Tribenuron metyl 565 SG, content: 14.6 g/kg tribenuron methyl and 565.0 g/kg MCPA as active ingredients batch number: 4.5/16 production date: 14.11.2016 expiry date: 14.11.2018
Biological test system:	Watermilfoil Myriophyllum spicatum Linne, dicotyledonous freshwater submerged plant, macrophyte, maintained in culture at the Institute of Industrial Organic Chemistry Branch Pszczyna, Department of Ecotoxicology, Laboratory of Aquatic Toxicology

The growth of watermilfoil Myriophyllum spicatum exposed to MCPA + Tribenuron metyl 565 SG for 14 days was studied in a water-sediment system, in static test design, in conditions required for the vege-

tative growth. The toxicity test consisted of a rooting phase (7 days) and an exposure phase (14 days). The plants (representative group) of the mean total shoot length 7.88 cm and of the mean fresh weight 159.54 mg were exposed in a set of nominal test item concentrations: 500, 156, 48.8, 15.26, 4.77, 1.49, 0.47, 0.15, 0.045 mg/L plus control. Three plants rooted in a pot with sediment were placed in a beaker and overlaid with test medium. The test item was applied into aqueous phase of water-sediment system. For each nominal test item concentration four replicates (i.e. 12 plants) and for the control six replicates (i.e. 18 plants) were used.

Results and discussions

Endpoint values were determined based on nominal test item concentrations, geometric means of determined concentrations of MCPA and tribenuron methyl. The ErCx, EyCx values were calculated using the probit method. The lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC) were determined on the basis of the results of statistical analyses.

Chemical determinations

The concentrations of MCPA and tribenuron methyl in aqueous phase, sediment and sediment pore water were determined in the collected samples of water-sediment system using a high-performance liquid chromatography method with UV-Vis detection. In samples collected from all test item concentrations at exposure initiation, the determined concentration of MCPA in aqueous phase was in the range of 82.8 – 114.5% of nominal concentration. In samples collected from the test item concentrations in the range of 0.15 – 500 mg/L, the determined concentration of tribenuron methyl in aqueous phase was in the range of 86.3 – 115.9% of nominal concentration. In sample collected from the test item concentration of 0.045 mg/L the analysed concentration of tribenuron methyl in aqueous phase was below LoQ. The results confirm that the test item concentrations were prepared correctly.
In samples collected from the test item concentration of 500 mg/L and the control at exposure initiation, the analysed concentration of MCPA and tribenuron methyl in sediment and sediment pore water was below LoD. In the sample collected from the test item concentration of 500 mg/L on day 7, the determined concentration of MCPA in aqueous phase was 101.1% of nominal concentration and the determined concentration of tribenuron methyl in aqueous phase was 81.2% of nominal concentration. The determined concentration of MCPA in sediment was 37.913 mg/kg and analysed concentration of tribenuron methyl was below LoD. The determined concentration of MCPA in sediment pore water
was 0.327 mg/L and determined concentration of tribenuron methyl was 0.0072 mg/L. In the sample collected from the control, the analysed concentrations of MCPA and tribenuron emthyl were below LoD in aqueous phase, sediment and sediment pore water. In samples collected from all test item concentrations at exposure termination, the determined concentration of MCPA in aqueous phase was in the range of 46.5 – 108.7% of nominal concentration. In samples collected from the test item concentrations in the range of 0.15 – 500 mg/L, the determined concentration of tribenuron methyl in aqueous phase was in the range of 51.4 – 74.5% of nominal concentration. In the sample collected from the test item concentration of 0.045 mg/L the analysed concentration of tribenuron methyl in aqueous phase was below LoD. In samples collected from the test item concentrations in the range of 4.77 – 500 mg/L, determined concentration of MCPA in sediment was in the range of 0.606 – 48.427 mg/kg. In samples collected from the test item concentrations in the range of 0.045 – 0.47 mg/L, analysed concentration of MCPA in sediment was below LoD and in the test item concentration of 1.49 mg/L, analysed concentration of MCPA was below LoQ. In samples collected from the test item concentration of 500 mg/L,
determined concentration of tribenuron methyl in sediment was 0.744 mg/kg. In samples collected from the test item concentrations in the range of 0.045 – 156 mg/L, analysed concentration of tribenuron methyl in sediment was below LoD. In samples collected from the test item concentrations in the range of 4.77 – 500 mg/L, determined concentration of MCPA in sediment pore water was in the range of 0.00734 – 0.492 mg/L. In samples collected from the test item concentrations in the range of 0.045 – 1.49 mg/L, analysed
concentration of MCPA in sediment pore water was below LoD. In samples collected from the test item concentrations of 156 and 500 mg/L, determined concentrations of tribenuron methyl in sediment pore water were 0.005 and 0.012 mg/L, respectively. In samples collected from the test item concentrations in

the range of 0.045 – 48.8 mg/L, analysed concentration of tribenuron methyl in sediment pore water was below LoD. Endpoint values were determined based on nominal test item concentrations, geometric means of determined concentrations of MCPA and tribenuron methyl.

Table 4. Concentration and stability of detected substances in system using spiking water, stability non-GLP test

Nominal test item concentration [mg/L]	Nominal concentration of detected substance [mg/L]	Average determined concentration of detected substance (n=3) in samples collected				
		in aqueous phase [mg/L] day 0	in aqueous phase [mg/L] (% of the nominal concentration) day 7	in sediment [mg/kg] day 7	in aqueous phase [mg/L] (% of the nominal concentration) day 14	in sediment [mg/kg] day 14
MCPA						
Control	0.00	<LoD	<LoD	<LoD	<LoD	<LoD
10	5.65	5.080 (89.9%)	4.844 (85.7%)	1.001	4.295 (76.0%)	1.063
Tribenuron methyl						
Control	0.00	<LoD	<LoD	<LoD	<LoD	<LoD
10	0.146	0.119 (81.2%)	0.128 (87.7%)	<LoD	0.130 (89.0%)	<LoD

MCPA and tribenuron methyl:

LoQ = 0.001 mg/L (aqueous phase)
LoD = 0.0003 mg/L (aqueous phase)

LoQ = 0.5 mg/kg (sediment)
LoD = 0.15 mg/kg (sediment)

Table 5. Concentration and stability of detected substances in system using spiking sediment, stability non-GLP test

Nominal test item concentration [mg/L]	Nominal concentration of detected substance [mg/L]	Average determined concentration of detected substance (n=3) in samples collected				
		in sediment [mg/kg] (% of the nominal concentration) day 0	in sediment [mg/kg] (% of the nominal concentration) day 7	in aqueous phase [mg/L] day 7	in sediment [mg/kg] (% of the nominal concentration) day 14	in aqueous phase [mg/L] day 14
MCPA						
Control	0.00	<LoD	<LoD	<LoD	<LoD	<LoD
10	5.65	4.585 (81.2%)	0.845 (15.0%)	0.954	<LoQ 0.427 (7.6%)	0.812
Tribenuron methyl						
Control	0.00	<LoD	<LoD	<LoD	<LoD	<LoD
10	0.146	<LoD 0.117 (80.1%)	<LoD	0.018	<LoD	0.014

MCPA and tribenuron methyl:

LoQ = 0.001 mg/L (aqueous phase)
LoD = 0.0003 mg/L (aqueous phase)

LoQ = 0.5 mg/kg (sediment)
LoD = 0.15 mg/kg (sediment)

Biological observations

Table 15. Observations of morphology at exposure termination, definitive test

Nominal test item concentration [mg/L]	Plant parts above sediment	Plant parts below sediment
Control	Normal shape of plants, green color of leaves and stems, no discoloration	Very good development of roots, anchored in sediment
0.045	No changes	Very good development of roots, anchored in sediment
0.15	Distorted apices	Very good development of roots, anchored in sediment
0.47	Distorted apices	Moderate root development (100%)
1.49	Distorted apices	Moderate root development (100%)
4.77	Distorted apices	Few roots (100%)
15.26	Distorted apices	Few roots (100%)
48.8	Distorted apices, loss of turgor around whorls	Few roots (100%)
156	Distorted apices, loss of turgor around whorls, chlorosis	Absent roots (100%)
500	Distorted apices, loss of turgor around whorls, necrosis	Absent roots (100%)

Results, Conclusion

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

Endpoint values calculated on the basis of the total shoot length:

The ErC50/14 d value is 15.919 mg/L (95% confidence limits: 12.059 – 21.214),
The NOEC/14 d value for growth rate is 0.045 mg/L,
The LOEC/14 d value for growth rate is 0.15 mg/L.
The EyC50/14 d value is 5.367 mg/L (95% confidence limits: 4.107 – 7.020),
The NOEC/14 d value for yield is lower than 0.045 mg/L,
The LOEC/14 d value for yield is lower than or equal to 0.045 mg/L.

Endpoint values calculated on the basis of the fresh weight:
The ErC50/14 d value is 39.575 mg/L (95% confidence limits: 23.552 – 72.174),
The EyC50/14 d value is 16.659 mg/L (95% confidence limits: 10.003 – 29.081),
The NOEC/14 d value for growth rate and yield is 0.045 mg/L,
The LOEC/14 d value for growth rate and yield is 0.15 mg/L.

Endpoint values calculated on the basis of the dry weight:
The ErC50/14 d value is 55.208 mg/L (95% confidence limits: 31.793 – 107.661),
The EyC50/14 d value is 11.067 mg/L (95% confidence limits: 6.586 – 19.231),
The NOEC/14 d value for growth rate and yield is 0.15 mg/L,
The LOEC/14 d value for growth rate and yield is 0.47 mg/L.

The endpoint values determined on the basis of geometric mean of determined concentrations of MCPA are given below:

Endpoint values calculated on the basis of the total shoot length:

The ErC50/14 d value is 8.269 mg/L (95% confidence limits: 6.171 – 11.181),
The NOEC/14 d value for growth rate is 0.017 mg/L,
The LOEC/14 d value for growth rate is 0.053 mg/L.
The EyC50/14 d value is 2.610 mg/L (95% confidence limits: 1.964 – 3.469),
The NOEC/14 d value for yield is lower than 0.017 mg/L,
The LOEC/14 d value for yield is lower than or equal to 0.017 mg/L.

Endpoint values calculated on the basis of the fresh weight:
The ErC50/14 d value is 21.520 mg/L (95% confidence limits: 12.489 – 40.385),
The EyC50/14 d value 8.642 mg/L (95% confidence limits: 5.042 – 15.548),
The NOEC/14 d value for growth rate and yield is 0.017 mg/L,
The LOEC/14 d value for growth rate and yield is 0.053 mg/L.
Endpoint values calculated on the basis of the dry weight:
The ErC50/14 d value is 30.496 mg/L (95% confidence limits: 17.082 – 61.537),
The EyC50/14 d value is 5.610 mg/L (95% confidence limits: 3.237 – 10.059),
The NOEC/14 d value for growth rate and yield is 0.053 mg/L

The endpoint values determined on the basis of geometric mean of determined concentrations of tribenuron methyl are given below:

Endpoint values calculated on the basis of the total shoot length:
The ErC50/14 d value is 0.17971 mg/L (95% confidence limits: 0.13588 – 0.23978),
The NOEC/14 d value for growth rate is 0.00039 mg/L,
The LOEC/14 d value for growth rate is 0.0016 mg/L.
The EyC50/14 d value is 0.05968 mg/L (95% confidence limits: 0.04545 – 0.07835),
The NOEC/14 d value for yield is lower than 0.00039 mg/L,
The LOEC/14 d value for yield is lower than or equal to 0.00039 mg/L.
Endpoint values calculated on the basis of the fresh weight:
The ErC50/14 d value is 0.44766 mg/L (95% confidence limits: 0.26661 – 0.81573),
The EyC50/14 d 0.18735 mg/L (95% confidence limits: 0.11196 – 0.32834),
The NOEC/14 d value for growth rate and yield is 0.00039 mg/L,
The LOEC/14 d value for growth rate and yield is 0.0016 mg/L.
Endpoint values calculated on the basis of the dry weight:
The ErC50/14 d value is 0.62266 mg/L (95% confidence limits: 0.35816 – 1.21756),
The EyC50/14 d value is 0.12363 mg/L (95% confidence limits: 0.07307 – 0.21605),
The NOEC/14 d value for growth rate and yield is 0.0016 mg/L,
The LOEC/14 d value for growth rate and yield is 0.0049 mg/L.

Table 20. Endpoint values for growth rate based on geometric means of determined concentrations of MCPA, definitive test

Endpoint value [mg/L]	Total shoot length		Fresh weight	Dry weight
	day 7	day 14	day 14	day 14
E_rC_{50}	37.768 (19.539 – 76.838)	8.269 (6.171 – 11.181)	21.520 (12.489 – 40.385)	30.496 (17.082 – 61.537)
E_rC_{20}	8.196 (1.588 – 16.500)	0.275 (0.162 – 0.427)	0.447 (0.155 – 0.941)	0.443 (0.147 – 0.958)
E_rC_{10}	3.688 (0.350 – 9.024)	0.046 (0.022 – 0.085)	0.059 (0.012 – 0.168)	0.048 (0.009 – 0.146)
LOEC	25.4	0.053	0.053	0.18
NOEC	9.0	0.017	0.017	0.053

(-) - 95% confidence limits
Calculations were made according to [6], [SOP/W/68].

Table 21. Endpoint values for yield based on geometric means of determined concentrations of MCPA, definitive test

Endpoint value [mg/L]	Total shoot length		Fresh weight	Dry weight
	day 7	day 14	day 14	day 14
E_yC_{50}	26.599 (12.360 – 59.642)	2.610 (1.964 – 3.469)	8.642 (5.042 – 15.548)	5.610 (3.237 – 10.059)
E_yC_{20}	4.956 (0.703 – 10.994)	0.064 (0.037 – 0.101)	0.112 (0.036 – 0.251)	0.057 (0.017 – 0.134)
E_yC_{10}	2.059 (0.125 – 5.694)	< 0.017 *calc. 0.009 (0.004 – 0.017)*	< 0.017 *calc. 0.012 (0.002 – 0.036)*	< 0.017 *calc. 0.005 (0.001 – 0.017)*
LOEC	86.3	≤ 0.017	0.053	0.18
NOEC	25.4	< 0.017	0.017	0.053

(-) - 95% confidence limits
Calculations were made according to [6], [SOP/W/68].

Table 22. Endpoint values for growth rate based on geometric means of determined concentrations of tribenuron methyl, definitive test

Endpoint value [mg/L]	Total shoot length		Fresh weight	Dry weight
	day 7	day 14	day 14	day 14
E_rC_{50}	0.74945 (0.40598 – 1.49275)	0.17971 (0.13588 – 0.23978)	0.44766 (0.26661 – 0.81573)	0.62266 (0.35816 – 1.21756)
E_rC_{20}	0.17808 (0.03707 – 0.34236)	0.00694 (0.00416 – 0.01057)	0.01124 (0.00408 – 0.02285)	0.01100 (0.00382 – 0.02299)
E_rC_{10}	0.08402 (0.00865 – 0.19450)	0.00127 (0.00062 – 0.00225)	0.00164 (0.00037 – 0.00444)	0.00133 (0.00027 – 0.00384)
LOEC	0.509	0.0016	0.0016	0.0049
NOEC	0.204	0.00039	0.00039	0.0016

(-) - 95% confidence limits
Calculations were made according to [6], [SOP/W/68].

Table 23. Endpoint values for yield based on geometric means of determined concentrations of tribenuron methyl, definitive test

Endpoint value [mg/L]	Total shoot length		Fresh weight	Dry weight
	day 7	day 14	day 14	day 14
E_yC_{50}	0.54098 (0.26347 – 1.17335)	0.05968 (0.04545 – 0.07835)	0.18735 (0.11196 – 0.32834)	0.12363 (0.07307 – 0.21605)
E_yC_{20}	0.11173 (0.01661 – 0.23618)	0.00171 (0.00101 – 0.00265)	0.00296 (0.00100 – 0.00641)	0.00153 (0.00048 – 0.00350)
E_yC_{10}	0.04899 (0.00313 – 0.12799)	< 0.00039 *calc. 0.00027 (0.00013 – 0.00049)*	< 0.00039 *calc. 0.00034 (0.00007 – 0.00100)*	< 0.00039 *calc. 0.00015 (0.00003 – 0.00049)*
LOEC	1.545	≤ 0.00039	0.0016	0.0049
NOEC	0.509	< 0.00039	0.00039	0.0016

(-) - 95% confidence limits
Calculations were made according to [6], [SOP/W/68].

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

A.2.2.2.1 Study 1

Comments of zRMS:	The study was conducted to OECD guideline 211 and according to the principles of GLP. Following deviations occurred: The range of recorded temperature was 3.9°C (higher than 2°C). Moreover, the temperature registered in the exposure was in range of 18.8 – 22.7°C (over the range of 18 - 22°C). The deviations did not have impact on the generated study results. Since 27% increase in mean cumulative offspring per parent in treated group compared to control was observed NOEC can not be consider as > 10 mg/L. In the Statistical analysis for determination of NOEC for Cumulative Offspring per Taking to consideration that positive effects on reproduction can also be considered adverse and achieved 27% thus only EC_{50} would be taken to consideration in risk assessment. All validity criteria were met: - the mortality of the parent *Daphnia magna* (defined as immobilization together with accidental and/or inadvertent immobilization) in the control was 0% and hence did not exceed 20% at exposure termination. - the mean number of living offspring produced per parent *Daphnia magna* surviving at exposure termination (defined as the number of living offspring produced per parent *Daphnia magna* alive at exposure termination, i.e. the mean cumulative number of offspring per survivor) in the control was higher than 60 (234.8) The analytical measurements demonstrated that the test item concentrations throughout the test was within 80-120% of nominal and for this reason endpoints are expressed as nominal concentrations. The study is reliable and suitable for the risk assessment. The study is considered to be reliable and suitable for the risk assessment. All results refer to nominal concentrations.

Reference:	KCP 10.2.2
Report	MCPA + Tribenuron metyl 565 SG *Daphnia magna*, Reproduction Test, Paweł Bąk, MSc, 2018, STUDY CODE: W/36/18, Institute of Industrial Organic Chemistry, Branch Pszczyna
Guideline(s):	Yes. According to the OECD Guideline No. 211 (2012)
Deviations:	Yes ~~No~~
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:	MCPA + Tribenuron metyl 565 SG, content: 14.6 g/kg tribenuron methyl and 565.0 g/kg MCPA as active ingredients batch number: 4.5/16 production date: 14.11.2016 expiry date: 14.11.2018
Biological test system:	Daphnia magna Straus (< 24 h old at the exposure initiation); not first brood progeny; range of parent Daphnia magna age: 21 – 25 days; test organisms collected from the laboratory culture cultivated at the Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicology, Laboratory of Aquatic Toxicology

Test design:	Semi-static system (renewal three times per week), test duration 21 days, 10 replicates per treatment with one parent *Daphnia magna* held individually.
Test item concentration:	10 mg/L plus the control.
Test conditions:	Medium: Elendt M7; illumination in daily cycle: 16 h light : 8 h darkness; temperature: 18.8 – 22.7 °C, pH of control: 7.56 – 8.57; dissolved oxygen concentration in control: 8.3 – 9.0 mg/L; mean light intensity: 1085 – 1315 lux; glass beakers with 100 mL of tested volume; one exposed *Daphnia magna* per replicate; daily feeding: algal suspension mixture: *Raphidocelis subcapitata* : *Desmodesmus subspicatus* (2:1, v/v) supplying 0.2 mg organic carbon per parent Daphnia per day; no aeration.
Chemical determinations:	The concentrations of MCPA and concentrations of tribenuron metyl were determined with a validated liquid chromatographic method with UV-Vis detection.
Statistics:	Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Two-sample Mann-Whitney U-test Procedure, Two-sample t-test Procedure.
Endpoint values:	EC_x, based on the number of living offspring produced per introduced and survived parent *Daphnia magna* at exposure termination, immobilisation of parent *Daphnia magna*, the final body size (body length) of parent *Daphnia magna* and the intrinsic rate of population growth.

Reproduction of young *Daphnia magna* exposed to the test item MT-565SG-OR2-C was investigated during a 21 days semi-static test. A single test item concentration of 10 mg/l and a control were used (limit test). During exposure Daphnia magna were held individually in 10 replicate glass beakers of 150 mL capacity, containing 100 mL of either the treatment or the control per replicate with a complete renewal three times per week. The observations on immobilization of each parent were reported daily. The body size of each alive parent was measured at exposure termination.

Results and discussions

In the control and in the test item concentration of 10 mg/l no immobilization was observed during exposure. The endpoints were determined on the basis of nominal test item concentration. In the test item concentration of 10 mg/l and in the control no immobilization of *Daphnia magna* was observed during exposure. Therefore, no statistical analysis was needed. The EC_{50} value after 21 days of exposure is higher than 10 mg/l. The LOEC/21d value is higher than 10mg/l. ~~The NOEC/21d value is higher than or equal to 10 mg/l.~~

Chemical Analysis

The concentrations of MCPA and the concentrations of tribenuron metyl determined in fresh samples collected at exposure initiation, on days: 7, 14 and 19 of exposure were in the ranges of 100.4 – 118.8% and 106.2 –117.8% of nominal concentration, respectively.
The concentrations of MCPA and the concentrations of tribenuron metyl determined in spent samples collected on days: 2, 9, 16 and 21 of exposure were in the ranges of 101.4 – 118.4% and 103.4 – 119.2% of nominal concentrations, respectively.
The chemical determinations confirmed stability of concentrations of MCPA and the concentrations of tribenuron metyl in periods between renewals in a semi-static test.
The endpoint values were determined based on nominal test item concentration.

Conclusion

In *Daphnia magna* reproduction semi-static test the endpoint values were determined based on the nominal test item concentration. ECx were based om the number of living offspring produced per introduced and survived parent Daphnia magna at exposure termination, immobilization of parent Daphnia magna, the final body size of parent Daphnia magna and the intrinsic rate of population growth:

Table 11. Compilation of results – definitive test

Nominal test item concentration [mg/L]	Control	10
Mean cumulative offspring number per parent	234.6	295.2*
Mean cumulative offspring number per parent as compared with the control [%]	100	125.8*
Reduction of Mean cumulative offspring number per parent [%]	0	-27.0*
Total of immobilized parent [%]	0	0
Mean body length of parent [mm]	3.96	4.19*
Mean body length of parent as compared with the control [%]	100	105.8*
Reduction of Mean body length of parent [%]	0	-5.8*
Mean intrinsic rate	0.400	0.425*
Mean intrinsic rate as compared with the control [%]	100	106.1*
Reduction of Mean intrinsic rate [%]	0	-6.1*

mean – arithmetic mean,
** value measured/counted is higher than measured/counted for the control.*

Number of living offspring produced per introduced parent Daphnia magna at exposure termination (i.e. the mean cumulative number of offspring per introduced parent):
EC10/21 d, EC20/21 d and EC50/21 d values are higher than 10 mg/L.
Number of living offspring produced per survived parent *Daphnia magna* at exposure termination (i.e. the mean cumulative number of offspring per survivor):
EC10/21 d, EC20/21 d and EC50/21 d values are higher than 10 mg/L.
Immobilisation of parent *Daphnia magna*:
EC10/21 d, EC20/21 d and EC50/21 d values are higher than 10 mg/L.
Final body size (body length) of parent Daphnia magna:
EC10/21 d, EC20/21 d and EC50/21 d values are higher than 10 mg/L.
Intrinsic rate of population growth:
EC10/21 d, EC20/21 d and EC50/21 d values are higher than 10 mg/L.

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

A 2.3.1.1.1.1 Study I

Comments of zRMS:	The study was conducted to OECD guideline 213 and according to the principles of GLP. Following deviation to the guideline were noted: deviation concerned replacing anaesthesia with mechanical immobilization. According to literature, carbon dioxide reduces life expectancy of honeybees. Moreover, the effects of interactions between carbon dioxide and other chemicals are not known. Considering 0.0% mortality in the control group it is certain that this deviation had no impact on the study results. In the definitive test all the validity criteria were met as follows. The study is reliable and suitable for the risk assessment. Overall, the study is considered acceptable with following endpoints: 48 h LD_{50} >95.3 µg/honeybee, corresponding to 1.4 µg of tribenuron methyl/bee and 53.9 µg of MCPA/bee.

Reference:	KCP 10.3.1/01
Report	MCPA + Tribenuron metyl 565 SG, Honeybees (*Apis mellifera* L.), Acute Oral Toxicity Test, N. Lemańska, B/09/17, 2018, Institute of Industrial Organic Chemistry Branch Pszczyna, Poland
Guideline(s):	Yes (OECD Guideline for the Testing of Chemicals No. 213 (1998): "Honeybees, Acute Oral Toxicity Test").
Deviations:	Yes ~~No~~
GLP:	Yes
Duplication (if vertebrate study)	No

Aim of the study
The aims of the study were to use a laboratory method to determine the acute oral toxicity of MCPA + Tribenuron methyl 565 SG to adult worker honeybees and to calculate the LD_{50} values.

Materials and methods

Test item:	MCPA + Tribenuron metyl 565 SG, content: 14.6 g/kg tribenuron methyl and 565.0 g/kg MCPA as active ingredients batch number: 4.5/16 production date: 14.11.2016 expiry date: 14.11.2018
Biological test system:	The honeybee, Apis mellifera L. strain: carnica source: an apiary at the Institute of Industrial Organic Chemistry, Branch Pszczyna [SOP/B/14] age: approximately 3 weeks
Test design:	- test item: exposure time: 48 hours number of doses: 5 doses and a control number of replicates: 3 replicates number of bees: 10 bees/replicate

	- reference item: exposure time: 24 hours number of doses: 3 doses number of replicates: 3 replicates number of bees: 10 bees/replicate
Test item doses:	6.25, 12.5, 25.0, 50.0 and 100.0 µg test item/bee and a control (0.0 µg/bee)
Reference item doses:	0.03, 0.06, and 0.12 µg a.i./bee
Test conditions:	temperature: 24 – 25°C, relative air humidity: 54 - 58% place: a dark room
Statistical method:	regression analysis using the log-probit method
Endpoints:	- honeybee mortality after 24 and 48 hours of exposure (LD50) - the LD50/24h of the reference item (dimethoate)

The acute oral toxicity study of MCPA + Tribenuron metyl 565 SG described in this Report was conducted to determine the LD_{50} values for honeybees. Five doses of the test item were used. These included: 6.25, 12.5, 25.0, 50.0 and 100.0 µg/honeybee. The range of the doses was selected on the basis of the preliminary test results.
Each group of 10 bees (3 replicates containing 10 bees each) was fed with 100 µL of a 50% sucrose solution, containing the test item at the doses enumerated above, using a micropipette. During the entire experiment, the insects were caged in groups of 10.
The general condition of the test honeybees and the reliability of the tests conducted on them were controlled using the recommended reference item - dimethoate.
After the administration, the insects were observed for mortality and other signs of toxicity. These observations were made 4 hours after the beginning of the treatment and then every 24 hours after the beginning of the treatment. The acute oral toxicity test ended after the 48-hour exposure.

Results and discussions

The acute oral toxicity study of the test item, MCPA + Tribenuron metyl 565 SG on honeybees (*Apis mellifera* L.) in the laboratory test are summarized below.

Dose			Number of tested bees [no.]	Mortality after 48 h		LD_{50} after 48 h		
	Tribenuron methyl	MCPA		Total				
[µg/bee][a]	[µg a.i./bee][b]			[no.]	[%]	[µg /bee][a]	Tribenuron methyl	MCPA
							[µg a.i./bee][b]	
0.0 (Control)			30	0	0.0	95.3	1.4	53.9
6.25	0.1	3.5	30	0	0.0			
12.5	0.2	7.1	30	0	0.0			
25.0	0.4	14.1	30	0	0.0			
50.0	0.7	28.3	30	0	0.0			
100.0	1.5	56.5	30	19	63.3			

[a]: µg test item/bee
[b]: µg active ingredient/bee

Mortality of the bees in the control group after 48 hours was 0.0%. After 48 hours of exposure to the test item at the doses of 6.25, 12.5, 25.0 and 50.0 µg/bee have not shown any lethal effect on the tested insects. On the other hand, there were 19 dead bees in the dose of 100 µg/bee and it corresponds to 63.3% mortality. No sublethal toxicity effects (behavioural abnormalities) such as excitement (uncoordinated movement, increased activity, intensive cleaning) or any signs of paralysis with respect to the test item and the control were observed over the 48 hours exposure.

The following validity criteria were met during the test:
- the average mortality for the total number of controls was 0.0% at the end of the experiment (criterion: it must not exceed 10%),
- the 24-hour LD_{50} of the reference item (dimethoate) was 0.10 µg a.i./bee (criterion: 0.10 - 0.35 µg a.i./bee).

Conclusion

The median lethal doses (LD_{50}/24h and LD_{50}/48h) of the test item are equal 95.3 µg/bee (1.4 µg of tribenuron methyl/bee and 53.9 µg of MCPA/bee).

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

A 2.3.1.1.2.1 Study I

Comments of zRMS:	The study was conducted to OECD guideline 214 and according to the principles of GLP. No deviations to the guideline were noted. In the definitive test all the validity criteria were met. The study is reliable and suitable for the risk assessment. Overall, the study is considered acceptable with following endpoints:

	48 h LD_{50} >200.0 µg/honeybee, corresponding to 2.8 µg of tribenuron methyl/bee and > 113 µg of MCPA/bee).

Reference:	KCP 10.3.1/02
Report	MCPA + Tribenuron metyl 565 SG Honeybees (*Apis mellifera* L.), Acute Contact Toxicity Test, N. Lemańska, B/10/17, 2018, Institute of Industrial Organic Chemistry Branch Pszczyna, Poland
Guideline(s):	Yes (OECD Guideline for the Testing of Chemicals No. 214 (1998): "Honeybees, Acute Contact Toxicity Test")
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:	MCPA + Tribenuron metyl 565 SG, content: 14.6 g/kg tribenuron methyl and 565.0 g/kg MCPA as active ingredients batch number: 4.5/16 production date: 14.11.2016 expiry date: 14.11.2018
Biological test system:	the honeybee, *Apis mellifera* L. strain: carnica source: an apiary at the Institute of Industrial Organic Chemistry, Branch Pszczyna age: approximately 3 weeks
Test design:	- test item: exposure time: 48 hours number of doses: 5 doses and a control number of replicates: 3 replicates number of bees: 10 bees/replicate - reference item: exposure time: 24 hours number of doses: 3 doses number of replicates: 3 replicates number of bees: 10 bees/replicate
Test item doses:	12.5, 25.0, 50.0, 100.0 and 200.0 µg test item/bee and a control (0.0 µg/bee)
Reference item doses:	0.1, 0.2, and 0.4 µg a.i./bee
Test conditions:	temperature: 24 – 25ºC, relative air humidity: 54 - 57% place: a dark room

Statistical method:	regression analysis using the log-probit method
Endpoints:	- honeybee mortality after 48 hours of exposure (LD50) - the 24/h LD50 of the reference item (dimethoate)

The acute contact toxicity study of MCPA + Tribenuron metyl 565 SG was conducted to determine the LD_{50}. Five doses of the test item were used. These included: 12.5, 25.0, 50.0, 100.0 and 200.0 µg test item/bee (and µg a.i./honeybee). The range of doses was selected on the basis of the preliminary test results.
A microapplicator was used to apply the test item. The volume was 1 µL/bee. During the experiment, the insects were caged in groups of 10.
The recommended reference item, i.e. dimethoate was used to verify the sensitivity of the honeybees and the precision of the test procedure.
After the application, the insects were observed for mortality and other signs of toxicity. These observations were made 4, 24, and 48 hours after the beginning of the treatment. The acute contact toxicity test finished after the 48-hour observation.

Results:
The median lethal doses (LD_{50}/24h and LD_{50}/48h) are higher than the maximum used dose, i.e. 200 µg test item/honeybee.

Dose			Number of tested bees [no.]	Mortality after 48 h		LD_{50} after 48 h		
				Total				
[µg/bee][a]	Tribenuron methyl	MCPA		[no.]	[%]	[µg /bee][a]	Tribenuron methyl	MCPA
	[µg a.i./bee][b]						[µg a.i./bee][b]	
0.0 (Control)			30	0	0.0	above 200	above 2.8	above 113.0
12.5	0.2	7.1	30	0	0.0			
25.0	0.4	14.1	30	0	0.0			
50.0	0.7	28.3	30	0	0.0			
100.0	1.5	56.5	30	0	0.0			
200.0	2.9	113.0	30	0	0.0			

[a]: µg test item/bee
[b]: µg active ingredient/bee

Mortality of the control group after 48 hours of exposure was 0.0%. There were no dead bees in groups with the test item at the rates of 12.5, 25.0, 100.0, 50.0 and 200.0 µg/honeybee. No signs of toxicity (behavioural abnormalities) such as excitement (uncoordinated movement. increased activity. or intensive cleaning) or paralysis were observed during the 48-hour exposure.

The following validity criteria were met during the test:
- the average mortality for the total number of controls was 0.0% after 48 h (criterion: it must not exceed 10%),

- the LD_{50}/24 h of the reference item (dimethoate) was 0.23 µg a.i./bee (criterion: 0.10 - 0.30 µg a.i./bee).

Conclusion

The median lethal doses (LD_{50}/24 h and LD_{50}/48 h) are higher than the maximum dose used in the test, i.e. 200 µg test item/bee.

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

A 2.3.1.2.1 Study I

Comments of zRMS:	The study was evaluated according to most recent OECD guideline 245 chronic oral toxicity test (10 day feeding test) and according to the principles of GLP. No deviations were noted. The following validity criteria were met during the test: - at the end of the experiment average mortality of the control groups was 3.3% (criterion: it must not exceed 15%) - after 10 days of exposure corrected mortality of the honeybees exposed to the reference item at the concentration of 0.015 µg/30 mg/day (0.016 µg/bee/day) was 93.1%. The study is considered acceptable.

Reference:	KCP 10.3.1/03
Report	MCPA + Tribenuron metyl 565 SG, Honeybees (*Apis mellifera* L.), Chronic Oral Toxicity Test, B/26/18, 2017, P. Parma, Institute of Industrial Organic Chemistry Branch Pszczyna, Poland
Guideline(s):	Yes (according to the OECD Guideline for the Testing of Chemicals No. 245 (2017))
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:	MCPA + Tribenuron metyl 565 SG batch number: 4.5/16 content: 565.0 g/kg MCPA, 14.6 g/k tribenuron-methyl production date: 14th November, 2016 expiry date: 14th November, 2018
Biological test system:	the honeybee, Apis mellifera L.; strain: carnica, source: an apiary at ŁUKASIEWICZ RESEARCH NETWORK – INSTITUTE OF INDUSTRIAL ORGANIC CHEMISTRY, BRANCH PSZCZYNA; age: freshly emerged worker honey-bees from the same queen-right colony

Design of the definitive test:	- the test item: exposure duration: 10 days; number of concentrations: 5 and a control; number of replicates: 3; number of insects: 10 bees/replicate - the reference item: exposure duration: 10 days; number of concentrations: 1; number of replicates: 3; number of insects: 10 bees/replicate
Concentrations of the test item:	0.42, 0.83, 1.67, 3.33 and 6.67 g/kg
Initial doses of the test item:	12.5, 25.0, 50.0, 100.0 and 200.0 µg/bee/day
Doses of the test item consumed by the bees:	15.1, 24.7, 40.4, 56.9 and 37.8 µg/bee/day
Concentration of the reference item (dimethoate):	0.5 mg/kg
Initial dose of the reference item (dimethoate):	0.015 µg/bee/day
Test conditions:	temperature: 31 – 34ºC; relative humidity: 53 – 62% Endpoints: - honeybee mortality after 10 days of exposure - LC_{50} and NOEC after 10 days of exposure - LDD_{50} and NOEDD after 10 days of exposure
Statistical analyses:	regression analysis using linear max. likelihood regression Step-down Cochran-Armitage Test Procedure

Test conditions:

All honeybees treated with the test item or the reference item (dimethoate), the control and control with acetone were caged in groups of 10. The size of the test cages (8 x 10 x 6 cm) provided adequate space. The honeybees ingested a treated or an untreated 50% sucrose solution via feeders. The caged honeybees during the test (without acclimatization period) were kept under the following conditions:
Temperature: 31 – 34ºC;
Relative humidity: 53 – 62%

The chronic oral toxicity study of MCPA + Tribenuron metyl 565 SG described in this Report was conducted to determine the median lethal concentration, i.e. the LC_{50}, the no observed effect concentration NOEC, median lethal dietary dose, i.e. LDD_{50} and no observed dietary dose NOEDD. Five concentrations of the test item were used.
The initial concentrations were 0.42, 0.83, 1.67, 3.33 and 6.67 g/kg (i.e. 12.5, 25.0, 50.0, 100.0 and 200.0 µg/bee/day) of MCPA + Tribenuron metyl 565 SG.
Daily doses, consumed by the bees in the groups treated with the test item at the concentrations mentioned above, were 15.1, 24.7, 40.4, 56.9 and 37.8 µg/bee/day, respectively.
Each group of bees (3 replicates/group; 10 bees/replicate) was fed with 2 mL of a 50% sucrose solution containing the test item at the concentrations mentioned previously for 10 days. Feeders were used. During the experiment, the insects were caged in groups of 10.
Dimethoate, which is a recommended reference item, was used to verify the sensitivity of the bees and the precision of the test procedure.
The insects were observed for mortality and behavioural abnormalities (signs of intoxication) at daily intervals up to 10 days of exposure. The chronic oral toxicity test finished after 10 days of exposure.

Results:

Chemical Analysis

Table 1. Concentrations and stability of MCPA + Tribenuron metyl 565 SG – definitive test

Nominal test item concentration [mg/kg]	Results					
	Day 0					
	MCPA concentration			Tribenuron methyl concentration		
	Nominal [mg/kg]	Average [mg/kg]	Recovery [%]	Nominal [mg/kg]	Average [mg/kg]	Recovery [%]
Control	–	–	–	–	–	–
420	237	258	**108.6**	6.132	5.43	**88.5**
6670	3769	4206	**111.6**	97.382	84.77	**87.0**

Honeybee mortality and the LDD_{50} and LC_{50}/10 d – definitive test

Initial dose	Consumed*		Number of tested bees [no]	Mortality		LC_{50}	LDD_{50}
	Concentration	Dose		Total			
[µg/bee/day]	[g/kg]	[µg/bee/day]		No.	[%]	[mg/kg]	[µg/bee/day]
MCPA + Tribenuron metyl 565 SG							
0.0 (Control)			30	1	3.3	4460 (n.d.)	100.62 (n.d.)
12.5	0.42	15.1	30	0	0.0[a]		
25.0	0.83	24.7	30	2	3.4[a]		
50.0	1.67	40.4	30	1	0.0[a]		
100.0	3.33	56.9	30	2	3.4[a]		
200.0	6.67+	37.8+	30	29	96.6[a]		
NOEC				3.33 [g/kg]			
NOEDD				56.9 [µg/bee/day]			
Initial dose	Consumed*		dimethoate				
[µg/bee/day]	concentration [mg/kg]	dose [µg/bee/day]					
0.015	0.5	0.016	30	28	93.1[a]	not determined	

*: consumed doses were calculated on the basis of the initial doses of the test item and average sucrose solution consumption
[a]: the control response of 3.3% was compensated using Abbott's formula [8]
+: statistically significant difference
n.d.: 95% confidence limits not determined due to mathematical reasons

Consumption of a 50% sucrose solution after 10 days of exposure – definitive test

Initial	Consumed		Consumption of a 50% sucrose solution[a] [mg/bee/day]			Average consumption [mg/bee/day]		
Dose	Concentration	Dose						
[µg/bee/day]	[mg/kg]	[µg/bee/day]	replicates					
			I	II	III			
MCPA + Tribenuron metyl 565 SG								
0.0 (Control)			32.4	30.2	37.2	**33.3**		
12.5	0.42	**15.1**	42.9	37.9	27.9	**36.2**		
25.0	0.83	**24.7**	27.7	32.8	28.6	**29.7**	**22.6**	
50.0	1.67	**40.4**	21.1	28.9	22.7	**24.2**		
100.0	3.33	**56.9**	14.5	15.1	21.6	**17.1**		**25.4**
200.0	6.67	**37.8**	0.3	0.4	16.3	**5.7**		
Initial dose	**Consumed**		**Dimethoate**					
	Concentration	**Dose**						
[µg/bee/day]	[mg/kg of food]	[µg/bee/day]						
0.015	0.5	**0.016**	21.9	37.5	36.5	**40.0**		

[a]: food consumption on each day of exposure (mg/bee/day) was determined by weighing the feeders with a sucrose solution and dividing the amount of food by the number of surviving bees substracting evaporation

a: food consumption on each day of exposure (mg/bee/day) was determined by weighing the feeders with a sucrose solution and dividing the amount of food by the number of surviving bees substracting evaporation

The insects were observed for mortality and behavioural abnormalities (signs of intoxication) at daily intervals up to 10 days of exposure. The chronic oral toxicity test finished after 10 days of exposure.

On the basis of the obtained mortality results, the LC_{50} value is equal to 4460 mg/kg and the NOEC value is equal to 3.33 g/kg. The LDD_{50} value is equal to 100.62 µg/bee/day and the NOEDD value is equal to 56.9 µg/bee/day.
The validity criterion concerning mortality was met, because mortality in the control was ≤ 15.0% (3.3%) after 10 days of exposure. In all study groups average consumption of a 50% sucrose solution was 25.4 mg/bee/day.
The percentages of corrected mortality of the honeybees exposed to the test item, MCPA + Tribenuron metyl 565 SG at the concentrations of 0.42, 0.83, 1.67, 3.33 and 6.67 g/kg (12.5, 25.0, 50.0, 100.0 and 200.0 µg/bee/day) were 0.0, 3.4, 0.0, 3.4 and 96.6% respectively.

A 2.3.1.3 KCP 10.3.1.3 Effects on honey bee development and other honey bee life stages (= larval toxicity)

A 2.3.1.3.1 Study I

Comments of zRMS:	The study was conducted to OECD guideline 239 and according to the principles of GLP. Following deviations were noted: Moreover, in course of the range-finding and definitive test, periodic deviation of temperature (required: 34-35°C) and humidity (required: 50-100%) occurred. It resulted from daily feedings and observations. These changes were short-termed, did not affect the condition of the test system. The deviations had no effect on the test results. The test met the validity criteria of the experiment listed in OECD GD 239 Guideline: - cumulative larval mortality in control on days 3-8 was 0.0% (required: ≤15%), - the adults emergence rate in control on day 22 was 88.9% (required: ≥70%), - the adults emergence rate in control on day 22 for fenoxycarb as a reference item was 0.0% (required: ≤20%). Overall, the study is considered acceptable.

Reference:	KCP 10.3.1/04
Report	Chronic Toxicity Test for Honey Bee Larvae according to OECD GD 239, MT-565SG-OR2-C, 0016/0102/E, 2020, W. Londzin, SORBOLAB Research Laboratory LLC, Poland
Guideline(s):	Yes (OECD GD 239 Guidance Document on Honey Bee (Apis Mellifera L.) Larval Toxicity Test, Repeated Exposure (2016)
Deviations:	Yes ~~No~~
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item

Name:	MT-565 SG-OR2-C
Test item description	light beige granules with characteristic scent
Appearance of the original packaging	(polyethylene) PE container
Date of delivery	17/06/2020
Batch No.	3/20
Name of active substances	MCPA Tribenuron methyl
Content of active substances	MCPA: about 550 g/kg (purity: 930 g/kg) Tribenuron methyl: about 15 g/kg (purity: 960 g/kg)

CAS of active substances	MCPA: 94-74-6 Tribenuron methyl: 101200-48-0
Production date:	08.01.2020
Expiry date:	08.01.2022

Test species

The study was carried out on larvae of honey bee *Apis mellifera L.* coming from a registered breeding of Mr. Wiesław Londzin in Poznań. The study used 1-day larvae of honey bee originated from 3 different, healthy, well-maintained breeding.

Test conditions

For larval stage (day 1-8): average temperature 34.186°C (minimal temperature 32.9°C; maximal temperature 35.1°C); average relative humidity 95.976% (minimal humidity 73.4%; maximal humidity 99.9%)
For pupal stage (day 8-15): average temperature 35.065°C (minimal temperature 33.6°C; maximal temperature 35.9°C); average relative humidity 78.547% (minimal humidity 68.4%; maximal humidity 97.4%)
For imago stage (day 15-22): average temperature 34.411°C (minimal temperature 33.0°C; maximal temperature 35.2°C); average relative humidity 76.554% (minimal humidity 59.7%; maximal humidity 84.2%).
The study was conducted in darkness

Test design

During range-finding test, one concentration of each reference item was used: fenoxycarb - 0.32 mg/kg of food; dimethoate - 48 mg/kg of food and control.
In the definitive test, were used following concentration of test item: 40.625 mg/kg; 81.25 mg/kg; 162.5 mg/kg; 325 mg/kg and 650 mg/kg of food. Each concentration and control were prepared in one replicate, 36 larvae per replicate on single breeding plate.
Parallel to definitive test, reference test was performed using fenoxycarb as reference item.
Based on the obtained data in definitive test, statistical analysis for following developmental stages was performed in accordance with OECD GD 239 Guideline, using ToxRat Professional version 3.3. statistical software.
The aim of the study was determination of the concentration causing 50% mortality of population (LC50 value) and the dose causing mortality of 50% of the population after 22 days (LD50 value). Values NOEC and NOED were calculated for the emerged bees on day 22.

Results and conclusions

In course of the experiment, the test item has shown apitoxic effect in mortality of following developmental stages of bees after 22 days of the test. At the end of the study, the concentration and the dose causing 50% mortality of the population in the test (LC50 and LD50 values) were determined, as well as LC10, LC20, LD10, LD20, NOEC and NOED values were determined at 22 day.

Table 19. Larval mortality – definitive test

Concentration [mg/kg of food]	Time [day]											
	3	4		5		6		7		8	
	Introduced larvae [pcs.]	Dead larvae*) [pcs.]	Intoxication signs	Dead larvae*) [pcs.]	Intoxication signs	Dead larvae*) [pcs.]	Intoxication signs	Dead larvae*) [pcs.]	Intoxication signs	Dead larvae*) [pcs.]	Intoxication signs
Control	36	0	none	0	none	0	none	0	none	0	stun. dev. – 3
40.626	36	0	none	0	none	0	stun. dev. – 1	0	stun. dev. – 2	0	stun. dev. – 2
81.25	36	0	none	0	none	0	none	0	stun. dev. – 1	0	stun. dev. – 1
162.5	36	0	stun. dev. – 2	0	stun. dev. – 3	1	stun. dev. – 2	3	stun. dev. – 3	4	stun. dev. – 4
325	36	0	stun. dev. – 5	1	stun. dev. – 5	3	stun. dev. – 6	3	stun. dev. – 7	4	stun. dev. – 6
650	36	1	stun. dev. – 7	2	stun. dev. – 6	2	stun. dev. – 9	4	stun. dev. – 9	5	stun. dev. – 8

*) cumulative amount
stun. dev. stunted development

Table 23. Number of emerged adults – definitive test

Concentration [mg/kg of food]	Time [day]					
	3	22				
	Introduced larvae [pcs.]	Emerged adults [pcs.]	Number of emerged adults [%]	Unemerged adults [pcs.]	Number of unemerged adults [%]	Statistical significance *)
Control	36	32	88.9	4	11.1	not
16.64	36	26	72.2	10	27.8	-
41.6	36	30	83.3	6	16.7	-
104	36	19	52.8	17	47.2	+
260	36	15	41.7	21	58.3	+
650	36	11	30.6	25	69.4	+

- statistically insignificant
+ statistically significant
**) values calculated using ToxRat Professional using Step-Down Cochran-Armitage Test with significance level p>0.05

Table 24. Larval mortality – fenoxycarb – definitive reference test

Concentration [mg/kg of food]	Time [day]					
	3	22				
	Introduced larvae [pcs.]	Emerged adults [pcs.]	Number of emerged adults [%]	Unemerged adults [pcs.]	Number of unemerged adults [%]	Statistical significance*)
Control	36	32	88.9	4	11.1	not applicable
0.32	36	0	0.0	36	100.0	+

+ statistically significant
*) values calculated using ToxRat Professional using Fisher Test with significance level p>0.05

The final results of the experiment are presented in Table below

Parameter	Concentration [mg of test item/kg of food]	Parameter	Dose [µg of test item/larva]
LC_{10}	35.430 (n.d. – 100,492)*	LD_{10}	5.451 (n.d. – 15.460)*
LC_{20}	75.113 (n.d. – 178.316)*	LD_{20}	11.556 (n.d. – 27.433)*
LC_{50}	316.262 (121.853 – 47 8987.531)*	LD_{50}	48.656 (18.747 – 73 655.047)*
NOEC	81.25	NOED	12.50

* upper and lower confidence limits (95%) given in the brackets
n.d. not determined
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%
NOEC the highest test item concentration not causing statistically significant differences in relations to the control
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%
NOED the highest test item dose not causing statistically significant differences in relations to the control

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

No new or additional studies have been submitted

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

No new or additional studies have been submitted

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

No new or additional studies have been submitted

A 2.3.2 KCP 10.3.2 Effects on arthropods other than bees

A 2.3.2.1 Study 1

Comments of zRMS:	The study follows the guideline specified by Blümel et al. (2000) and according to the principles of GLP. No deviations to the guideline were noted. In the definitive test all the validity criteria were met Considering the current test guideline (Blümel et al., 2000) the study is considered valid.

Reference:	KCP 10.3.2/01
Report	A laboratory test for evaluating the effects of MCPA + Tribenuron metyl 565 SG on the predatory mite, *Typhlodromus pyri* (Sch.), A. Glanas, B/12/17, 2018, Institute of Industrial Organic Chemistry Branch Pszczyna, Poland
Guideline(s):	Yes (ESCORT 1 (Barrett K.L. et al., 1994), ESCORT 2 (Candolfi M.P. et al., 2001) IOBC, BART and EPPO Joint Initiative (Blümel S. et al., 2000))
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Summary

The aim of the laboratory test was to evaluate the effects of the test item, MCPA + Tribenuron metyl 565 SG on mortality and reproduction of the predatory mite, *T. pyri*(Sch.).
On the basis of the preliminary test results, it was decided to use three rates of the test item in the definitive test. These were 0.25, 0.50 and 1.00 kg/ha (i.e. 141.3 g MCPA/ha + 3.7 g tribenuron methyl/ha, 282.5 g MCPA/ha + 7.3 g tribenuron methyl/ha and 565 g MCPA/ha + 14.6 g tribenuron methyl/ha).
The mites, *T. pyri* at the protonymphal stage (24 hours old) were exposed to the test item applied to discs. The mites were fed with pine pollen (*Pinus sp.*). Mortality observations were made after 7 days of the treatment. Observations of reproduction of the control group and all groups treated with the test item were made after 8, 12, and 14 days of the treatment.
Mortality of *T.pyri* after 7 days of the treatment and the reproduction reduction (Pr) after 14 days of the treatment were test endpoints.
To verify the sensitivity of the mites and the precision of the test procedure, an insecticide, Danadim 400 EC (400 g dimethoate/L) was used as a reference item. The rate of the reference item was 9.0 mL/ha

(3.6 g a.i./ha). The control group was treated with distilled water.

Materials and methods

1. Test material:
Name: MCPA + Tribenuron metyl 565 SG
Batch number: 4.5/16
Manufacturing date: 14th November, 2016
Expiry date: 14th November, 2018
Appearance: brown granules
Active substance: 14.6 g/kg tribenuron methyl, 565.0 g/kg MCPA

2. Species: the predatory mite, *Typhlodromus pyri* Scheuten (Acari: *Phytoseiidae*)
age: 24-hour-old protonymph. Source: a laboratory culture at the Institute of Industrial Organic Chemistry, Branch Pszczyna the culture was obtained from the Research Institute of Pomology and Floriculture, Skierniewice, Poland.

3. Test Units:
The study was performed according to the 'island method' described by Joisten M. Each test set consisted of a glass tray filled with water and a glass bench containing 5 test units. Plastic discs (ϕ 45 mm) were floating on the water surface in glass Petri dishes ('island dishes', ϕ 54 mm) with central holes at the bottom (ϕ 6 mm). Water in the test units prevented the mites from escaping.
Temperature: 23 – 27°C
Humidity: 64 – 83%
Light regime: 16 (622 lux) : 8

Experimental design:	5 study groups: - a control group (0.0 g/ha) – - MCPA + Tribenuron metyl 565 SG at the rate of 0.25 kg/ha (141.3 g MCPA/ha + 3.7 g tribenuron methyl/ha); – - MCPA + Tribenuron metyl 565 SG at the rate of 0.50 kg/ha (282.5 g MCPA/ha + 7.3 g tribenuron methyl/ha), – - MCPA + Tribenuron metyl 565 SG at the rate of 1.0 kg/ha (565 g MCPA/ha + 14.6 g tribenuron methyl/ha), - Danadim 400 EC at the rate of 9.0 mL/ha (3.6 g a.i./ha) number of replicates: 3; number of mites in each replicate: 20
Statistical analysis:	probit analysis using linear max. likelihood regression, Step-down Cochran-Armitage Test Procedure, Shapiro-Wilk's test on normal distribution, Levene's test on variance homogeneity (with Residuals), Williams Multiple Sequential t-test Procedure
Endpoints:	– mite mortality after 7 days of the treatment (LR50 and NOERmortality) – reproduction reduction (Pr) after 14 days of the treatment (ER50 and NOERreproduction)

Results and discussions

The effects of MT-565SG-OR2-C on mortality and reproduction of *Typhlodromus pyri* in the definitive test are summarized below:

Study group [application rate]			Parameter (endpoint)								
			Mortality					Reproduction			
Test item			Total [%]	Corre-cted*	LR_{50}			Mean number of eggs/ female (Rr) [no.]	Repro-duction reduction Pr [%]	ER_{50}		
[kg/ha][a]	[g a.i./ha][b]				[kg/ha] [a]	[g a.i./ha] [b]				[kg/ha] [a]	[g a.i./ha][b]	
	a.i. [d]	a.i. [e]				a.i. [d]	a.i. [e]				a.i. [d]	a.i. [e]
Control (0.0)			3.3	-	-			4.4	-	-		
0.25	141.3	3.7	3.3	0.0				2.2+	**49.2**			
0.50	282.5	7.3	3.3	0.0	**> 1.0**	> 565.0	> 14.6	1.7+	**60.4**	**< 0.25**	< 141.3	< 3.7
1.00	565.0	14.6	0.0	(-3.5)				1.4+	**67.4**			
$NOER_{mortality}$			**≥ 1.0**	≥ 565.0	≥ 14.6	$NOER_{reproduction}$			**< 0.25**	< 141.3	< 3.7	
Reference item			—									
[mL/ha][c]	[g a.i./ha] [b]											
9.0	**3.6**		88.3	87.9	not determined			not assessed				

[a]: [kg test item/ha]
[b]: [g active ingredient/ha]
[c]: [mL reference item/ha]
[d]: MCPA
[e]: tribenuron metyl
*: mortality corrected using the formula of Abbott [1]
+: statistically significant difference

In the definitive test, mortality of the control group after 7 days of exposure was 3.3%. After 7 days of exposure to MCPA + Tribenuron methyl 565 SG at the rates of 0.25, 0.50 and 1.00 kg/ha (i.e. 141.3 g MCPA/ha + 3.7 g tribenuron methyl/ha, 282.5 g MCPA/ha + 7.3 g tribenuron methyl/ha and 565 g MCPA/ha + 14.6 g tribenuron methyl/ha) the percentages of mortality of *T. pyri*, were 0.0, 0.0 and (-3.5) %, respectively.

After 7 days of exposure to Danadim 400 EC at the rate of 9.0 mL/ha (3.6 g a.i./ha), mortality of the mites, was 88.3%. Therefore, the validity criterion specified in the Method description was met. The results obtained in the reference item group showed that the test organisms were sensitive to dimethoate.
The mean reproduction rate (Rr) in the control group was 4.1 eggs/female. The mean reproduction rates after 14 days of exposure to D-500SC-OR2-C at the rates of 0.05, 0.1, 0.2, 0.4 and 0.8 L/ha were 3.1, 3.6, 3.5, 4.5 and 3.3 eggs/female, respectively.
The percentages of reproduction reduction (Pr) caused by D-500SC-OR2-C at the rates of 0.05, 0.1, 0.2, 0.4 and 0.8 L/ha were 22.9, 11.6, 14.3, (-10.8) and 18.8%, respectively. The negative value shows that the reproduction was higher than in the control group.

The following validity criteria were met during the study:
– mortality of the control group was 3.3% on day 7 of exposure (criterion: a maximum of 20%),
– corrected mortality of the mites exposed to the reference item at the rate of 9.0 mL/ha was 87.9% on day 7 of exposure (criterion: from 50 to 100%),
– the mean number of eggs per female in the control group was 4.4 (required: ≥ 4 eggs per female).

Conclusion

On the basis of the obtained mortality results, the LR50and $NOER_{mortality}$ values could not be estimated.

It can only be concluded that the LR50value is higher than the highest tested rate, i.e. > 1.00 kg/ha (565 g MCPA/ha + 14.6 g tribenuron methyl/ha)and $NOER_{mortality}$ is higher than or equal 1.00 kg/ha (565 g MCPA/ha + 14.6 g tribenuron methyl/ha).

On the basis of the obtained results, the ER50 and the $NOER_{reproduction}$ value could not be estimated. The ER50is lower than the lowerrate used in the experiment, i.e. < 0.25 kg/ha (< 141.3 g MCPA/ha + 3.7 g tribenuron methyl/ha) and the $NOER_{reproduction}$ is lower than the lower rate 0.25 kg/ha (i.e. 141.3 g MCPA/ha + 3.7 g tribenuron methyl/ha).

A 2.3.2.2 Study 2

Comments of zRMS:	The study follows the guideline specified by Mead Briggs M.A. et al. (2000) and according to the principles of GLP. No deviations to the guideline were noted. In the definitive test all the validity criteria were met. Considering the current test guideline (Mead Briggs M.A. et al, 2000) the study is considered valid.

Reference:	KCP 10.3.2/02
Report	A laboratory test for evaluating the effects of MCPA + Tribenuron metyl 565 SG on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani - Perez), A. Glanas, B/11/17, 2018, Institute of Industrial Organic Chemistry Branch Pszczyna, Poland
Guideline(s):	Yes (ESCORT 1 (Barrett K.L. et al., 1994), ESCORT 2 (Candolfi M.P. et al., 2001) IOBC, BART and EPPO Joint Initiative (Mead-Briggs M.A et al., 2000)).
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

SUMMARY

The laboratory test involved the evaluation of the effects of the test item, MCPA + Tribenuron metyl 565 SG on mortality and fecundity of the parasitic wasp, *Aphidius rhopalosiphi.* On the basis of the results of the preliminary test, the definitive test was performed as a single-rate limit test. The application rate of 1.0 kg/ha (565 g MCPA + 14.6 tribenuron methyl/ha) was used. Adult wasps were exposed to the test item applied to glass plates. Mortality assessments were made 2, 24, and 48 hours after the introduction of the wasps to the test arenas.
Then, females which survived 48-hour exposure to MCPA + Tribenuron metyl 565 SG and the ones from the control group were subjected to fecundity assessments. To allow the oviposition, fifteen female wasps from the groups treated with MCPA + Tribenuron metyl 565 SG at the rate of 1.0 kg/ha (565 g MCPA + 14.6 tribenuron methyl/ha) and the control group were individually introduced into fecundity units containing barley plants infested with the aphid, *Rhopalosiphum padi*. After the 24- hour oviposition, the wasps were removed from the test arenas. After 12 days, the number of mummies (parasitized aphids in which wasp pupae were developing) was recorded.
Mortality of the wasps after 48 hours of exposure and the percentage of fecundity reduction (Pr) 12 days after the oviposition were the endpoints. To verify the sensitivity of the test system and the precision of the test procedure, an insecticide, i.e. Danadim 400 EC (400 g dimethoate/L) was used as a reference

item. The rate of the reference item was 0.1 mL/ha (0.04 g dimethoate/ha). The control group was treated with distilled water.

Materials and methods

1. Test material:
Name: MCPA + Tribenuron metyl 565 SG;
Content: 14.6 g/kg tribenuron methyl and 565.0 g/kg MCPA as an active ingredient;
Batch number: 4.5/16;
Manufacturing date: 14th November, 2016,
Expiry date: 14th November, 2018

2. Species: the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez); Hymenoptera: Braconidae, Aphiidinae, **age:** adult females (24 - 48 hours after emerging from mummies)
Source: a laboratory culture at the Institute of Industrial Organic Chemistry, Branch Pszczyna; the culture was obtained from Katz Biotech AG (Baruth, Germany).

Experimental design:	3 test groups: – a control group (0.0 L/ha) – MCPA + Tribenuron metyl 565 SG at the rate of 1.0 kg/ha (565 g MCPA + 14.6 tribenuron methyl/ha)

3. Test Units:

The test unit for mortality assessment (exposure unit) consisted of two glass plates (12 x 12 cm) fitted with rubbers to a stainless steel frame. On the side walls, there were ten holes covered with finegauge mesh providing ventilation for the insects and two holes to introduce the wasps to the test units. Later these holes were sealed with cotton bungs soaked with a 1:3 v/v solution of honey in water used as a source of food.
For fecundity assessment, the test unit consisted of a transparent PMMA cylinder (isolator) with a diameter of 11 cm and a height of 20 cm put on a plastic pot with a diameter of 12 cm. The pots contained 20 untreated seedlings of 7-day-old barley infested with the bird cherry-oat aphid, *Rhopalosiphum padi* (more than 100 aphids per pot). To provide good ventilation, the apex of each cylinder and two longitudinal openings on its two sides were covered with metal netting. There was a hole in the cylinder enabling introduction of the wasps to the test unit. The hole was filled with a cotton bung soaked with a 1:3 v/v solution of honey in water.

– temperature: 19 - 21°C
– relative air humidity: 65 - 73%
– photoperiod: 16 hours light (mortality assessment and oviposition: 2827 lx; fecundity assessment: 4287 lx) : 8 hours dark

Statistical analyses:	Shapiro-Wilk's test on normal distribution, Levene's test on variance homogeneity, Two-sample t-test procedure.
Endpoints:	– wasp mortality after 48 hours of exposure – determination of the LR50 and the NOERmortality – reduction in fecundity (Pr) of surviving female wasps exposed MCPA + Tribenuron metyl 565 SG, recorded 12 days after the oviposition period – determination of the ER50 and the NOERfecundity

Results and discussions

The effects of MCPA + Tribenuron metyl 565 SG on mortality and fecundity of *Aphidius rhopalosiphi* in the laboratory test are summarized below:

Study group [application rate]			Parameter (endpoint)								
			Mortality after 48 h				Fecundity				
Test item			Total [%]	LR_{50}			mean no. of mummies/ female	Fecundity reduction Pr [%]	ER_{50}		
[kg/ha][a]	[g a.i./ha][c]			[kg/ha][a]	[g a.i./ha][c]				[kg/ha][a]	[g a.i./ha][c]	
	a.i.[d]	a.i.[e]			a.i.[d]	a.i.[e]				a.i.[d]	a.i.[e]
Control (0.0)			0.0	-			18.1	-	-		
1.0	565.0	14.6	0.0	> 1.0	>565.0	>14.6	11.5	36.2*	> 1.0	>565.0	>14.6
$NOER_{mortality}$				≥ 1.0	≥565.0	≥14.6	$NOER_{fecundity}$		> 1.0	>565.0	>14.6
Reference item			Mortality after 48 h				—				
[mL/ha][b]	[g a.i./ha][c]										
0.1	0.04		78.6	not determined			not assessed				

[a]: [kg test item/ha]
[b]: [mL reference item/ha]
[c]: [g active ingredient/ha]
[d]: MCPA
[e]: Tribenuron methyl
*: statistically significant differences

After 48 hours mortality of the control wasps and mortality in the group exposed to MCPA + Tribenuron metyl 565 SG at the rate of 1.00 kg/ha (i.e. 565 g MCPA/ha+ 14.6 g tribenuron methyl) were 0.0 %. On the basis of the obtained mortality results, the LR_{50} and the $NOER_{mortality}$ values could not be estimated. It can only be concluded that the LR_{50} value is higher than the highest tested rate, i.e. > 1.00 kg/ha (565 g MCPA/ha + 14.6 g tribenuron methyl/ha) and NOER mortality is equal to or higher than the highest tested rate, i.e. 1.00 kg/ha (≥ 565 g MCPA/ha + 14.6 g tribenuron methyl/ha).
Mortality of the wasps exposed to Danadim 400 EC at the rate of 0.1 mL/ha was 78.6% after 48 hours. Therefore, the validity criterion specified in the Method description was met. The results showed that the test organisms were sensitive to dimethoate.
The fecundity assessment showed that the mean number of mummies per female in the control group was 18.1. As for the wasps treated with MCPA + Tribenuron metyl 565 SG at the rate of 1.00 kg/ha (565 g MCPA/ha + 14.6 g tribenuron methyl/ha), the number of mummies/female was 11.5.
Fecundity reduction (Pr) in the group treated with MCPA + Tribenuron metyl 565 SG at the rates of 1.00 kg/ha (565 g MCPA/ha + 14.6 g tribenuron methyl/ha) were 36.2%. At the significance level of 0.05, there were statistically significant differences in fecundity between the wasps exposed to the test item at the rate of 1.00 kg/ha (565 g MCPA/ha + 14.6 g tribenuron methyl/ha) and control group (Two-sample t-test Procedure, $p(t) < 0.05$).

The following validity criteria were met during the study:
– after 48 hours mortality of the control group was 0.0% (criterion: a maximum of 13.0%),

– after 48 hours the Abbott corrected mortality of the group treated with the reference item at the rate of 0.1 mL/ha was 78.6% (criterion: from 75 to 100%),
– the mean number of mummies per female in the control group was 18.1 (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).

Conclusion

On the basis of the obtained mortality results, the LR_{50} and the $NOER_{mortality}$ values could not be estimated. It can only be concluded that the LR_{50} value is higher than the highest tested rate, i.e. > 1.00 kg/ha (565 g MCPA/ha + 14.6 g tribenuron methyl/ha) and $NOER_{mortality}$ is equal to or higher than the highest tested rate, i.e. 1.00 kg/ha (≥ 565 g MCPA/ha + 14.6 g tribenuron methyl/ha).

A 2.3.2.2 Study 3

Comments of zRMS:	The study follows the guideline specified by Blümel et al. (2000) and according to the principles of GLP. No deviations to the guideline were noted. In the definitive test all the validity criteria were met Considering the current test guideline (Blümel et al., 2000) the study is considered valid.

Reference:	KCP 10.3.2/03
Report	An extended laboratory test for evaluating the effects of MCPA + Tribenuron metyl 565 SG on the predatory mite, Typhlodromus pyri (Sch.), STUDY CODE: B/39/18, Institute of Industrial Organic Chemistry Branch Pszczyna Department of Ecotoxicology Doświadczalna 27 43-200 Pszczyna, Poland
Guideline(s):	according to the ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Blümel S. *et al.*, 2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Summary

The aim of the extended laboratory test was to evaluate the effects of the test item, MCPA + Tribenuron metyl 565 SG on mortality and reproduction of the predatory mite, *T. pyri* (Sch.).
On the basis of the preliminary test results it was decided to use four rates of the test item in the definitive test. These were: 0.125, 0.25, 0.5 and 1.0 kg/ha. The mites, *T. pyri* at the protonymphal stage (24 hours old) were exposed to the test item applied to bean leaf discs. The mites were fed with pine pollen (*Pinus sp.*). Mortality observations were made after 7 days of the treatment. Observations of reproduction of the control group and groups treated with the test item at the rates of 0.125, 0.25, 0.5 and 1.0 kg/ha were made after 8, 11, and 14 days of the treatment.
Mortality of *T.pyri* after 7 days of the treatment and the reproduction reduction (Pr) after 14 days of the treatment were test endpoints.
To verify the sensitivity of the mites and the precision of the test procedure, an insecticide, Danadim 400 EC (400 g dimethoate/L) was used as a reference item. The rate of the reference item was 9.0 mL/ha (3.6 g a.i./ha). The control group was treated with distilled water.

Materials and methods:

Test item:	name: MCPA + Tribenuron metyl 565 SG content: 14.6 g/kg tribenuron metyl, 565.0 g/kg MCPA batch number: 4.5/16 production date: 14st November, 2016 expiry date: 14st November, 2018
Biological test system:	the predatory mite, *Typhlodromus pyri* (Sch.) (Acari: *Phytoseiidae*)
– age:	24-hour-old protonymphs
– source:	a laboratory culture at the Institute of Industrial Organic Chemistry, Branch Pszczyna; the culture was obtained from the Research Institute of Pomology and Floriculture, Skierniewice, Poland and renewed from commercial supplier, Katz Biotech
Experimental design:	– 6 study groups: – a control group (0.0 L/ha) – MCPA + Tribenuron metyl 565 SG at the rate of 0.125 kg/ha – MCPA + Tribenuron metyl 565 SG at the rate of 0.25 kg/ha – MCPA + Tribenuron metyl 565 SG at the rate of 0.5 kg/ha – MCPA + Tribenuron metyl 565 SG at the rate of 1.0 kg/ha – Danadim 400 EC at the rate of 9.0 mL/ha (3.6 g a.i./ha) number of replicates: 3; number of mites in each replicate: 20
Test conditions:	
– temperature:	24 – 27°C
– relative air humidity:	64 – 88%
– photoperiod:	16 h light (899 lux) : 8 h dark
Statistical analysis:	regression analysis using the log-probit method, Step-down Cochran-Armitage Test Procedure, Shapiro-Wilk's test on normal distribution, Levene's test on variance homogeneity, Williams Multiple Sequantial t-test Procedure
Endpoints:	– mite mortality after 7 days of the treatment – LR50 and NOERmortality – reproduction reduction (Pr) after 14 days of the treatment – ER50 and NOERreproduction

RESULTS

Mortality of *Typhlodromus pyri*

In the definitive test, mortality of the control group after 7 days of exposure was 1.7%. After 7 days of exposure to MCPA + Tribenuron metyl 565 SG at the rates of 0.125, 0.25, 0.5 and 1.0 kg/ha the percent-

ages of mortality of *T. pyri*, corrected using the formula of Abbott, were 1.7, 13.6, 11.9 and 15.3%, respectively.
On the basis of the obtained mortality results, the LR50 and the NOERmortality values were estimated. The LR50 value is higher than 1.0 kg/ha and the NOERmortality is 0.125 kg/ha.
At the significance level of 0.1, there were no statistically significant differences in mortality between the group treated with the test item at the rate of 0.125 kg/ha. However, there were statistically significant differences in mortality between the group treated with the test item at the rates of 0.25, 0.5 and 1.0 kg/ha (Step-down Cochran-Armitage Test Procedure (p(trend) > 0.1).
After 7 days of exposure to Danadim 400 EC at the rate of 9.0 mL/ha (3.6 g a.i./ha), mortality of the mites, corrected using the formula of Abbott, was 93.2%. Therefore, the validity criterion specified in the Method description was met. The results obtained in the reference item group showed that the test organisms were sensitive to dimethoate.

Table 1. Mortality of T. pyri after 7 days of exposure definitive test

Study group /rate [kg/ha]	Number of tested mites [no.]	Mortality					
		Number of dead mites [no.] Replicates			Total		Corrected[a]
		I	II	III	[no.]	[%]	[%]
Control / 0.0	60	0	0	1	1	1.7	–
MCPA + Tribenuron metyl 565 SG							
0.125	60	0	1	1	2	3.3	1.7
0.25	60	2	4	3	9	15.0	13.6+
0.5	60	1	4	3	8	13.3	11.9+
1.0	60	3	4	3	10	16.7	15.3+
LR_{50}		> 1.0 kg/ha					
$NOER_{mortality}$		0.125 kg/ha					
[mL/ha]	[g a.i./ha]	Danadim 400 EC					
9.0	3.6	60	18	20	18	56	93.3	93.2

The definitive test was performed between 29.06 – 13.07.2018
[a]: mortality corrected using the formula of Abbott [1]
+: statistically significant differences [SOP/B/67], [10]

Reproduction of *Typhlodromus pyri*
The mean reproduction rate (Rr) in the control group was 5.0 eggs/female. The mean reproduction rates after 14 days of exposure to MCPA + Tribenuron metyl 565 SG at the rates 0.125, 0.25, 0.5 and 1.0 kg/ha were 3.9, 3.1, 2.3 and 1.6 eggs/female, respectively. The percentages of reproduction reduction (Pr) caused by MCPA + Tribenuron metyl 565 SG at the rates of 0.125, 0.25, 0.5 and 1.0 kg/ha were 20.4, 38.1, 54.2 and 67.7% respectively.
At the significance level $\alpha \leq 0.1$, there were statistically significant differences in reproduction between the group treated with the test item at the rates of 0.125, 0.25, 0.5 and 1.0 kg/ha and the control group (Williams Multiple Sequantial t-test Procedure, $|t|>|t^*|$).
The ER_{50} value is 0.448 kg/ha and the $NOER_{reproduction}$ is lower than 0.125 kg/ha.

Table 2. Reproduction of T. pyri – definitive test

Study group [rate]	Replicates (X)	Developmental stages of the mites	Observation period DAT 8	DAT 11	DAT 14	RrX	Rr	Pr [%]
Control [0.0 kg/ha]	I	Eggs	3	32	24	5.2	5.0	-
		Larvae	0	1	3			
		Males	7	7	7			
		Females	13	12	11			
	II	Eggs	0	29	22	4.3		
		Larvae	0	4	1			
		Males	5	5	5			
		Females	13	13	13			
	III	Eggs	6	35	26	5.4		
		Larvae	0	2	0			
		Males	6	6	6			
		Females	13	13	12			
MCPA + Tribenuron metyl 565 SG [0.125 kg/ha]	I	Eggs	5	29	27	4.3	3.9	20.4+
		Larvae	0	0	1			
		Males	5	5	4			
		Females	15	14	14			
	II	Eggs	0	26	20	4.1		
		Larvae	0	2	2			
		Males	6	6	6			
		Females	13	12	12			
	III	Eggs	1	22	19	3.5		
		Larvae	0	3	1			
		Males	5	5	5			
		Females	14	13	13			
MCPA + Tribenuron metyl 565 SG [0.25 kg/ha]	I	Eggs	2	16	17	3.1	3.1	38.1+
		Larvae	0	0	0			
		Males	5	4	4			
		Females	12	11	11			
	II	Eggs	1	20	12	3.1		
		Larvae	0	0	1			
		Males	5	5	5			
		Female	11	11	11			
	III	Eggs	0	14	14	3.0		
		Larvae	0	1	3			
		Males	5	5	5			
		Females	12	10	10			
MCPA + Tribenuron metyl 565 SG [0.50 kg/ha]	I	Eggs	1	15	14	2.7	2.3	54.2+
		Larvae	0	0	0			
		Males	4	4	4			
		Females	14	11	9			
	II	Eggs	0	12	10	2.3		
		Larvae	0	1	1			
		Males	5	5	3			
		Females	11	10	10			
	III	Eggs	0	10	9	1.8		
		Larvae	0	0	0			
		Males	4	4	3			
		Females	13	10	9			
MCPA + Tribenuron metyl 565 SG [1.0 kg/ha]	I	Eggs	0	6	5	1.6	1.6	67.7+
		Larvae	0	0	2			
		Males	3	3	3			
		Females	9	8	8			
	II	Eggs	1	6	6	1.5		
		Larvae	0	1	0			
		Males	4	3	3			
		Females	11	10	6			
	III	Eggs	0	9	6	1.8		
		Larvae	0	0	4			
		Males	4	4	4			
		Females	12	12	6			
ER_{50}			0.448 kg/ha[a] (0.305 – 0.588)					
$NOER_{reproduction}$			< 0.125 kg/ha					

DAT: days after treatment
RrX: the reproduction rate for each replicate (X) of a given study group after 14 days, calculated according to equation no. 2, point 5.2
Rr: the mean reproduction rate in a given study group after 14 days
Pr: the percentage of reproduction reduction calculated according to equation no. 3, point 5.2
+: statistically significant difference [SOP/B/67], [10]
a: the ER_{50} value (with 95% confidence limits)

The following validity criteria were met during the study:
– mortality of the control group was 1.7% on day 7 of exposure (criterion: a maximum of 20%),
– corrected mortality of the mites exposed to the reference item at the rate of 9.0 mL/ha was 93.2% on day 7 of exposure (criterion: a minimum of 50%),
– the mean number of eggs per female in the control group was 5.0 (required: ≥ 4 eggs per female).

Conclusion

On the basis of the obtained results it can be concluded that MCPA + Tribenuron metyl 565 SG at the rates of 0.25, 0.5 and 1.0 kg/ha has adverse effect on mortality of the mites.
The test item at the rates of 0.125, 0.25, 0.5 and 1.0 kg/ha has adverse effect on reproduction of the mites.

The effects of MCPA + Tribenuron metyl 565 SG on mortality and reproduction of *Typhlodromus pyri* in the definitive test are summarized below.

Study group [application rate]	Parameter (endpoint)					
	Mortality			Reproduction		
Test item	Total		LR_{50}	Mean number of eggs/ female (Rr) [no.]	Repro-duction reduction Pr [%]	ER_{50}
[kg/ha]	[%]	Corrected [%][a]	[kg/ha]			[kg/ha]
Control (0.0)	1.7	–	–	5.0	–	–
0.125	3.3	1.7	> 1.0	3.9	20.4+	0.448[b] (0.305 – 0.588)
0.25	15.0	13.6+		3.1	38.1+	
0.5	13.3	11.9+		2.3	54.2+	
1.0	16.7	15.3+		1.6	67.7+	
$NOER_{mortality}$			0.125	$NOER_{reproduction}$		< 0.125
Reference item			–			
[mL/ha]	[g a.i./ha]					
9.0	3.6	93.3	93.2	–		

[a]: mortality corrected using the formula of Abbott
[b]: the ER_{50} value (with 95% confidence limits)
+: statistically significant differences

A 2.3.2.3 KCP 10.4 Effects on non-target soil meso- and macrofauna

A 2.3.3 KCP 10.4.1 Earthworms

A 2.3.3.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

Comments of zRMS:	The study was conducted to OECD guideline 222 and according to the principles of GLP. No deviation has been noted in the study. In the definitive test all the validity criteria were met. The study is reliable and suitable for the risk assessment. Overall, the study is considered acceptable with following endpoints: NOEC = 180 mg test item/kg dry weight of the artificial soil EC_{10} = 102.653 mg test item/kg dry weight of the artificial soil

Reference: KCP 10.4.1.1

Report	MCPA + TRIBENURON METYL 565 SG Earthworm Reproduction Test *(Eisenia andrei)*, Aneta Gierbuszewska, 2018, Study code G/158/17, Institute of Industrial Organic Chemistry Branch Pszczyna, Poland
Guideline(s):	Yes. According to the OECD Guideline for the Testing of Chemicals No. 222 (2016)
Deviations:	No deviations occurred.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

1. Test material: MCPA + TRIBENURON METYL 565 SG
   Batch number: 4.5/16
   Concentration of the MCPA: 565 g/kg, Concentration of the Tribenuron-methyl 14.6 g/kg

2. Test organism: adult (about 2 months old) the earthworms, *Eisenia andrei* (body weight between 290 – 459 mg) *obtained* from a standard laboratory culture cultivated at the Institute of Industrial Organic Chemistry Branch Pszczyna, Department of Ecotoxicology, Laboratory of Soil Toxicology

3. Test design: test duration: 8 weeks; number of replicates: 4 replicates/concentration + 8 replicates/control; number of earthworms: 10 earthworms/replicate

4. Concentrations of the test item: control, 18, 32, 56, 100, 180, 320, 560, and 1000 mg/kg dry soil

5. Composition of artificial soil: 10% sphagnum peat, 20% kaolin clay, 70% industrial sand

Test conditions:	temperature: 19 – 22°C; pH at the beginning of the experiment: 5.63 – 5.67; pH at the end of the experiment: 5.65 – 5.67; soil moisture content at the beginning of the experiment: 20.0 – 21.4% (41.3 – 44.2% of the maximum water holding capacity); soil moisture content at the end of the experiment: 20.0 – 21.9% (41.3 – 45.3% of the maximum water holding capacity); light-dark cycle: 16h : 8h; light intensity: 525 – 690 lux
Statistical analysis:	EC10, EC20, EC50 – the probit method NOEC (reproduction) – Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Williams Multiple Sequential t-test Procedure NOEC (survival) – Fisher's Exact Binomial Test with Bonferroni Correction
Endpoints:	EC10, EC20, EC50, NOEC

The aims of the study were to assess the impact of MCPA + TRIBENURON METYL 565 SG on reproduction of the earthworm, *Eisenia andrei* and to determine the EC10, EC20, EC50, and NOEC. The test item in the form of an aqueous solution was mixed with a suitable amount of the artificial soil. The concentrations of the test item were 18, 32, 56, 100, 180, 320, 560, and 1000 mg/kg dry soil. Each of them was divided into four replicates. There were also untreated control group (with deionized water and without test item) divided into eight replicates. The experiment lasted 8 weeks. After 4 weeks, all adult earthworms were removed from the test containers and observed. All changes in their behavior and morphology were recorded. The number of earthworms and their body weights were also determined. The impact

of the test item on reproduction was evaluated after an additional 4-week period on the basis of the number of juveniles hatched from cocoons during the experiment.

Results and discussions

On the basis of the results, it was concluded that after 4 weeks, at the control group there was mortality of adult earthworm noticed. It was equal to 1.3%. At concentrations ranging from 18 to 1000 mg/kg dry weight of artificial soil, after 4 weeks of exposure to the test item, mortality of the adult earthworms was ranging from 0.0 to 45.0%. However, mortality of one earthworm at the concentration equal to 18 mg/kg dry weight of the artificial soil and one earthworm at the concentration equal to 56 mg/kg dry weight of the artificial soil are not connected with the test item.

Concentration [mg/kg dry soil]	Replicate	Number of tested earthworms [no.]	Number of alive earthworms [no.]	Total mortality	
				no.	%
0	1	10	9	1	1.3
	2	10	10		
	3	10	10		
	4	10	10		
	5	10	10		
	6	10	10		
	7	10	10		
	8	10	10		
18	1	10	10	1	2.5
	2	10	10		
	3	10	9		
	4	10	10		
32	1	10	10	0	0.0
	2	10	10		
	3	10	10		
	4	10	10		
56	1	10	10	1	2.5
	2	10	9		
	3	10	10		
	4	10	10		
100	1	10	10	0	0.0
	2	10	10		
	3	10	10		
	4	10	10		
180	1	10	10	1	2.5
	2	10	9		
	3	10	10		
	4	10	10		
320	1	10	10	1	2.5
	2	10	9		
	3	10	10		
	4	10	10		
560	1	10	10	1	2.5
	2	10	9		
	3	10	10		
	4	10	10		
1000	1	10	4	18*	45.0
	2	10	7		
	3	10	3		
	4	10	8		

* - statistically significant difference (Fisher's Exact Binomial Test with Bonferroni Correction, $\alpha = 0.05$)

No changes in the appearance (morphology) and behaviour of the earthworms were noticed.

Concentration [mg/kg dry soil]	Replicate	Number of tested earthworms [no.]	Changes in behaviour and in morphology
0	1	10	9 nc 1 d
	2	10	10 nc
	3	10	10 nc
	4	10	10 nc
	5	10	10 nc
	6	10	10 nc
	7	10	10 nc
	8	10	10 nc
18	1	10	10 nc
	2	10	10 nc
	3	10	9 nc 1 d
	4	10	10 nc
32	1	10	10 nc
	2	10	10 nc
	3	10	10 nc
	4	10	10 nc
56	1	10	10 nc
	2	10	9 nc 1 d
	3	10	10 nc
	4	10	10 nc
100	1	10	10 nc
	2	10	10 nc
	3	10	10 nc
	4	10	10 nc
180	1	10	10 nc
	2	10	9 nc 1 d
	3	10	10 nc
	4	10	10 nc
320	1	10	10 nc
	2	10	9 nc 1 d
	3	10	10 nc
	4	10	10 nc
560	1	10	10 nc
	2	10	9 nc 1 d
	3	10	10 nc
	4	10	10 nc
1000	1	10	4 nc 6 d
	2	10	7 nc 3 d
	3	10	3 nc 7 d
	4	10	8 nc 2 d

nc – no changes;
d – dead

After the application of the test item at the concentrations ranging from 18 to 1000 mg/kg dry soil, the body weight increase was between 1.7 to 72.2%. As for the control group, it was equal to 51.9%.

Concentration [mg/kg dry soil]	Replicate	At the beginning of the experiment		After 4 weeks of the experiment		Body weight increase		Mean body weight increase	
		Number of earthworms [no.]	Mean weight of 1 earthworm [mg]	Number of earthworms [no.]	Mean weight of 1 earthworm [mg]	mg	%	mg	%
0	1	10	338	9	534	196	58.1	183.6	51.9
	2	10	432	10	617	185	42.8		
	3	10	366	10	577	211	57.7		
	4	10	346	10	503	157	45.4		
	5	10	383	10	524	141	36.8		
	6	10	332	10	534	202	60.8		
	7	10	327	10	510	183	56.0		
	8	10	335	10	528	193	57.6		
18	1	10	397	10	553	156	39.3	217.7	64.6
	2	10	301	10	580	279	92.7		
	3	10	351	9	628	277	78.9		
	4	10	333	10	492	159	47.7		
32	1	10	392	10	574	182	46.4	186.0	52.0
	2	10	400	10	580	180	45.0		
	3	10	385	10	565	180	46.8		
	4	10	290	10	492	202	69.7		
56	1	10	389	10	551	162	41.6	188.0	52.5
	2	10	383	9	619	236	61.6		
	3	10	358	10	501	143	39.9		
	4	10	315	10	526	211	67.0		
100	1	10	434	10	614	180	41.5	172.8	48.8
	2	10	408	10	552	144	35.3		
	3	10	393	10	440	47	12.0		
	4	10	301	10	621	320	106.3		
180	1	10	410	10	601	191	46.6	213.3	55.8
	2	10	389	9	671	282	72.5		
	3	10	385	10	572	187	48.6		
	4	10	348	10	541	193	55.5		
320	1	10	398	10	624	226	56.8	270.0	72.2
	2	10	413	9	789	376	91.0		
	3	10	344	10	579	235	68.3		
	4	10	335	10	578	243	72.5		
560	1	10	459	10	424	-35	-7.6	172.6	51.6
	2	10	350	9	583	233	66.7		
	3	10	330	10	611	281	85.2		
	4	10	339	10	550	211	62.2		
1000	1	10	386	4	393	7	1.7	0.8	1.7
	2	10	380	7	259	-121	-32.0		
	3	10	318	3	367	49	15.3		
	4	10	318	8	388	70	21.9		

After 8 weeks of the experiment, it was concluded that **MCPA + TRIBENURON METYL 565 SG** had an impact on reproduction of the earthworms in concentrations ranging from 320 to 1000 mg/kg dry weight of the artificial soil.

Concentration [mg/kg dry soil]	Replicate	Number of juveniles [no.]	Mean ±SD	Comparison to the control [%]	CV* [%]
0	1 2 3 4 5 6 7 8	37 37 34 39 45 31 47 51	**40.1 ± 6.9**	100.0	17.1
18	1 2 3 4	40 35 43 53	**42.8 ± 7.6**	106.5	17.8
32	1 2 3 4	36 32 38 45	**37.8 ± 5.4**	94.1	14.4
56	1 2 3 4	40 35 38 39	**38.0 ± 2.2**	94.7	5.7
100	1 2 3 4	40 36 37 35	**37.0 ± 2.2**	92.2	5.8
180	1 2 3 4	27 37 45 38	**36.8 ± 7.4**	91.6	20.2
320	1 2 3 4	35 27 14 32	**27.0** ± 9.3**	67.3	34.3
560	1 2 3 4	24 27 25 31	**26.8** ± 3.1**	66.7	11.6
1000	1 2 3 4	18 22 35 22	**24.3** ± 7.4**	60.4	30.6

* - coefficient of variation
** - statistically significant difference (Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Williams Multiple Sequential t-test Procedure, α = 0.05)

After 8 weeks of the experiment, the juveniles of earthworms did not exhibit any changes in appearance and behaviour.

ECx values for the number of offspring, survival of adult earthworms as well as the NOEC and LOEC values were calculated using the ToxRat Professional statistical program and are presented below:

Parameter	Value [mg of test item/kg dry weight of artificial soil]
EC_{10}	102.653 (27.702 – 178.700)
EC_{20}	254.906 (131.366 – 376.313)
EC_{50}	> 1000
NOEC	180
LOEC	320
LC_{50}	> 1000

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

- each replicate produced 40.1 juveniles (mean) at the end of the experiment - (criterion: ≥ 30 juveniles by the end of the experiment),
- the coefficient of variation of reproduction was 17.1% (criterion: ≤ 30%),
- adult mortality over the initial 4 weeks of the experiment was 1.3% (criterion: ≤ 10%).

Conclusion:
In the course of this experiment and the statistical calculations performed, it was shown that test material does not affect the survival and growth (expressed in mass change) of adults in 4 weeks of experiment. But it affects reproduction expressed in the number of young earthworms under the end of the study in the concentration range 320 to 1000 mg/kg dry weight of the artificial soil. The NOEC for reproduction was determined as 180 mg/kg dry weight of the artificial soil.

A 2.3.3.2 KCP 10.4.1.2 Earthworms - field studies

A 2.3.4 KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

A 2.3.4.1 KCP 10.4.2.1 Species level testing

Comments of zRMS:	The study was conducted to OECD guideline 232 and according to the principles of GLP. All the validity criteria were met. The study is reliable and suitable for the risk assessment.

Reference:	KCP 10.4.2.1/01
Report	MCPA + Tribenuron metyl 565 SG Collembolan *(Folsomia candida)* Reproduction Test, Agnieszka Stalmach, 2018, Study code G/135/18, Institute of Industrial Organic Chemistry, Branch Pszczyna
Guideline(s):	Yes. According to the OECD Guideline for the Testing of Chemicals No. 232 (2016)
Deviations:	Deviations from the OECD Guideline No. 232 (2016): 1. The end of the test the soil moisture content was determined by drying small sample of the artificial soil in 105°C instead of weighing the test vessels as it is mentioned in OECD Guideline No. 232 (2016). 2. Physiological or pathological symptoms or distinct changes in behavior were not described. The deviations did not affect the study results
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

1. Test material: MCPA + TRIBENURON METYL 565 SG
   Batch number: 4.5/16
   Concentration of the MCPA: 565 g/kg, Concentration of the Tribenuron-methyl 14.6 g/kg

2. Test organism: the collembolan, *Folsomia candida* obtained from a standard laboratory culture at the Institute of Industrial Organic Chemistry, Branch Pszczyna

3. Test design: test duration: 28 days; number of replicates: 4 replicates / concentration + 8 replicates / control; number of collembolans: 10 / replicate

4. Concentrations of the test item: control, 5,6; 10; 18; 32; 56; 100; 180; 320; 560 and 1000 mg/kg dry soil

5. Composition of artificial soil: 5% sphagnum peat (a particle size of 2 ± 1 mm), 20% kaolin clay, 75% air-dried industrial sand with more than 50% of the particles between 50 and 200 μm.

Test conditions:	temperature: 18.0 – 20.5°C; pH at the beginning of the test: 6.44 – 6.49; pH at the end of the test: 6.44 – 6.50; soil moisture content at the beginning of the test: 13.3 – 13.9% (43.81 – 45.96% of the maximum water holding capacity); soil moisture content at the end of the test: 12.6 – 14.3% (41.68 – 47.23% of the maximum water holding capacity); lighting: 16 h light and 8h dark; light intensity at the beginning of the experiment: 594 - 665 lux light intensity at the end of the experiment: 591 - 678 lux
Statistical analysis:	EC10, EC20, and EC50 – Weibull analysis using linear max, likelihood regression LC10, LC20, and LC50 - Weibull analysis using linear max, likelihood regression NOEC (number of juveniles): - Shapiro-Wilk's Test on Normal Distribution,

	- Bartlett's Test Procedure on Variance Homogeneity, - Welch-t, test for Inhomogeneous Variances with Bonferroni-Holm Adjustment NOEC (survival): - Fisher's Exact Binomial Test with Bonferroni Correction LOEC – a value suggested by the program
Endpoints:	EC10, EC20, EC50, NOEC, LOEC LC10, LC20, LC50, NOEC, LOEC

The aims of the study were to assess the impact of MCPA + TRIBENURON METYL 565 SG on reproduction of the collembolans, *Folsomia candida* and to determine the EC10, EC20, EC50, and NOEC. Ten concentrations of the test item were used. These were 5,6; 10; 18; 32; 56; 100; 180; 320; 560 and 1000 mg of the test item/kg of dry weight of the artificial soil. Each concentration was divided into four replicates. There was also an untreated control group divided into eight replicates. The test item in form of aqueous solution was mixed with the artificial soil. The control artificial soil was mixed with deionized water alone. The experiment lasted 28 days. After that, the collembolans were extracted from the artificial soil. The numbers of adults and juveniles were determined separately.

Results and discussions

Chemical analysis

The analytical measurements of the artificial soil treated with the test item at the highest concentration (1000 mg/kg dry weight of the artificial soil) were provided at the beginning, during (after 2 weeks) and at the end of the test. There were 3 additional vessels provided for analysed concentration and control group.

Table 3. MCPA + TRIBENURON METHYL 565 SG – Results of the determination of MCPA in the test samples

Date of analysis	Concentration of MCPA [mg/kg]	Concentration determined in particular replicates mg/kg dry weight			Average [mg/kg]	SD [mg/kg]	RSD [%]	Recovery [%]
		1	2	3				
04.07.2018	0.00	ND	ND	ND	ND	0.00	-	-
Day 0	565.00	608.51	585.18	587.22	593.64	11.36	1.9	105.1
18.07.2018	0.00	ND	ND	ND	ND	0.00	-	-
Day 14	565.00	622.78	599.45	621.84	614.69	11.59	1.9	108.8
01.08.2018	0.00	ND	ND	ND	ND	0.00	-	-
Day 28	565.00	450.15	427.18	492.61	456.65	29.24	6.4	80.8

ND – not detected

Table 4. MCPA + TRIBENURON METHYL 565 SG – Results of the determination of tribenuron methyl in the test samples

Date of analysis	Concentration of tribenuron methyl [mg/kg]	Concentration determined in particular replicates mg/kg dry weight			Average [mg/kg]	SD [mg/kg]	RSD [%]	Recovery [%]
		1	2	3				
04.07.2018	0.00	ND	ND	ND	ND	0.00	-	-
Day 0	14.60	12.705	11.759	12.263	12.243	0.416	3.4	83.9
18.07.2018	0.00	ND	ND	ND	ND	0.00	-	-
Day 14	14.60	6.906	6.899	6.578	6.794	0.165	2.4	46.5
01.08.2018	0.00	ND	ND	ND	ND	0.00	-	-
Day 28	14.60	3.732	3.856	3.817	3.802	0.056	1.5	26.0

ND – not detected

Mortality at the concentrations ranging from 5.6 to 1000 mg/kg dry weight of the artificial soil ranged from 7.5 to 67.5%. As for the control group, it was equal to 16.3%.

Table 5. 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	8 7 7 10 8 7 10 10	13	16.3
5.6	1 2 3 4	10 10 10 10	6 10 10 6	8	20.0
10	1 2 3 4	10 10 10 10	9 7 5 10	9	22.5
18	1 2 3 4	10 10 10 10	7 7 10 10	6	15.0
32	1 2 3 4	10 10 10 10	10 9 7 10	4	10.0
56	1 2 3 4	10 10 10 10	9 7 9 10	5	12.5
100	1 2 3 4	10 10 10 10	10 9 10 8	3	7.5
180	1 2 3 4	10 10 10 10	6 9 9 10	6	15.0
320	1 2 3 4	10 10 10 10	5 10 5 6	14	35.0
560	1 2 3 4	10 10 10 10	5 5 4 8	18*	45.0
1000	1 2 3 4	10 10 10 10	2 2 3 6	27*	67.5

* - statistically significant difference between the control and the treatment group (Fisher's Exact Binomial Test with Bonferroni Correction , significance level = 0.05, one-sided smaller)

The concentration of the test item causing a 50% mortality of adults within the exposure period (LC_{50}) is equal 910.3 mg/kg dry weight of the artificial soil (514.3 mg MCPA/ kg dry weight of the artificial soil and 13.3 mg tribenuron methyl/ kg dry weight of the artificial soil). The endpoint values showing the impact of the test item on the survival of adult collembolans are presented in the table given below.

Endpoint values - the impact of the test item on the mortality of adult collembolans *(Folsomia candida)*.

Endpoint	Value [mg test item /kg dry weight of the artificial soil]	Value [mg a.s./kg dry weight of the artificial soil] MCPA	Value [mg a.s./kg dry weight of the artificial soil] tribenuron methyl
LC_{10}	**156.8** (0.0 – 386.0)	**88.6** (0.0 – 218.1)	**2.3** (0.0 – 5.6)
LC_{20}	**316.0** (0.1 – >1000.0)	**178.5** (0.1 – 569.3)	**4.6** (0.0 – 14.7)
LC_{50}	**910.3** (366.1 – >1000)	**514.3** (206.8 – >1000.0)	**13.3** (5.3 – >1000.0)
NOEC	**320.0**	**180.8**	**4.7**
LOEC	**560.0**	**316.4**	**8.2**

After the exposure of collembolans to the test item at the concentrations ranging from 5.6 to 1000 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 7.3 – 813.8 per replicate. As for the control group, the number of juveniles was equal to 861.3 per replicate.

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

Concentration [mg/kg dry weight of the artificial soil]	Replicate	Number of juveniles	Mean ±SD	Comparison to the control [%]	CV* [%]
0 (control)	1 2 3 4 5 6 7 8	745 721 984 784 783 853 968 1052	861.3 ± 124.32	**100**	14.4
5.6	1 2 3 4	610 716 1072 857	813.8 ± 199.69	**94.5**	24.5
10	1 2 3 4	769 646 869 959	810.8 ± 134.48	**94.1**	16.6
18	1 2 3 4	774 761 686 839	765.0 ± 62.75	**88.8**	8.2
32	1 2 3 4	871 719 439 1081	777.5 ± 270.10	**90.3**	34.7
56	1 2 3 4	283 453 535 564	458.8+ ± 126.24	**53.3**	27.5
100	1 2 3 4	562 464 504 525	513.8+ ± 40.93	**59.7**	8.0
180	1 2 3 4	212 307 176 404	274.8+ ± 102.36	**31.9**	37.3
320	1 2 3 4	174 247 107 32	140.0+ ± 91.94	**16.3**	65.7
560	1 2 3 4	29 26 0 42	24.3+ ± 17.59	**2.8**	72.6
1000	1 2 3 4	0 8 11 10	7.3+ ± 4.99	**0.8**	68.9

* CV – coefficient of variation

\+ - statistically significant difference between the control and the treatment group (Welch-t test for Inhomogeneous Variances with Bonferroni-Holm Adjustment, significance level = 0.05, one-sided smaller)

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 a.s./kg dry weight of the artificial soil] MCPA	Value [mg a.s./kg dry weight of the artificial soil] tribenuron methyl
EC_{10}	**14.5** (5.5 – 25.1)	**8.2** (3.1 – 14.2)	**0.2** (0.1 – 0.4)
EC_{20}	**32.5** (16.8 – 47.7)	**18.4** (9.5 – 27.0)	**0.5** (0.2 – 0.7)
EC_{50}	**109.0** (82.1 – 141.6)	**61.6** (46.4 – 80.0)	**1.6** (1.2 – 2.1)
NOEC	**32.0**	**18.1**	**0.5**
LOEC	**56.0**	**31.6**	**0.8**

The results are considered valid because the following criteria were satisfied in the controls:
- mean adult mortality: 16.3% (criterion: ≤ 20%),
- the mean number of juveniles per vessel at the end of the test: 861.34 (criterion: ≥ 100 juveniles at the end of the test),
- the coefficient of variation calculated for the number of juveniles: 14.4 (criterion: ≤ 30%).

Conclusion:

After the application of the test item at the concentrations ranging from 5.6 to 1000 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 7.3 – 813.8 per replicate. As for the control group, the number of juveniles was equal to 861.3 per replicate. The highest concentration at which the test item is observed to have no statistically significant effects on collembolan reproduction (NOEC) is equal to 32 mg/kg dry weight of the artificial soil.

Comments of zRMS:	The study was conducted to OECD guideline 226 and according to the principles of GLP. All the validity criteria were met. The study is reliable and suitable for the risk assessment.
Reference:	KCP 10.4.2.1/02
Report	MCPA + Tribenuron metyl 565 SG, Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil, Agnieszka Stalmach, 2018, Study code G/136/18, Institute of Industrial Organic Chemistry, Branch Pszczyna
Guideline(s):	Yes. According to the OECD Guideline for the Testing of Chemicals No. 226 (2016)
Deviations:	Deviations from the OECD Guideline No. 226 (2016): 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 re-watering the vessels periodically. In the study to maintain proper moisture content, a small sample of soil was drying at 105°C and re-weighing at the beginning, and after 7 days of the test. 2. Due to the use of the temperature extraction method, there was no need for euthanasia of the extracted organisms since the mites are fixed in a 70% ethanol solution.

	3. Due to the use of the temperature extraction method, there was no impossible to record the symptoms with behavioral and morphology changes of the extracted predatory mites. The deviations did not affect the study results
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Summary

The aims of the study were to assess the impact of MCPA + TRIBENURON METYL 565 SG on reproduction of the predatory mite, *Hypoaspis (Geolaelaps) aculeifer* and to determine the EC_{10}, EC_{20}, EC_{50}, LOEC and NOEC. Ten concentrations of the test item were used. These included: 5,6; 10; 18; 32; 56; 100; 180; 320; 560 and 1000 mg/kg dry weight of the artificial soil. Each concentration was divided into four replicates. There was also an untreated control group divided into eight replicates. The test item in the form of aqueous solution was mixed with the artificial soil. The control artificial soil was mixed with deionized water alone. The experiment lasted 14 days. After that, the mites were extracted from the artificial soil (48-hour extraction). The numbers of adults and juveniles were determined separately.

Materials and methods

1. Test material: MCPA + TRIBENURON METYL 565 SG
   Batch number: 4.5/16
   Concentration of the MCPA: 565 g/kg, Concentration of the Tribenuron-methyl 14.6 g/kg

2. Test organism: the predatory mites, *Hypoaspis (Geolaelaps) aculeifer* (adult female mites from a synchronized cohort) obtained from a standard laboratory culture at the Institute of Industrial Organic Chemistry, Branch Pszczyna. The mites were introduced 7-14 days after becoming adult.

3. Test design: test duration: 14 days; number of replicates: 4 replicates / concentration + 8 replicates / control; number of mites: 10 mites / replicate

4. Concentrations of the test item: control, 5,6; 10; 18; 32; 56; 100; 180; 320; 560 and 1000 mg/kg dry soil

5. Composition of artificial soil: 5% sphagnum peat, 20% kaolin clay, and 75% air-dried industrial sand

Test conditions:	temperature: 18.0 – 20.5°C pH at the beginning of the test: 6.33 – 6.40 pH at the end of the test: 6.40 – 6.49 soil moisture content at the beginning of the test: 13.0 – 13.9% (42.9 – 46.1% of the maximum water holding capacity) soil moisture content at the end of the test: 13.0 – 14.2% (43.1 – 46.9% of the maximum water holding capacity) light-dark cycle: 16 h light and 8 h dark light intensity at the beginning of the test: 569 – 672 lux light intensity at end of the test: 632 - 684 lux
Statistical analysis:	EC10, EC20, EC50 – a probit analysis LC10, LC20, LC50 – a probit analysis NOEC - Shapiro-Wilk's Test on Normal Distribution, Bartlett's Test Procedure

	on Variance Homogeneity, Williams Multiple Sequential t-test Procedure (number of juveniles) and Fisher's Exact Binomial Test with Bonferroni Correction (survival)
Endpoints:	EC10, EC20, EC50, NOEC, LOEC LC10, LC20, LC50, NOEC, LOEC

Results and discussions

Chemical analysis

The analytical measurements of the artificial soil treated with the test item at the highest concentration (1000 mg/kg dry weight of the artificial soil) was provided at the beginning, during (after 7 days) and at the end of the test. The control and the treated group (1000 mg/kg dry weight of the artificial soil) for chemical analysis were divided into 2 replicates, therefore there were 6 additional containers for analysis concentration and control group.

Table 3. MCPA + TRIBENURON METHYL 565 SG – Results of the determination of MCPA in the test samples

Date of analysis	Concentration of MCPA [mg/kg]	Concentration determined in particular replicates mg/kg dry weight			Average [mg/kg]	SD [mg/kg]	RSD [%]	Recovery [%]
		1	*2*	*3*				
04.07.2018	0.00	ND	ND	ND	ND	0.00	-	-
Day 0	565.00	564.19	570.48	618.83	584.50	26.40	4.5	103.5
11.07.2018	0.00	ND	ND	ND	ND	0.00	-	-
Day 7	565.00	558.14	611.90	573.02	581.02	24.45	4.2	102.8
18.07.2018	0.00	ND	ND	ND	ND	0.00	-	-
Day 14	565.00	443.627	434.885	390.134	422.882	25.194	6.0	74.8

ND – not detected

Table 4. MCPA + TRIBENURON METHYL 565 SG – Results of the determination of tribenuron methyl in the test samples

Date of analysis	Concentration of tribenuron methyl [mg/kg]	Concentration determined in particular replicates mg/kg dry weight			Average [mg/kg]	SD [mg/kg]	RSD [%]	Recovery [%]
		1	*2*	*3*				
04.07.2018	0.00	ND	ND	ND	ND	0.00	-	-
Day 0	14.60	13.713	13.317	13.744	13.592	0.210	1.5	93.1
11.07.2018	0.00	ND	ND	ND	ND	0.00	-	-
Day 7	14.60	5.580	5.243	4.540	5.121	0.468	9.1	35.1
18.07.2018	0.00	ND	ND	ND	ND	0.00	-	-
Day 14	14.60	4.547	3.729	3.426	3.901	0.509	13.0	26.7

ND – not detected

Mortality of the predatory mites exposed to the test item at the concentrations ranging from 5.6 to 1000 mg/kg dry weight of the artificial soil was between 0.0% and 7.5%. Mortality of the control group was equal to 5.0%. After the application of the test item at the concentrations ranging from 5.6 to 1000 mg/kg dry weight of the artificial soil the mean number of juveniles was between 68.5 – 76.0 per replicate. The mean number of juveniles in the control group was equal to 71.8 per replicate.

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

Concentration [mg/kg dry weight of soil]	Replicate	Number of juvenile mites	Mean ±SD	Comparison to the control [%]	CV [%]
control	1 2 3 4 5 6 7 8	81 64 89 59 71 76 61 73	71.8 ± 10.3	100.0	14.3
5.6	1 2 3 4	72 70 89 68	74.8 ± 9.6	104.2	12.9
10	1 2 3 4	67 72 69 66	68.5 ± 2.6	95.5	3.9
18	1 2 3 4	71 65 88 61	71.3 ±11.9	99.3	16.7
32	1 2 3 4	68 79 75 82	76.0 ± 6.1	105.9	8.0
56	1 2 3 4	73 72 70 70	71.3 ± 1.5	99.3	2.1
100	1 2 3 4	66 69 80 78	73.3 ± 6.8	102.1	9.3
180	1 2 3 4	73 63 66 87	72.3 ± 10.7	100.7	14.8
320	1 2 3 4	61 93 60 61	68.8 ± 16.2	95.8	23.5
560	1 2 3 4	68 78 64 83	73.3 ± 8.8	102.1	12.0
1000	1 2 3 4	63 82 63 67	68.8 ± 9.0	95.8	13.1

The results are summarized in the table given below.

Concentration [mg/kg dry weight of the artificial soil]	Adult mites		Number of juveniles (mean)
	Number of tested mites	Number of dead mites after 14 days	
Control	80	4	71.8
5.6	40	0	74.8
10	40	2	68.5
18	40	3	71.3
32	40	1	76.0
56	40	2	71.3
100	40	3	73.3
180	40	2	72.3
320	40	2	68.8
560	40	3	73.3
1000	40	1	68.8

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

Endpoint	Value [mg/kg dry weight of the artificial soil]	Value [mg MCPA /kg dry weight of the artificial soil]	Value [mg tribenuron methyl /kg dry weight of the artificial soil]
EC_{10}	>1000	>565	>14.6
EC_{20}	>1000	>565	>14.6
EC_{50}	>1000	>565	>14.6
NOEC	≥1000	≥565	≥14.6
LOEC	>1000	>565	>14.6
LC_{10}	>1000	>565	>14.6
LC_{20}	>1000	>565	>14.6
LC_{50}	>1000	>565	>14.6
NOEC	≥1000	≥565	≥14.6
LOEC	>1000	>565	>14.6

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

- mean adult mortality: 5% (criterion: ≤ 20%);
- the mean number of juveniles per vessel at the end of the test: 71.8 (criterion: ≥ 50 juveniles at the end of the test);
- the coefficient of variation for the number of juveniles: 14.3 (criterion: ≤ 30%).

Conclusion:

Obtained results show no effect of the test item on reproduction of predatory mites (*Hypoaspis aculeifer*) at tested concentration. EC_{10}, EC_{20}, EC_{50}, LOEC and NOEC are beyond the range of maximal concentration used in the study.

A 2.3.4.2 KCP 10.4.2.2 Higher tier testing

Not needed.

A 2.4 KCP 10.5 Effects on soil nitrogen transformation

Comments of zRMS:	The study was conducted to OECD guideline 216 and according to the principles of GLP. In the definitive test all the validity criteria were met as follows:

	The coefficients of variation (CV) in the control were: 3.03; 5.49; 0.32; 1.68; 0.16; 1.94 % The validity criterion was met, because the variation between replicate control samples is less than ± 15%. The soil nitrate formation rates were below the 25% trigger value given by the OECD 216 guideline. On the basis of the results, it was concluded that HAKSAR TOP 565 at the concentrations of 2.70 mg formulation/kg dry weight of the soil and 13.50 mg formulation/kg dry weight of the soil, did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.
Reference:	KCP 10.5
Report	Study of impact on soil microorganisms - nitrogen transformation test according to Guideline OECD 216. Agnieszka Woźniak, Study code: 0016/0103/E, SORBOLAB Research Laboratory LLC
Guideline(s):	Yes. According to the OECD Guideline for the Testing of Chemicals No. 216 (2000) / EU Method C.21.
Deviations:	Deviations from the OECD Guideline No. 2126 (2000): 1. On day 7, for 5 PEC, repeat 3, maximum water capacity was slightly below than recommended (40-60% maximum WHC requirement). According to the literature data (Roberts et al. 2010) the recommended % of the maximum water holding capacity is lower and amounts to 20-50%. During the experiment the % of the maximum WHC was kept at the lower limit i.e. approx. 40% of maximum WHC. 2. Nitrate content of the control decreased on day 7, which is related to the stabilization of the experimental conditions in the container after the addition of water. In the following days an increase in the content of nitrates was observed. The above deviations did not affect the results of the study. All validity criteria were met.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

1. Test material: MT-565 SG-OR2-C
   Batch number: 01/2020
   Concentration of MCPA: 527 g/kg; tribenuron methyl: 15.7 g/kg

2. Soil: Agricultural soil collected from Fraunhofer Institute for Molecular Biology and Applied Ecology in Germany.

3. Test design:

Test duration: 56 days.
In the test, following test item concentrations were used:

- control (without test item additive)

- 1PEC: 2.70 mg of test item/kg dry weight of soil

- 5PEC: 5-times the maximal single dose recommended by the producer, i.e 13.50 mg test item/kg dry weight of soil.

Each concentration and control was prepared in three replicates. The soil was enriched with the organic substrate. i.e. lucerne at dose of 5 g/kg dry weight of soil.

Study conditions	on days 0-28 - average temperature 20.281°C (minimal temperature 20.10°C, maximal temperature 20.50°C) – average humidity: 63.756% (minimal humidity 46.20%, maximal humidity 74.30%) – photoperiod: 24 h darkness on days 0-56 - average temperature 20.269°C (minimal temperature 20.10°C, maximal temperature 20.50°C) – average humidity: 62.396% (minimal humidity 46.10%, maximal humidity 74.30%) – photoperiod: 24 h darkness
Substrate	soil obtained from agricultural areas, not ploughed for the last six months, which have not been treated with plant protection products for a minimum of one year before being used for testing, nor have they been fertilized with any fertilizer for at least six months

A study of the test item MT-565 SG-OR2-C effects on the activity of soil microorganisms responsible for nitrogen transformations occurring in aerobic soils in accordance to the guideline OECD 216. The study consisted of comparing the rate of nitrate production in the soil exposed to the test item with the rate of nitrate production in the control soil. The study was conducted in compliance with GLP.

Results and discussions

The percent deviation from the control calculated on the basis of the nitrate formation rate of the soil treated with the test item at both concentrations (1 PEC and 5 PEC) did not exceed 25% after 56 day of the analysis.

The results were statistically analyzed using ToxRat Professional. The Shapiro-Wilk test was used to check the normality of the distribution, Levene's test to check the homogeneity of variance and the student's t-test (statistical significance at the level of 5%).

The tested item at the concentration of 1PEC and 5PEC (2.70 mg of the test item/kg of dry weight of soil and 13.50 mg of the test item/kg of dry weight of soil, respectively) did not statistically significantly affect the production rate of nitrates after 56 days of the test.

The results of the test are presented in Table 1 and Table 2.

Table 1. Nitrate production rate calculated by comparison to the previous determination

Calculated using the ToxRat Professional statistical program							
Time of observation	Control	1PEC*)			5PEC**)		
	Average rate of nitrate production [mg of nitrate/kg dry weight of soil/day]	Average rate of nitrate production [mg of nitrate/kg dry weight of soil/day]	Stimulation in relation to control [%]	Statistical significance***)	Average rate of nitrate production [mg of nitrate/kg dry weight of soil/day]	Stimulation in relation to control [%]	Statistical significance***)
after 7 days	-1.220	0.790	164.7	-	3.972	425.7	-
after 14 days	3.635	2.841	-21.8	+	6.072	67.0	-
after 28 days	4.453	4.349	-2.3	-	3.995	-10.3	+
after 42 days	3.815	3.583	-6.1	-	2.888	-24.3	+
after 56 days	3.415	3.509	2.8	-	3.548	3.9	-

*) (Predicted Environmental Concentration): maximum predicted effective concentration in soil (2.70 mg of test item/kg dry weight of soil
**) (Predicted Environmental Concentration): 5 times the maximum expected effective concentration in soil (13.50 mg of test item/kg dry weight of soil)
***) significance calculated by ToxRat Professional using the Student's t test at the significance level of p≤0.05
- statistically insignificant
+ statistically significant

Table 2. Nitrate production rate calculated by comparison to day 0

Calculated using the ToxRat Professional statistical program							
Time of observation	Control	1PEC*)			5PEC**)		
	Average rate of nitrate production [mg of nitrate/kg dry weight of soil/day]	Average rate of nitrate production [mg of nitrate/kg dry weight of soil/day]	Stimulation in relation to control [%]	Statistical significance***)	Average rate of nitrate production [mg of nitrate/kg dry weight of soil/day]	Stimulation in relation to control [%]	Statistical significance***)
after 7 days	-1.220	0.790	164.7	-	3.972	425.7	-
after 14 days	1.208	1.816	50.3	-	5.022	315.8	-
after 28 days	2.830	3.082	8.9	-	4.508	59.3	-
after 42 days	3.158	3.249	2.9	-	3.968	25.6	-
after 56 days	3.222	3.314	2.8	-	3.863	19.9	-

*) (Predicted Environmental Concentration): maximum predicted effective concentration in soil (2.70 mg of test item/kg dry weight of soil
**) (Predicted Environmental Concentration): 5 times the maximum expected effective concentration in soil (13.50 mg of test item/kg dry weight of soil)
***) significance calculated by ToxRat Professional using the Student's t test at the significance level of p≤0.05
- statistically insignificant
+ statistically significant

Validity criteria:

The validity criteria were met in accordance with Guideline OECD 216. The coefficients of variance for the nitrate content in the individual replicate samples in the control were as follows: 3.03; 5.49; 0.32; 1.68; 0.16; 1.94 % with the maximum of 5.49% and thus, below the acceptable level of 15%.

Conclusion:
As regards to the obtained results, it was concluded that MT-565 SG-OR2-C (HAKSAR TOP 565 SG) at the concentrations corresponding to the 2.70 mg /kg of soil and 13.50 mg /kg of soil can be perceived as having no long-term influence on nitrogen transformations in soil.

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

A 2.5.1 KCP 10.6.1 Summary of screening data

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A 2.5.2 KCP 10.6.2 Testing on non-target plants

Comments of zRMS:	The study on the Effects on the seedling emergence and growth of ten non-target terrestrial plant species was performed in line with requirements of OECD 208 and according to the principles of GLP. All the validity criteria were fulfilled according to OECD 208 Guideline:

Reference: KCP 10.6.2/01

Report	MCPA + TRIBENURON METYL 565 SG Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test, Aneta Gierbuszewska, 2018, Study code G/160/17, Institute of Industrial Organic Chemistry Branch Pszczyna, Poland
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:	• According to OECD Guideline No. 208 (2006), the light intensity should be 350 ± 50µE/m2/s. However, these values are recommended for tests conducted in greenhouses. The experiment was conducted in a test room, where only artificial lighting was used. The light intensity was between 110.5 – 131.4 µE/m2/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing. • The number of seeds per pot is a deviation from the OECD Guideline No. 208. Nevertheless, in the course of statistical evaluation of the results the increased number of seeds per replicate significantly decreased the variability between replicates. Therefore, this deviation from the guideline recommendation ensures an optimal and more reliable statistical analysis with a high discriminatory power.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

SUMMARY
The aimed at evaluating the effect of **MCPA + TRIBENURON METYL 565 SG** on seedling emergence.
and seedling growth of 10 terrestrial plants was conducted on 6 dicotyledonous and 4 monocotyledonous test plants. The test item was sprayed onto the soil surface. For cabbage, pea, tomato, perennial ryegrass, onion, bean, wheat and oats, seven application rates were used. There was also a concurrent control group. For sunflower, nine application rates were used. There was also a concurrent control group. Each application rate and the control group were divided into four replicates. The experiment was conducted in a special room. Suitable environmental conditions for each test species were provided. During the experiment, the plants were observed for emergence (every day and then every 2 – 3 days) and visual phytotoxicity (after 7 and 14 days). The experiment finished 14 days after the emergence of 50% of the control seedlings. At the end of the experiment, the number of surviving plants was determined. Next, the plants were cut down, measured, dried to a constant weight at 60ºC, and weighed. The results concerning the emergence, the shoot length, and the dry weight were statistically analyzed in order to determine the ER_{25}, ER_{50}, NOER.

Materials and methods

1. Test material: MCPA + TRIBENURON METYL 565 SG
   Batch number: 4.5/16
   Concentration of the MCPA: 565 g/kg, Concentration of the Tribenuron-methyl 14.6 g/kg

2. Test organism: Ten plant species were used. These were carrot *(Daucus carota)*, sunflower *(Helianthus annuus)*, cabbage *(Brassica olerace var. capitata)*, pea *(Pisum sativum)*, bean *(Phaseolus vulgaris)*, tomato *(Solanum lycopersicon)*, onion *(Allium cepa)*, perennial ryegrass *(Lolium perenne)*, oats *(Avena sativa)*, wheat *(Triticum aestivum)*.

3. Test design:
Seven or nine application rates of the test item were used in the experiment. They were determined on the basis of the non-GLP range – finding test results. In cultivation of cabbage, pea, tomato, perennial ryegrass, onion, bean, carrot, wheat, and oats seven application rates were used i.e. 1.37, 4.12, 12.35, 37.04, 111.10, 333.30, and 1000.00 g of test item/ha.
In cultivation of sunflower nine application rates were used i.e. 0.15, 0.46, 1.37, 4.12, 12.35, 37.04, 111.10, 333.30, and 1000.00 g of test item/ha. A separation factor was 3.0. The volume of deionized water used to prepare the test item at the highest rate corresponded to 300 L/ha. One untreated control group was used for each species. Four replicates were used, both for control and treated groups. The test system was the same for all test species. There were 5 seeds in each pot. A pot was defined as the replicate. There were four replicates/rate. The total number of seeds/rates was twenty.

The concentration of MCPA and tribenuron methyl in water was determined with a validated analytical method. The test item was sprayed onto the soil using a suitable spraying chamber. The experiment finished 14 days after the emergence of 50% of the control seedlings. During the experiment, the plants were observed for emergence (every day and then every 2 – 3 days) and visual phytotoxicity (7 and 14 days after the emergence of 50% of the control seedlings). At the end of the experiment, the plants were counted, cut down, measured, dried to a constant weight at 60°C, and weighed.

Soil: sandy loam

Test conditions: temperature: 14.1 – 30.7°C; humidity: 45.0 – 89.4%; lighting: 16 h light : 8 h dark; light intensity: 110.5 – 131.4 μE/m2/s; carbon dioxide concentration:
335 – 357 ppm

Statistical analysis: ER25, ER50 – probit analyses
NOER:
- emergence - Fisher's Exact Binomial Test with Bonferroni Correction, Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Williams Multiple Sequential t-test Procedure.
- post-emergence - Fisher's Exact Binomial Test with Bonferroni Correction.
- shoot length - Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Williams Multiple Sequential t-test Procedure or Welch-t test for Inhomogeneous Variances with Bonferroni-Holm Adjustment.
- shoot weight - Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals) or Bartlett's Test Procedure on Variance Homogeneity, Williams Multiple Sequential t-test Procedure or Welch-t test for Inhomogeneous Variances with Bonferroni-Holm Adjustment.

Results and discussions:

The test item i.e. MCPA + TRIBENURON METHYL 565 SG had no impact on the growth and seedling emergence of bean. The test item slightly impacted the growth of oats and the test item impacted of the growth of carrot, sunflower, cabbage, pea, tomato, onion, perennial ryegrass, and wheat.
After the application of the test item at the rates ranging from 0.15 to 1000.00 g/ha test plant species such as sunflower emerged. After the application of the test item at the rates ranging from 1.37 to 1000.00 g/ha test plant species such as cabbage, bean, tomato, pea, onion, perennial ryegrass, oats, and wheat also emerged. After the application of the test item at the rates from 1.37 to 333.30 g/ha test plant species such as carrot also emerged. Only seeds of carrot and onion did not emerge on the top application rate equal to 1000 g/ha.

The death of five pea plants were noticed at application rates equal to 1000 g/ha.

The following phytotoxic symptoms were observed;

- stunted growth - carrot, sunflower, cabbage, pea, bean, tomato, onion, perennial ryegrass, oats, and wheat;
- deformations - sunflower, and pea;
- death of plants - carrot, and pea.

On the basis of NOER, ER25 and ER50 values determined from the shoot length, it was proved that the test item did not inhibit the process of growth of bean and oats. The shoot length was inhibited for carrot, sunflower, cabbage, pea, tomato, onion, perennial ryegrass and wheat.
On the basis of NOER, ER25 and ER50 values determined from the shoot dry weight, it was proved that the test item inhibited the process of growth of all tested plant species.

The EC50 and NOER values determined on the basis of plant number, shoot length and dry shoot weight measurements expressed as g of the test item / ha for all test species are given below.

	Carrot *Daucus carota*	Sunflower *Helianthus annuus*	Cabbage *Brassica oleracea var. capitata*	Pea *Pisum sativum*	Bean *Phaseolus vulgaris*	Tomato *Solanum lycopersicon*
Plant number at the end of the experiment						
ER_{50}	82.1 (15.3 - 996.5)	> 1000.0	192.3 (102.2 - 452.0)	627.9 (472.6 - 858.6)	> 1000.0	> 1000.0
NOER	12.4	333.3	37.0	333.3	≥ 1000.0	111.1
Shoot length (plants without roots)						
ER_{50}	26.7 (16.4 - 42.4)	642.3 (539.9 - 766.7)	234.6 (144.3 - 421.1)	575.2 (257.9 - > 1000.0)	> 1000.0	969.6 (635.4 - > 1000.0)
NOER	4.1	37.0	37.0	333.3	≥ 1000.0	37.0
Plant weight (plants without roots)						
ER_{50}	96.5 (38.9 - 485.5)	> 1000.0	49.3 (22.0 - 113.9)	163.2 (116.3 - 233.7)	> 1000.0	435.4 (321.9 - 627.1)
NOER	4.1	111.1	1.4	12.4	111.1	37.0

	Onion *Allium cepa*	Perennial ryegrass *Lolium perenne*	Oats *Avena sativa*	Wheat *Triticum aestivum*
Plant number at the end of the experiment				
ER_{50}	122.8	> 1000.0	> 1000.0	> 1000.0
NOER	37.0	≥ 1000.0	≥ 1000.0	≥ 1000.0
Shoot length (plants without roots)				
ER_{50}	54.9 (43.8 - 68.8)	> 1000.0	> 1000.0	> 1000.0
NOER	12.4	111.1	≥ 1000.0	111.1
Plant weight (plants without roots)				
ER_{50}	528.8 (411.0 - 715.3)	671.6 (619.9 - 725.5)	> 1000.0	516.8 (454.6 - 592.1)
NOER	37.0	111.1	111.1	111.1

Validity of the test:

- Seedling emergence in the control was at least 70% as follows:

90% - carrot,
100% - sunflower,
100% - cabbage,
70% – pea,
90% – bean,
75% – tomato,

85% – onion,
80% - perennial ryegrass,
95% – oats,
90% - wheat;

- 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 ER50 value determined on the basis of shoot length was equal to 26.7 g/ha and it was for Carrot.
The following order of the test plant sensitivity was noticed:
carrot > onion > cabbage > pea > tomato > sunflower > wheat > perennial ryegrass > bean, oats

Comments of zRMS:	The study on effects on the vegetative vigour on ten non-target terrestrial plant species was performed in line with requirements of OECD 227 and according to the principles of GLP. In the definitive test all the validity criteria were met. The study is considered acceptable for the risk assessment purposes.

Reference:	KCP 10.6.2/02
Report	MCPA + TRIBENURON METYL 565 SG, Terrestrial Plant Test: Vegetative Vigour Test, Weronika Dec, 2018, Study code G/161/17, Institute of Industrial Organic Chemistry Branch Pszczyna, Poland
Guideline(s):	Yes. According to the OECD Guideline for the Testing of Chemicals No. 227 "Terrestrial Plant Test: Vegetative Vigour Test".
Deviations:	According to OECD Guideline No. 227 (2006), the light intensity should be 350 ± 50µE/m2/s. However, these values are recommended for tests conducted in greenhouses. The experiment was conducted in a test room, where only artificial lighting was used. The light intensity was between 117.2 – 150.0 µE/m2/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing. The number of seeds per pot is a deviation from the OECD Guideline No. 227. Nevertheless, in the course of statistical evaluation of the results the increased number of seeds per replicate significantly decreased the variability between replicates. Therefore, this deviation from the guideline recommendation ensures an optimal and more reliable statistical analysis with a high discriminatory power.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

SUMMARY

The study, aimed at evaluating the effect of **MCPA + TRIBENURON METYL 565 SG** on vegetative vigour of 10 terrestrial plants, was conducted on 6 dicotyledonous and 4 monocotyledonous species. Seeds of the test plant species were sown in plastic pots (10 seeds/pot). The plants were grown to the 2- to 4- true leaf stage. Then, some of them were removed. As a result there were five plants per pot (replicate)

left. The test item was sprayed onto the plants. Six, seven or eight rates of the test item were used in the experiment. They were determined on the basis of the non-GLP range - finding test results. Untreated control group was conducted simultaneously. The treated and the control groups were divided into four replicates. The experiment was conducted in a plant growth room where suitable environmental conditions for each test species were provided. During the experiment, the plants were observed for visual phytotoxicity (7, 14 and 21 days after the test item application). The experiment finished 21 days after the spraying. At the end of the experiment, the number of surviving plants was counted. Next, the plants were cut down, and the lengths of their shoots were determined. Finally, they were dried at 60ºC to a constant weight and weighed.

The results concerning the shoot length, the dry weight, and the number of plants at the end of the experiment were statistically analysed to determine the ER_{25}, ER_{50}, and NOER.

Materials and methods

1. Test material: MCPA + TRIBENURON METYL 565 SG
   Batch number: 4.5/16
   Concentration of the MCPA: 565 g/kg, Concentration of the Tribenuron-methyl 14.6 g/kg

2. Test organism: Ten plant species were used. These were carrot *(Daucus carota)*, sunflower *(Helianthus annuus)*, cabbage *(Brassica olerace var. capitata)*, pea *(Pisum sativum)*, bean *(Phaseolus vulgaris)*, tomato *(Solanum lycopersicon)*, onion *(Allium cepa)*, perennial ryegrass *(Lolium perenne)*, oats *(Avena sativa)*, wheat *(Triticum aestivum).*

3. Test design:
Six, seven or eight rates of the test item were used in the experiment. They were determined on the basis of the non-GLP range - finding test results. In cultivation of cabbage, sunflower and carrot eight application rates were used i.e. 0.5, 1.4, 4.1, 12.3, 37.0, 111.1, 333.3 and 1000 g test item/ha.
In cultivation of tomato, bean, pea and onion seven application rates were used i.e. 1.4, 4.1, 12.3, 37.0, 111.1, 333.3 and 1000 g test item/ha. In cultivation of perennial ryegrass, wheat, and oats six application rates will be used i.e. 4.1, 12.3, 37.0, 111.1, 333.3 and 1000 g test item/ha.
A separation factor was 3.0. The volume of deionized water used to prepare the test item at the highest rate corresponded to 300 L/ha.
One untreated control group was used for each species. Four replicates were used, both for control and treated groups. The test system was the same for all test species.

Soil: sandy loam

Test conditions: temperature: 18.2 – 26.1°C, humidity: 45.8 – 92.0%, controlled light – dark cycles (16h:8h), light intensity: 117.2 – 150.0 µE/m2/s, carbon dioxide concentration: 320 – 360 ppm.

Statistical analysis: ER25, ER50 – probit analysis or logit analysis or weibull analysis NOER - Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Williams Multiple Sequential t-test Procedure or Dunnett's Multiple l t-test Procedure or Welch-t test for Inhomogeneous Variances with Bonferroni-Holm Adjustment.

Seeds of the test plant species were sown in plastic pots (pot's diameter – 15 cm, pot's surface area – about 177 cm2) containing the test soil. There were 10 seeds in each pot. The replicate is defined as a pot. The plants were grown to the 2- to 4- true leaf stage. Then, some of them were removed.
As a result, there were 5 plants/pot. The total number of plants per application rate was twenty. Next, the test item was sprayed onto the plants with calibrated spraying equipment. The concentration of MCPA and tribenuron metyl in water was determined with a validated analytical method.

Results and discussions:

The test item, i.e. MCPA + TRIBENURON METYL 565 SG applied at rates ranging from 0.5 to 1000 g/ha had a varied impact on vegetative vigour of the test plant species. The impact depended on the rate and species.

The test item caused mortality of: carrot and sunflower at rates ranging from 37.0 to 1000 g/ha, tomato at the rates ranging from 111.1 to 1000 g/ha, onion at the rates equal to 333.3 and 1000 g/ha, and also perennial ryegrass at the highest rate equal to 1000 g/ha.
Mortality of carrot ranged from 5 to 60%.
Mortality of sunflower ranged from 5 to 100%.
Mortality of tomato ranged from 10 to 100%.
Mortality of onion ranged from 25 to 50%.
Mortality of perennial ryegrass was equal to 10%.
There was no mortality observed for cabbage, bean, pea, oats and wheat.

On the basis of NOER, ER25 and ER50 values determined from the shoot length and shoot dry weight, it was observed that the test item caused inhibition of growth of all tested species.

Some phototoxic symptoms were observed after 21 days of the exposure:
- stunted growth (all tested species),
- wilting (bean, tomato),
- chlorosis (carrot, sunflower, cabbage, pea, bean, tomato)
- deformations (carrot, bean),
- spots (sunflower, cabbage),
- necrosis (carrot, sunflower, cabbage, pea, tomato, onion)
- mortality of plants (carrot, sunflower, tomato, onion, perennial ryegrass).

In case of wheat and oats no phytotoxic symptoms were observed.

The following order of the test plant sensitivity was noticed:
sunflower > carrot, tomato, onion > cabbage > pea, bean > perennial ryegrass > wheat > oats.

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

	Carrot *Daucus carota*	**Sunflower** *Helianthus annuus*	**Cabbage** *Brassica olerace var. capitata*	**Pea** *Pisum sativum*	**Bean** *Phaseolus vulgaris*	**Tomato** *Solanum lycopersicon*
Plant number at the end of the experiment						
ER_{50}	812.7 (535.4 – > 1000*)	100.1 (75.6 – 133.9)	> 1000	> 1000	> 1000	401.2 (295.0 – 526.6)
NOER	111.1	37.0	> 1000	> 1000	> 1000	111.1
Shoot length (plants without roots)						
ER_{50}	60.8 (19.3 – 245.3)	28.2 (15.1 – 64.8)	945.1 (588.6 – > 1000*)	584.9 (421.2 – 910.8)	> 1000	46.4 (23.3 – 93.1)
NOER	4.1	1.4	4.1	37.0	37.0	1.4
Plant dry weight (plants without roots)						
ER_{50}	9.3 (4.4 – 19.6)	18.4 (11.8 – 28.9)	199.9 (130.6 – 320.7)	243.9 (112.7 – 616.9)	290.8 (144.9 – 827.7)	31.2 (18.8 – 51.8)
NOER	1.4	0.5	12.3	37.0	12.3	4.1

* value determined out of the range of the tested application rates

	Onion *Allium cepa*	Perennial ryegrass *Lolium perenne*	Oats *Avena sativa*	Wheat *Triticum aestivum*
Plant number at the end of the experiment				
ER_{50}	906.0 (591.6 – > 1000*)	> 1000	> 1000	> 1000
NOER	111.1	≥ 1000	> 1000	> 1000
Shoot length (plants without roots)				
ER_{50}	54.3 (37.1– 80.2)	> 1000* (828.8 – > 1000*)	> 1000	> 1000
NOER	1.4	37.0	37.0	12.3
Plant dry weight (plants without roots)				
ER_{50}	29.6 (20.8 – 42.0)	456.7 (276.6 – 927.0)	> 1000* (641.1 – n.d.)	> 1000* (852.3 – > 1000*)
NOER	1.4	37.0	111.1	111.1

* value determined out of the range of the tested application rates

n.d. – not determined

Validity of the test:

- Seedling emergence in the control was at least 70% as follows:

80.0 – 90.0 – carrot,
87.5 – 100.0 – sunflower,
85.0 – 95.0 – cabbage,
85.0 – 100.0 – pea,
87.5 – 100 – bean,
90.0 – 97.5 – tomato,
80.0 – 90.0 – onion,
90.0 – 95.0 – perennial ryegrass,
80.0 – 95.0 – oats,
90.0 – 100.0 - wheat

- 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} value determined on the basis of plant dry weight was equal to 9.3 g/ha and it was for Carrot.

The following order of the test plant sensitivity was noticed:

sunflower > carrot, tomato, onion > cabbage > pea, bean > perennial ryegrass > wheat > oats.

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

Not needed.

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

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A 2.7 KCP 10.8 Monitoring data

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