FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: SHA 4307 A

Product name: PRIMARY MX

Chemical active substances:

Rimsulfuron, 30 g/kg

Nicosulfuron, 120 g/kg

Mesotrione 360 g/kg

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Applicant: SHARDA Cropchem España S.L.

Submission date: 02.2020

Update date: 03.2021, 07.2021, 08.2021, 10.2021

MS Finalisation date: 07.2022, 12.2022; 03.2023

Version history

When	What
March 2021	Applicant update
July 2021	Applicant update
August 2021	Applicant update
October 2021	Applicant update
July 2022	zRMS first evaluation
November 2022	Applicant update
December 2022	Applicant update
December 2022	Applicant update
December 2022	Final zRMS assessment.
March 2023	zRMS corrected

Table of Contents

9 Ecotoxicology (KCP 10)

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use-No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: devel-opmental stages of the pest or pest group)	**Application**				**Application rate**			PHI (days)	Remarks: e.g. g safener/ synergist per ha	**Conclusion**						
					Method / Kind	Timing / Growth stage of crop & season	Max. num-ber a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthro-'	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	CEU	**Maize**	F	Broadleaved and grass weeds	Foliar spray	BBCH 12-18	a) 1 b) 1	NA	~~a) 0.33~~ ~~b) 0.33~~ 0.25	a) 0.0099 rimsulfuron + 0.0396 nicosulfuron + 0.118 mesotrione b) 0.0099 rimsulfuron + 0.0396 nicosulfuron + 0.118 mesotrione	200-400	-	In order to get accepta-ble risk on mammals applicant proposes reduction of rate to 0.25 kg equivalent to 0.09 kg mesotrione/ha							

* 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 “Conclusions”

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

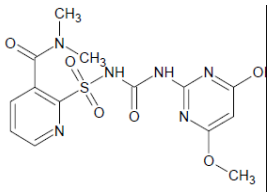
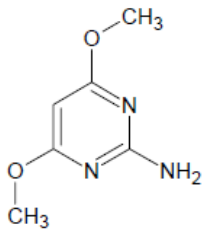
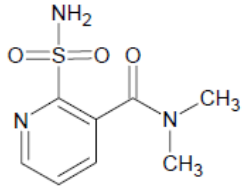
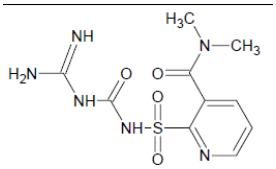
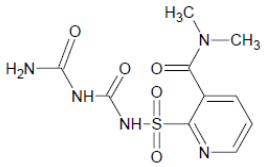
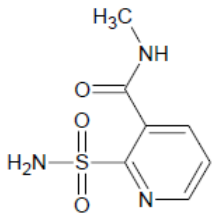
Remarks table:

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

9.1.1 Overall conclusions

9.1.1.1

9.1.1.2 Table 9.1-3 Metabolites of Nicosulfuron

9.1.1.3 Metabolite	Molar mass	Chemical structure	Maximum occurrence in compartments	Risk assessment required?
HMUD (2-{[(4-hydroxy-6-methoxypyrimidin-2-yl)carbamoyl]sulfamoyl}-N-N-dimethylpyridine-3-carboxamide)	396.4 g/mol		Soil:14.4% Water: 14.1% Sediment: 5.7% Water/sediment: 19.3%	Aquatic organisms Erthworms and other soil meso- and marco-fauna Microbial activity
ADMP (4,6-dimethoxypyrimidin-2-amine)	155.2 g/mol		Soil: 9.8% Water: 23.1%*	Aquatic organisms Erthworms and other soil meso- and marco-fauna Microbial activity
ASDM (N,N-dimethyl-2-sulfamoylpyridine-3-carboxmide)	229.2 g/mol		Soil: 63.4% Water: 61%* Sediment: 4.4% Water/sediment: 61%*	Aquatic organisms Erthworms and other soil meso- and marco-fauna Microbial activity
AUSN (2-[(carbamimidoylcarbamoyl)sulfamoyl]-N,N-dimethylpyridine-3-carboxamide	314.3 g/mol		Soil: 26.8% Water: 9.1% Sediment: 2.4% Water/sediment: 11.1%	Aquatic organisms Erthworms and other soil meso- and marco-fauna Microbial activity
UCSN (2-[(carbamoylcarbamoyl)sulfamoyl]-N,N-dimethylpyridine-3-carboxamide	315.3 g/mol		Soil: 11% Water: 5.4% Sediment: 1.4% Water/sediment: 6.5%	Aquatic organisms Erthworms and other soil meso- and marco-fauna Microbial activity
MU-466 (N-methyl-2-sulfamoylpyridine-3-carboxamide)	215.2 g/mol			-

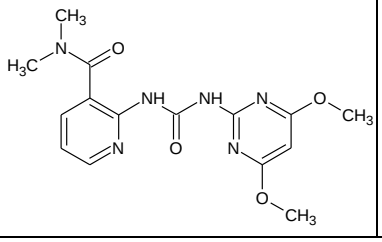
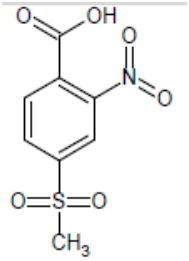
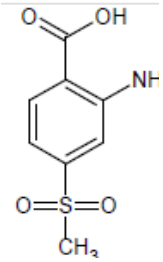
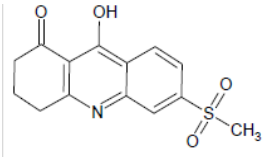
9.1.1.3 Metabolite	Molar mass	Chemical structure	Maximum occurrence in compartments	Risk assessment required?
DUDN 2-{[(4,6-dimethoxypyrimidin-2-yl)carbamoyl]amino}-N,N-dimethylpyridine-3-carboxamide	346.3 g/mol		Soil: 1 x 10^{-10}% Water: 22.3%*	Aquatic organisms

Table 9.1-4 Metabolites of Mesotrione

Metabolite	Molar mass	Chemical structure	Maximum occurrence in compartments	Risk assessment required?
MNBA (4-(methylsulfonyl)-2-nitrobenzoic acid)	245 g/mol		Soil: 57.2 % Water: 7.9% Sediment: <1% Water/sediment: 7.9%	Aquatic organisms Erthworms and other soil meso- and marco-fauna Microbial activity
AMBA (2-amino-4-(methylsulfonyl)benzoic acid)	215 g/mol		Soil: 9.7% Water: 15.8% Sediment: 8.8% Water/sediment: 24.6%	Aquatic organisms Erthworms and other soil meso- and marco-fauna Microbial activity
SYN546974 (9-hydroxy-6-(methylsulfonyl)-3,4-dihydroacridin-1(2H)-one)	291 g/mol		Soil: <1 x 10^{-10}% Water: 9.4% Sediment: 25.6% Water/sediment: 33%	Aquatic organisms

9.1.1.4 Effects on birds (KCP 10.1.1), Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)

- Birds:

Risk assessment for birds concludes in a low acute and long-term risk as well as for drinking water exposures and secondary poisoning. Therefore, no unacceptable acute and long-term risks are expected for birds. According to results, no unacceptable acute and long-term risk due to combined exposure are obtained in according to the proposed GAP.

- Mammals:

According to the screening assessment for maize, the TER_a and TER_{lt} values for the active substances Rimsulfuron and Nicosulfuron are greater than the Annex VI trigger of 10 and 5, respectively. After screening and first-tier assessment for active substance Mesotrione, the TER_a value is greater than the Annex VI trigger of 10 whereas TER_{lt} values are lower than the Annex VI trigger of 5 for the use on maize, indicating that PRIMARY MX presents an unacceptable long-term risk to mammals. A refinement of the risk was done by selecting the two focal species european brown hare and wood mouse, using a PT value of ~~0.98~~ 0.139 for wood mouse and 0.62 for hare, ~~a refined endpoint, the specific deposition factor of the crop and~~ a refined TWA for maize and also a reduction in rate to 90 g mesotrione/ha; in addition, a refinement of RUD for maize was presented but was not used by zRMS in the risk assessment. Therefore, there is no unacceptable acute and long-term risk for mammals as well as for drinking water exposures and secondary poisoning. According to results, no unacceptable acute and long-term risk due to combined exposure are obtained according to the proposed GAP.

9.1.1.5 Effects on aquatic organisms (KCP 10.2)

Rimsulfuron

All PEC/RAC values for Rimsulfuron and its metabolite are below the trigger value of 1 at step 3, indicating that Rimsulfuron poses a low risk to aquatic organisms, as well as for IN-70941, IN-70942 and IN-E9260 metabolite.

Nicosulfuron

For the intended uses on maize, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for higher plant as characterised by an EC_{50} for *Lemna gibba* of 1.7 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. Based on the results of the risk assessment at step 4, the following conclusions regarding buffer zones and vegetative buffer strips may be drawn for maize use:

- D3 ditch~~,~~ ~~D4 stream, D5 stream and D6 ditch~~ scenarios: A 5 m no spray buffer zone is required.
- R1 stream scenario: A ~~15~~10 m no spray buffer zone and a ~~15~~10 m vegetative buffer strip are required.
- R2 stream, R3 stream and R4 stream: A 20 m no spray buffer zone and a 20 m vegetative buffer strip are reduction are not enough for acceptable risk. ~~After the refinement with the results of the recovery phase of the study on *Lemna* conducted with nicosulfuron (RAC equal to 0.74 µg nicosulfuron/L), the risk is considered acceptable with an unsprayed vegetated buffer zone of 15~~10 ~~m for R3 and R4 stream and an unsprayed vegetated buffer zone of 5 m for R2 stream.~~
- After the refinement with the value agreed at EU level, based on 7 d E_rC_{50} of 2.7 µg/L (RAC equal to 0.27 µg nicosulfuron/L), the risk is considered acceptable with an unsprayed vegetated buffer zone of 5 m.

For ASDM, AUSN, HMUD, ADMP and UCSN metabolites, all PEC/RAC values are below the trigger value of 1 at step 1-2. Therefore, no further assessment is necessary.

Mesotrione

For the intended uses on maize, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for higher plant as characterised by an EC_{50} for *Lemna gibba* of 7.7 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. Based on the results of the risk assessment at step 4, the following conclusions regarding buffer zones and vegetative buffer strips may be drawn for maize use:

- R2 stream (pH 5.1) ~~Linear:~~ A ~~10~~5m no spray buffer zone and a ~~10~~5m vegetative buffer strip are required.

For MNBA, AMBA and SYN546974 metabolites, all PEC/RAC values are below the trigger value of 1 at step 1-2. Therefore, no further assessment is necessary.

PRIMARY MX

For the endpoints from formulated product PRIMARY MX, any spray buffer zone with 50% of nozzles reduction OR a 5 m no spray buffer zone is enough for acceptable risk. In addition, for the combined exposure the risk is considered acceptable with an unsprayed vegetated buffer zone of 5 m.

Conclusion

Maize – SPe 3: *To protect aquatic organisms respect an unsprayed vegetated buffer zone of 5 m to surface water bodies.*

9.1.1.6 Effects on bees (KCP 10.3.1)

First-tier assessments indicate that no unacceptable risk for bees exposed to PRIMARY MX is expected according to the proposed intended uses.

9.1.1.7 Effects on arthropods other than bees (KCP 10.3.2)

The results of the risk assessment for non-target arthropods showed an acceptable in-field and off-field risk after the application of PRIMARY MX. ~~The absence of risk in the in-field area has been demonstrated according to the data from the monograph.~~

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

The TER values for earthworms and other soil macro- and mesofauna for PRIMARY MX were above the relevant Annex VI trigger of 10 and 5, respectively. Therefore, it is concluded that active substance Rimsulfuron, Nicosulfuron and Mesotrione do not pose acute and chronic risk to earth-worms and other soil macro- and mesofauna.

Risk assessments conducted with relevant PEC_{soil} for the active substance Rimsulfuron, Nicosulfuron and Mesotrione indicate a low risk to soil microorganisms when applied according to the proposed use rates. The use of PRIMARY MX at the proposed rates poses no unacceptable risk to non-target soil microorganisms.

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

~~Risk assessment conducted with relevant toxicity data on non-target terrestrial plants for Rimsulfuron, Nicosulfuron and Mesotrione shows that the Annex VI trigger value of 5 is exceeded. Therefore, mitigation measures are needed. When there is 5m buffer zone with 75% nozzle reduction OR 10 m buffer zone with 50% nozzle reduction, PRIMARY MX poses a low risk to non-target plants when applied according to the proposed use rates.~~

~~***Maize – SPe 3:*** *To protect non-target plants respect an unsprayed buffer zone of 5m with 75% drift reducing nozzles OR 10m with 50% drift reducing nozzles to non-agricultural land.~~

Risk assessment conducted with relevant toxicity data on non-target terrestrial plants for Rimsulfuron, Nicosulfuron and Mesotrione shows that the Annex VI trigger value of 5 is exceeded. Therefore, mitigation measures are needed. When there is 90% nozzle reduction OR 3m buffer zone with ~~75~~50% nozzle reduction ~~OR 5m buffer zone with 50% nozzle reduction~~ OR 10 m buffer zone, PRIMARY MX poses a low risk to non-target plants when applied according to the proposed use rates.

Maize – SPe 3: *To protect non-target plants respect 90% drift reducing nozzles OR an unsprayed buffer zone of 3m with ~~75~~50% drift reducing nozzles OR an unsprayed buffer zone of 5m with 50% drift reducing nozzles OR an unsprayed buffer zone of 10m to non-agricultural land.*

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

Rimsulfuron:
Data from a test with activated sludge are available and indicate that the risk to biological methods of sewage treatment plants is low.

Nicosulfuron:
Effects on biological methods for sewage treatment

Test type/organism	End point
Activated sludge	--
Pseudomonas putida	Nicosulfuron EC_{50} > 250 mg as/L (no reported effects) ASDM, AUSN, UCSN, MU-466, HMUD > 100 mg metabolite/L (no significant inhibition)

Mesotrione:
Effects on biological methods for sewage treatment

Test type/organism	End point
Activated sludge	$EC_{50} \geq 160$ mg as/L
Pseudomonas sp.	NOEC = 100 mg as/L

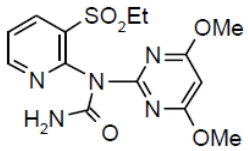
9.1.2 Grouping of intended uses for risk assessment

Not relevant.

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 PRIMARY MX is indicated in the table.

Table 9.1-2 Metabolites of Rimsulfuron

Metabolite	Molar mass	Chemical structure	Maximum occurrence in compartments	Risk assessment required?
IN-70941 (N-(4.6-dimethoxy-2-pyrimidinyl)-N-[3-(ethylsuflonyl)-2-pyridinyl] urea)	367.4 g/mol		Soil: 54.5% Total system: 87.2%	Aquatic organisms Erthworms and other soil meso- and marco-fauna Microbial activity

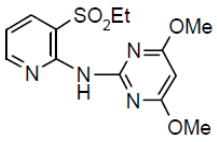
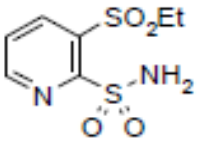
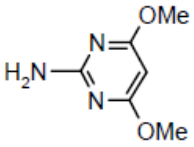
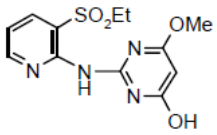
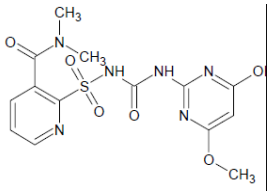
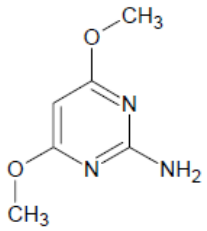
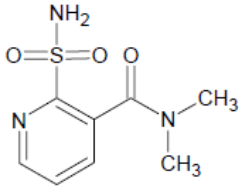
Metabolite	Molar mass	Chemical structure	Maximum occurrence in compartments	Risk assessment required?
IN-70942 (N-[3-(ethylsulfuronyl)-2-pyridinyl]-4,6-dimethoxy-2-pyrimiinamine)	324.36 g/mol		Soil: 23.5% Total system: 83.8%*	Aquatic organisms Erthworms and other soil meso- and marco-fauna Microbial activity
IN-E9260 (3-(ethylsulfuonyl)-2-pyridinesulfonamide)	250.30 g/mol		Soil: 18.9% Total system: 16.2%**	Aquatic organisms Erthworms and other soil meso- and marco-fauna Microbial activity
IN-J0290 (4,6-dimethoxy-2-pyrimidinamine) a.k.a ADMP	155.20 g/mol		Soil: 12.7%*** Total system: 19.1%**	Aquatic organisms Erthworms and other soil meso- and marco-fauna Microbial activity
IN-JF999 (2-[[3-ethylsulfonyl)-2-pyridinyl]amino]-6-methoxy-4(1H)-pyrimidinone)	310.33 g/mol		Soil: 1 x 10^{-10}% Total system: 24.5%	Aquatic organisms

Table 9.1-3 Metabolites of Nicosulfuron

Metabolite	Molar mass	Chemical structure	Maximum occurrence in compartments	Risk assessment required?
HMUD (2-{[(4-hydroxy-6-methoxypyrimidin-2-yl)carbamoyl]sulfamoyl}-N-N-dimethylpyridine-3-carboxamide)	396.4 g/mol		Soil:14.4% Water: 14.1% Sediment: 5.7% Water/sediment: 19.3%	Aquatic organisms Erthworms and other soil meso- and marco-fauna Microbial activity
ADMP (4,6-dimethoxypyrimidin-2-amine)	155.2 g/mol		Soil: 9.8% Water: 23.1%*	Aquatic organisms Erthworms and other soil meso- and marco-fauna Microbial activity
ASDM (N,N-dimethyl-2-sulfamoylpyridine-3-carboxmide)	229.2 g/mol		Soil: 63.4% Water: 61%* Sediment: 4.4% Water/sediment: 61%*	Aquatic organisms Erthworms and other soil meso- and marco-fauna Microbial activity

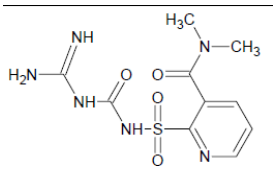
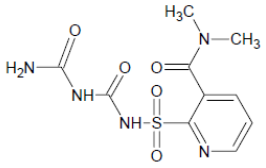
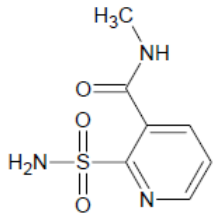
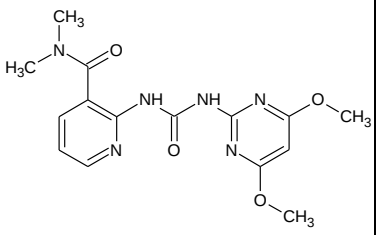
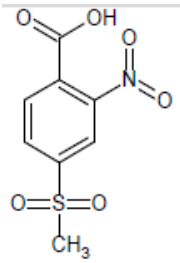
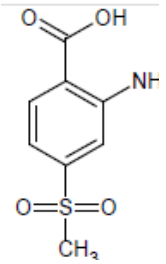
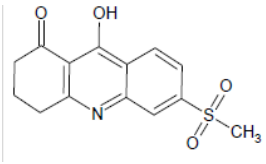
Metabolite	Molar mass	Chemical structure	Maximum occurrence in compartments	Risk assessment required?
AUSN (2-[(carbamimidoylcarbamoyl)sulfamoyl]-N,N-dimethylpyridine-3-carboxamide	314.3 g/mol		Soil: 26.8% Water: 9.1% Sediment: 2.4% Water/sediment: 11.1%	Aquatic organisms Erthworms and other soil meso- and marco-fauna Microbial activity
UCSN (2-[(carbamoylcarbamoyl)sulfamoyl]-N,N-dimethylpyridine-3-carboxamide	315.3 g/mol		Soil: 11% Water: 5.4% Sediment: 1.4% Water/sediment: 6.5%	Aquatic organisms Erthworms and other soil meso- and marco-fauna Microbial activity
MU-466 (N-methyl-2-sulfamoylpyridine-3-carboxamide)	215.2 g/mol			-
DUDN 2-{[(4,6-dimethoxypyrimidin-2-yl)carbamoyl]amino}-N,N-dimethylpyridine-3-carboxamide	346.3 g/mol		Soil: 1 x 10^{-10}% Water: 22.3%*	Aquatic organisms

Table 9.1-4 Metabolites of Mesotrione

Metabolite	Molar mass	Chemical structure	Maximum occurrence in compartments	Risk assessment required?
MNBA (4-(methylsulfonyl)-2-nitrobenzoic acid)	245 g/mol		Soil: 57.2 % Water: 7.9% Sediment: <1% Water/sediment: 7.9%	Aquatic organisms Erthworms and other soil meso- and marco-fauna Microbial activity
AMBA (2-amino-4-(methylsulfonyl)benzoic acid)	215 g/mol		Soil: 9.7% Water: 15.8% Sediment: 8.8% Water/sediment: 24.6%	Aquatic organisms Erthworms and other soil meso- and marco-fauna Microbial activity

Metabolite	Molar mass	Chemical structure	Maximum occurrence in compartments	Risk assessment required?
SYN546974 (9-hydroxy-6-(methylsulfonyl)-3,4-dihydroacridin-1(2H)-one)	291 g/mol		Soil: <1 x 10^{-10}% Water: 9.4% Sediment: 25.6% Water/sediment: 33%	Aquatic organisms

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with Rimsulfuron, Nicosulfuron, Mesotrione and their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on birds of PRIMARY MX (Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG) were not evaluated as part of the EU assessment of Rimsulfuron, Nicosulfuron and Mesotrione. However, the provision of further data on the formulation PRIMARY MX is not considered essential, because risk to mammals may be sufficiently assessed using the EU agreed endpoints and new studies should not be conducted in regards of animal welfare (EFSA Journal 2009; 7(12):1438).

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

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

Species	Substance	Exposure System	Results	Reference
Mallard duck *Anas platyrhynchos*	Rimsulfuron	Acute	**LD_{50} > 2250** mg a.s./kg bw/day**	EFSA Scientific Report 2005; 45; 1-61
Bobwhite quail *Colinus viriginianus*	Rimsulfuron	Acute	LD_{50} > 2250** mg a.s./kg bw/day	
Mallard duck *Anas platyrhynchos*	Rimsulfuron	Dietary toxicity (short-term)	LC_{50} > 5620 mg as/kg food NOEC = 5620 mg as/kg food LD_{50} > 1610 mg a.s./kg bw/day	
Bobwhite quail *Colinus viriginianus*	Rimsulfuron	Dietary toxicity (short-term)	LC_{50} > 5620 mg as/kg food NOEC = 5620 mg as/kg food	
Mallard duck *Anas platyrhynchos*	Rimsulfuron	Reproductive toxicity (long-term)	NOAEL = 1250 mg as/kg food	
Bobwhite quail *Colinus viriginianus*	Rimsulfuron	Reproductive toxicity (long-term)	NOAEL = 1250 mg as/kg food **NOAED = 142 mg a.s./kg bw/day**	
Bobwhite quail *Colinus viriginianus*	Nicosulfuron tech.	Acute	**LD_{50} > 2000* mg a.s./kg bw/day** NOEL = 2000 mg a.s./kg bw/day	EFSA Scientific Report 2007; 120;

Species	Substance	Exposure System	Results	Reference
Mallard duck *Anas platyrhynchos*	Nicosulfuron tech.	Acute	LD_{50} > 2000* mg a.s./kg bw/day NOEL = 2000 mg a.s./kg bw/day	1-91
Bobwhite quail *Colinus viriginianus*	SL-950 4% SC	Acute	LD_{50} > 2000 mg a.s./kg bw/day NOEL = 2000 mg a.s./kg bw/day	
Mallard duck *Anas platyrhynchos*	SL-950 4% SC	Acute	LD_{50} > 2000 mg a.s./kg bw/day NOEL = 2000 mg a.s./kg bw/day	
Mallard duck *Anas platyrhynchos*	Nicosulfuron	Dietary 5 d	LD_{50} > 5000 mg/kg food NOEL = 5000 mg/kg food LD_{50} > 911 mg a.s./kg bw/day NOEL = 911 mg a.s./kg bw/day	
Bobwhite quail *Colinus viriginianus*	Nicosulfuron	Dietary 5 d	LD_{50} > 5000 mg/kg food NOEL = 5000 mg/kg food LD_{50} > 1603 mg a.s./kg bw/day NOEL = 1603 mg a.s./kg bw/day	
Japanese quail *Coturnix japonica*	Nicosulfuron	Reproductive toxicity (long-term)	NOEC = 1000 mg a.s./kg food **NOEC = 171 mg a.s./kg bw/day**	
Bobwhite quail *Colinus viriginianus*	Mesotrione	Oral 1 d Acute	**LD_{50} > 2000 mg a.s./kg bw** **NOEL = 2000 mg a.s./kg bw**	EFSA Journal 2016;14(3):4419
Mallard duck *Anas platyrhynchos* & Bobwhite quail *Colinus viriginianus*	Mesotrione	Dietary Short-term	LC_{50} > 5200 mg/kg diet NOEC = 5200 mg/kg diet	
Bobwhite quail *Colinus viriginianus*	Mesotrione	Long-term	NOEC = 300 mg a.s./kg diet	
Mallard duck *Anas platyrhynchos*	Mesotrione	Long-term	**NOEL = 120 mg a.s./kg diet = 20.6 mg a.s./kg bw/d**	

* Nicosulfuron dietary LDD_{50} of >911 mg as/kg bw/d is formally lower than acute LD_{50} of >2000 mg as/kg bw, however under test conditions it corresponds to the maximum tested dose of 5000 mg as/kg feed, and this concentration was determined as the NOEL. Therefore, it is justified to assess acute risk with the acute LD_{50} of >2000 mg as/kg bw

** Rimsulfuron dietary LDD_{50} of >1610 mg as/kg bw/d is formally lower than acute LD_{50} of >2250 mg as/kg bw, however under test conditions it corresponds to the maximum tested dose of 5620 mg as/kg feed, and this concentration was determined as the NOEL. Therefore, it is justified to assess acute risk with the acute LD_{50} of >2250 mg as/kg bw

zRMS comment:

Avian toxicity data presented in Table 9.2-1 are in general in line with EU agreed endpoints reported in EFSA Journal 2016;14(3):4419 for mesotrione, EFSA Scientific Report 2005; 45; 1-61 for rimsulfuron and EFSA Scientific Report 2007; 120; 1-91 for nicosulfuron.

It is noted that the acute toxicity study for SHA4307/PRIMARY MX for birds is not provided.

However, the vertebrate toxicity testing must be performed only when crucial for the evaluation.

Therefore, the provision of further data on the formulation PRIMARY MX is not considered essential,

because risk to birds may be sufficiently assessed using the EU agreed endpoints and new studies should not be conducted in regards of animal welfare.

9.2.1.1 Justification for new endpoints

The used endpoints are the EU agreed ones.

9.2.2 Risk assessment for spray applications

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

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

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

Table 9.2-2: First-tier assessment of the acute and long-term/reproductive risk for birds regarding Rimsulfuron due to the use of PRIMARY MX in maize

Intended use	**Maize**				
Active substance/product	Rimsulfuron				
Application rate (g/ha)	1 x 9.9				
Acute toxicity (mg/kg bw)	2250				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize	Indicator species for screening	158.8	1.0	1.57	1431.2
Reprod. toxicity (mg/kg bw/d)	142				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize	Indicator species for screening	64.8	1.0 x 0.53	0.34	417.6

Table 9.2-3: First-tier assessment of the acute and long-term/reproductive risk for birds regarding Nicosulfuron due to the use of PRIMARY MX in maize

Intended use	**Maize**
Active substance/product	Nicosulfuron
Application rate (g/ha)	1 x 39.6
Acute toxicity (mg/kg bw)	2000
TER criterion	10

Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize	Indicator species for screening	158.8	1.0	6.29	318.0
Reprod. toxicity (mg/kg bw/d)	171				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize	Indicator species for screening	64.8	1.0 x 0.53	1.36	125.7

Table 9.2-4: First-tier assessment of the acute and long-term/reproductive risk for birds regarding Mesotrione due to the use of PRIMARY MX in maize

Intended use	**Maize**				
Active substance/product	Mesotrione				
Application rate (g/ha)	1 x 118				
Acute toxicity (mg/kg bw)	2000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize	Indicator species for screening	158.8	1.0	18.74	106.7
Reprod. toxicity (mg/kg bw/d)	20.6				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize	Indicator species for screening	64.8	1.0 x 0.53	4.05	5.1

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.

The risk assessment for mesotrione has been re-done considering a reduction on rate from 118 g a.s./ha to 90 g a.s./ha.

Table 9.2-5: First-tier assessment of the acute and long-term/reproductive risk for birds regarding Mesotrione due to the use of PRIMARY MX in maize

Intended use	**Maize**				
Active substance/product	Mesotrione				
Application rate (g/ha)	1 x 90				
Acute toxicity (mg/kg bw)	2000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize	Indicator species for screening	158.8	1.0	14.29	140.0
Reprod. toxicity (mg/kg bw/d)	20.6				
TER criterion	5				
Crop scenario	**Indicator/generic focal species**	**SV_m**	**MAF_m ×**	**DDD_m**	**TER_{lt}**

Growth stage			**TWA**	**(mg/kg bw/d)**	
Maize	Indicator species for screening	64.8	1.0 x 0.53	3.09	6.67

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.

Risk Assessment for combined exposure

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

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

With:

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

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

Acute risks from combined exposure

The active substance content of the formulation PRIMARY MX addressed in this dossier is Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG, making up a total of 510 g a.s./Kg product. According to GAP, the maximum application rate is 0.250 ~~0.33~~ kg product/ha, therefore, an application rate of 127.5 ~~168.3~~ g a.s./ha was considered in the assessment.

Table 9.2-7 shows the calculation of the predicted LD_{50} (mix) of Rimsulfuron, Nicosulfuron and Mesotrione when mixed in these proportions (step 1 in Appendix B to the EFSA GD 2009).

Table 9.2-6: Avian LD_{50} (mix) for Rimsulfuron, Nicosulfuron and Mesotrione when combined as PRIMARY MX (step 1 in EFSA GD 2009, Appendix B)

	Rimsulfuron	Nicosulfuron	Mesotrione
Content in the formulation PRIMARY MX	3%	12%	36%
Fraction in the a.s. mixture	0.06	0.24	0.71
LD_{50} of a.s. [mg/kg bw]	> 2250	>2000	>2000
Fraction / LD_{50}	0.000026	0.00012	0.00035
Sum	0.0005		
1/ sum = predicted LD_{50} (mix)	2013.16 mg mix/kg bw		

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

Table 9.2-7: Avian "tox per fraction" for the PRIMARY MX (step 1 in EFSA GD 2009, Appendix B)

	Rimsulfuron	Nicosulfuron	Mesotrione	"mix"
Content in the formulation PRIMARY MX	3%	12%	36%	51%
Fraction in mixture	0.06	0.24	0.71	1.0
LD_{50} (mg/kg bw)	> 2250	>2000	>2000	2013.16
Tox per fraction	38250	8500	2833.3	2013.16
Contribution to predicted toxicity	5.26%	23.68%	71.05%	

Rimsulfuron contributes to 5.26% to mixture toxicity, nicosulfuron have an impact on the predicted risk of 23.68% and mesotrione of 71.05%, therefore, surrogate LD_{50} was used in the acute risk assessment.

Table 9.2-8: Screening step assessment of the acute risk for birds due to the use of PRIMARY MX in all crops

Intended use	Maize				
Active substance/product	PRIMARY MX				
Application rate (g/ha)	1 x 127.5 ~~168.3~~				
LD_{50} (mix) (mg/kg bw)	2013.16				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening	Indicator species for screening	158.8	1.0	20.25 ~~26.73~~	99.42 ~~75.33~~

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.

According to reults, no unacceptable acute risk due to combined exposure are obtained in acording to the proposed GAP.

zRMS comments:

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

The presented above birds risk assessment is agreed by the zRMS. All TER values exceed the relevant triggers indicating that SHA4307/PRIMARY MX does not pose an unacceptable risk to birds following applications according to recommended use pattern. On the basis of performed calculations, acceptable acute and long-term risk to birds may be concluded from proposed uses of PRIMARY MX.

Regarding chronic risk assessment, the Applicant considers that, according to EFSA/2009/1438, the calculation of a combined toxicity is not applicable to the risk assessment for reproductive effect. Due to differences in evaluated endpoints and the dependency of the derived NOEL of the test design, any calculated TER_{mix} value can only be used for illustrating purposes. Hence, in the case of an unacceptable TER_{mix}, it has to be discussed if the results of the toxicity studies present any evidence for a possible concentration additivity of the effects and risks.

In addition, the combined toxicological effect of these two active substances has not been investigated with regard to repeated dose toxicity. Possibly, the combined exposure to these active substances may lead to a different toxicological profile than the profile(s) based on the individual substances.

Despite all of this, the reproductive risk from combined exposure has been performed by the Applicant:

Reproductive risks from combined exposure

Table 9.2-9: Avian NOEL (mix) for Rimsulfuron, Nicosulfuron and Mesotrione when combined as PRIMARY MX (step 1 in EFSA GD 2009, Appendix B)

	Rimsulfuron	Nicosulfuron	Mesotrione
Content in the formulation PRIMARY MX	3%	12%	36%
Fraction in the a.s. mixture	0.06	0.24	0.71
NOEL of a.s. [mg/kg bw]	142	171	20.6
Fraction / NOEL	0.0004	0.0014	0.0342
Sum	0.0361		
1/ sum = predicted NOEL (mix)	27.73 mg mix/kg bw		

It is obvious from the comparison of the (low) long- term oral toxicity of the active substances, and their relative proportions of the formulated product PRIMARY MX, that any risk of long-term effects would very much be similar to toxicity of the three active substances.

Table 9.2-10: Avian "tox per fraction" for the PRIMARY MX (step 1 in EFSA GD 2009, Appendix B)

	Rimsulfuron	Nicosulfuron	Mesotrione	"mix"
Content in the formulation PRIMARY MX	3%	12%	36%	51%
Fraction in mixture	0.06	0.24	0.71	1.0
NOEL (mg/kg bw)	142	171	20.6	27.73
Tox per fraction	2414	726.75	29.18	27.73
Contribution to predicted toxicity	1.15%	3.82%	95.03%	

The tox per fraction is 2414 for Rimsulfuron, 726.75 for Nicosulfuron and 29.18 for Mesotrione. The NOEL for Mesotrione and surrogate NOEL are very similar this indicates that this active substance will contribute to ≥ 90 % to mixture toxicity, while the other components of the mixture will only have a marginal impact on the predicted risk. Consequently, the risk assessment can be performed for the most toxic active substance alone. No further considerations according to Steps 2 - 4 are necessary.

According to reults, no unacceptable long-term risk due to combined exposure are obtained in acording to the proposed GAP.

zRMS comments:

The presented risk assessment for birds based on combined chronic exposure is agreed by the zRMS.

The surrogate NOEL indicates that active substance mesotrione will contribute to ≥ 90 % to mixture toxicity, while the other components of the mixture will only have a marginal impact on the predicted risk. Consequently, the risk assessment can be performed for the most toxic active substance – mesotrione alone.

9.2.2.2 Higher-tier risk assessment

Not necessary.

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

Puddle scenario

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

With a K(f)oc of 42.4 (geometric mean, n = 4 (EFSA Scientific Report (2005) 45, 1-61)), Rimsulfuron belongs to the group of less sorptive substances.

Effective application rate (g/ha)	=	9.9		
Acute toxicity (mg/kg bw)	=	2250	quotient =	< 0.01
Reprod. toxicity (mg/kg bw/d)	=	142	quotient =	0.07

As the ratios of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) do not exceed the value of 50 for Rimsulfuron, it is not necessary to conduct a drinking water risk assessment for birds.

With a K(f)oc of 20.7 (EFSA Scientific Report (2007) 120, 1-91), Nicosulfuron belongs to the group of less sorptive substances.

Effective application rate (g/ha)	=	39.6		
Acute toxicity (mg/kg bw)	=	2000	quotient =	0.02
Reprod. toxicity (mg/kg bw/d)	=	171	quotient =	0.23

As the ratios of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) do not exceed the value of 50 for Nicosulfuron, it is not necessary to conduct a drinking water risk assessment for birds.

With a K(f)oc of 14 (EFSA Journal 2016;14(3):4419), Mesotrione belongs to the group of less sorptive substances.

Effective application rate (g/ha) =	118-90		
Acute toxicity (mg/kg bw) =	2000	quotient =	0.06-0.045
Reprod. toxicity (mg/kg bw/d) =	20.6	quotient =	5.73-4.37

As the ratios of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) do not exceed the value of 50 for Mesotrione, it is not necessary to conduct a drinking water risk assessment for birds.

zRMS comments:

Screening evaluation of the risk resulting from exposure to mesotrione (90 g a.s./ha) , rimsulfuron and nicosulfuron via drinking water is agreed by the zRMS. It is not necessary to conduct a drinking water risk assessment for birds.

9.2.2.4 Effects of secondary poisoning

The log P_{ow} of Rimsulfuron amounts to -1.46 at pH 7 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

The log P_{ow} of Nicosulfuron amounts to 0.61 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

The log P_{ow} of Mesotrione amounts to < -1 at pH 7 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

Risk assessment for earthworm-eating birds via secondary poisoning

Not required.

zRMS comments:

The evaluation of the risk of secondary poisoning for earthworm-eating birds for mesotrione, rimsulfuron and nicosulfuron is not triggered due to log Pow being <3.

Risk assessment for fish-eating birds via secondary poisoning

Not required.

zRMS comments:

The evaluation of the risk of secondary poisoning for fish-eating birds for mesotrione, rimsulfuron and nicosulfuron is not triggered due to log Pow being <3.

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.2.4 Overall conclusions

Risk assessment for birds concludes in a low acute and long-term risk as well as for drinking water exposures and secondary poisoning. Therefore, no unacceptable acute and long-term risks are expected for birds. According to results, no unacceptable acute and long-term risk due to combined exposure are obtained in according to the proposed GAP.

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 Rimsulfuron, Nicosulfuron, Mesotrione and their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on mammals of PRIMARY MX were not evaluated as part of the EU assessment of Rimsulfuron, Nicosulfuron and Mesotrione. However, the provision of further data on the formulation PRIMARY MX is not considered essential, because risk may be reliably assessed using the EU-agreed endpoints only and new studies should not be conducted in regards of animal welfare (EFSA Journal 2009; 7(12):1438).

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

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

Species	Substance	Exposure System	Results	Reference
Rat	Rimsulfuron	Oral 1 d Acute	**LD_{50} = 5000 mg/kg bw**	EFSA Scientific Report (2005) 45, 1-61
Rat	Rimsulfuron	Chronic, multigeneration	NOAEL = 3000 mg a.s./kg diet **NOAED = 11.8 mg a.s./kg bw/day**	
Rat	Nicosulfuron	Oral 1 d Acute	**LD_{50} > 5000 mg/kg bw**	EFSA Scientific Report (2007) 120, 1-91
Mouse	Nicosulfuron	Oral 1 d Acute	**LD_{50} > 5000 mg/kg bw**	

Species	Substance	Exposure System	Results	Reference
Rat	ASDM	Oral 1 d Acute	LD_{50} > 5000 mg/kg bw	
Rat	AUSN	Oral 1 d Acute	LD_{50} > 2000 mg/kg bw	
Rat	Nicosulfuron	Long-term	**NOAEL = 3861 (male)*** & 4404 (female)* **mg/kg bw/d**	
Rat	Mesotrione	Oral 1 d Acute	**LD_{50} > 5000 mg/kg bw**	EFSA Journal 2016;14(3):4419
Rat	MNBA	Oral 1 d Acute	LD_{50} > 5000 mg/kg bw	
Rat	AMBA	Oral 1 d Acute	LD_{50} > 5000 mg/kg bw	
Rat	Mesotrione	Long-term	**NOEL = 2.5 mg/kg diet = 0.3 mg/kg bw/d**	

* Based on highest treatment dose – no significant adverse effects in study

9.3.1.1 Justification for new endpoints

The used endpoints were the EU agreed ones.

9.3.2 Risk assessment for spray applications

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

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

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

Table 9.3-2: First-tier assessment of the acute and long-term/reproductive risk for mammals regarding Rimsulfuron due to the use of PRIMARY MX in maize

Intended use	Maize				
Active substance/product	Rimsulfuron				
Application rate (g/ha)	1 x 9.9				
Acute toxicity (mg/kg bw)	5000				
TER criterion	10				
Crop scenario	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90}**	**TER_a**

Growth stage				**(mg/kg bw/d)**	
Maize	Small omnivorous mammal	136.4	1.0	1.35	3702.7
Reprod. toxicity (mg/kg bw/d)	11.8				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize	Small omnivorous mammal	72.3	1.0 x 0.53	0.38	31.1

Table 9.3-3: First-tier assessment of the acute and long-term/reproductive risk for mammals regarding Nicosulfuron due to the use of PRIMARY MX in maize

Intended use	**Maize**				
Active substance/product	Nicosulfuron				
Application rate (g/ha)	1 x 39.6				
Acute toxicity (mg/kg bw)	> 5000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize	Small omnivorous mammal	136.4	1.0	5.40	925.7
Reprod. toxicity (mg/kg bw/d)	3861				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize	Small omnivorous mammal	72.3	1.0 x 0.53	1.52	2544.4

Table 9.3-4: First-tier assessment of the acute and long-term/reproductive risk for mammals regarding Mesotrione due to the use of PRIMARY MX in maize

Intended use	**Maize**				
Active substance/product	Mesotrione				
Application rate (g/ha)	1 x 118				
Acute toxicity (mg/kg bw)	> 5000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize	Small omnivorous mammal	136.4	1.0	16.10	310.7
Reprod. toxicity (mg/kg bw/d)	0.3				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**

Maize BBCH 10-19	Small insectivorous mammal "shrew". 100% ground arthropods. Ground dwelling invertebrates without interception.	4.2	1.0 x 0.53	0.26	**1.1**
Maize BBCH 10-29	Small herbivorous mammal "vole". All maize shoots + later grass. Grass + cereals	72.3	1.0 x 0.53	4.52	**0.1**
Maize BBCH 10-29	Small omnivorous mammal "mouse". 25% weeds 50% weed seeds 25% ground arthropods. Combination (invertebrates with interception)	7.8	1.0 x 0.53	0.49	**0.6**

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 for mesotrione has been re-done considering a reduction on rate from 118 g a.s./ha to 90 g a.s./ha.

Table 9.3-5: First-tier assessment of the acute and long-term/reproductive risk for mammals regarding Mesotrione due to the use of PRIMARY MX in maize

Intended use	**Maize**				
Active substance/product	Mesotrione				
Application rate (g/ha)	1 x 90				
Acute toxicity (mg/kg bw)	> 5000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize	Small omnivorous mammal	136.4	1.0	12.28	407.17
Reprod. toxicity (mg/kg bw/d)	0.3				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize BBCH 10-19	Small insectivorous mammal "shrew". 100% ground arthropods. Ground dwelling invertebrates without interception.	4.2	1.0 x 0.53	0.20	**1.5**
Maize BBCH 10-29	Small herbivorous mammal "vole". All maize shoots + later grass. Grass + cereals	72.3	1.0 x 0.53	3.45	**0.09**
Maize BBCH 10-29	Small omnivorous mammal "mouse". 25% weeds 50% weed seeds 25% ground arthropods. Combination (invertebrates with interception)	7.8	1.0 x 0.53	0.37	**0.81**

zRMS comments:

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

The presented above mammal's risk assessment is agreed by the zRMS. All TER_A values exceed the relevant triggers indicating that SHA4307/PRIMARY MX does not pose an unacceptable acute risk to mammals following applications according to recommended use pattern. In case of the long-term risk the TER_{LT} values for rimsulfuron as well as for nicosulfuron exceed the relevant triggers indicating an acceptable risk. However, for mesotrione the TER_{LT} values were below relevant triggers indicating an un acceptable risk. Therefore, further refinement is needed.

Risk Assessment for combined exposure

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

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

With:

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

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

Acute risks from combined exposure

The active substance content of the formulation PRIMARY MX addressed in this dossier is Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG, making up a total of 510 g a.s./Kg product. According to GAP, the maximum application rate is 0.250 ~~0.33~~ kg product/ha, therefore, an application rate of 127.5 ~~168.3~~ g a.s./ha was considered in the assessment.

Table 9.3-7 shows the calculation of the predicted LD_{50} (mix) of Rimsulfuron, Nicosulfuron and Mesotrione when mixed in these proportions (step 1 in Appendix B to the EFSA GD 2009).

Table 9.3-6: Mammalian LD_{50} (mix) for Rimsulfuron, Nicosulfuron and Mesotrione when combined as PRIMARY MX (step 1 in EFSA GD 2009, Appendix B)

	Rimsulfuron	Nicosulfuron	Mesotrione
Content in the formulation PRIMARY MX	3%	12%	36%
Fraction in the a.s. mixture	0.06	0.24	0.71
LD_{50} of a.s. [mg/kg bw]	>5000	>5000	>5000
Fraction / LD_{50}	0.000012	0.000047	0.000141
Sum	0.0002		

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

1/ sum = predicted LD_{50} (mix)	5000 mg mix/kg bw

Table 9.3-7: Mammalian "tox per fraction" for the PRIMARY MX (step 1 in EFSA GD 2009, Appendix B)

	Rimsulfuron	Nicosulfuron	Mesotrione	"mix"
Content in the formulation PRIMARY MX	3%	12%	36%	51%
Fraction in mixture	0.06	0.24	0.71	1.0
LD_{50} (mg/kg bw)	>5000	>5000	>5000	5000
Tox per fraction	85000	21250	7083.3	5000
Contribution to predicted toxicity	5.88%	23.53%	70.59%	

Rimsulfuron contributes to 5.88% to mixture toxicity, nicosulfuron have an impact on the predicted risk of 23.53% and mesotrione of 70.59%, therefore, surrogate LD_{50} was used in the acute risk assessment.

Table 9.3-8: Screening step assessment of the acute risk for mammals due to the use of PRIMARY MX in all crops

Intended use	Maize				
Active substance/product	PRIMARY MX				
Application rate (g/ha)	1 x 127.5 ~~168.3~~				
LD_{50} (mix) (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**
Screening	Indicator species for screening	136.4	1.0	17.39 ~~22.96~~	287.52 ~~217.81~~

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.

According to reults, no unacceptable acute risk is obtained for combined exposure acording to the proposed GAP.

zRMS comments:

The presented risk assessment for mammals based on acute combined exposure is agreed by the zRMS.

Regarding chronic risk assessment, the Applicant considers that, according to EFSA/2009/1438, the calculation of a combined toxicity is not applicable to the risk assessment for reproductive effect. Due to differences in evaluated endpoints and the dependency of the derived NOEL of the test design, any calculated TERmix value can only be used for illustrating purposes. Hence, in the case of an unacceptable TERmix, it has to be discussed if the results of the toxicity studies present any evidence for a possible concentration additivity of the effects and risks.

In addition, the combined toxicological effect of these two active substances has not been investigated with regard to repeated dose toxicity. Possibly, the combined exposure to these active substances may lead to a different toxicological profile than the profile(s) based on the individual substances.

Despite all of this, the reproductive risk from combined exposure has been performed by the Applicant:

Reproductive risks from combined exposure

Table 9.3-9: Avian NOEL (mix) for Rimsulfuron, Nicosulfuron and Mesotrione when combined as PRIMARY MX (step 1 in EFSA GD 2009, Appendix B)

	Rimsulfuron	Nicosulfuron	Mesotrione
Content in the formulation PRIMARY MX	3%	12%	36%
Fraction in the a.s. mixture	0.06	0.24	0.71
NOEL of a.s. [mg/kg bw]	11.8	3861	0.3
Fraction / NOEL	0.00499	0.00006	2.35294
Sum	2.35799		
1/ sum = predicted NOEL (mix)	0.4241 mg mix/kg bw		

Table 9.3-10: Avian "tox per fraction" for the PRIMARY MX (step 1 in EFSA GD 2009, Appendix B)

	Rimsulfuron	Nicosulfuron	Mesotrione	"mix"
Content in the formulation PRIMARY MX	3%	12%	36%	51%
Fraction in mixture	0.06	0.24	0.71	1.0
NOEL (mg/kg bw)	11.8	3861	0.3	0.4241
Tox per fraction	200.6	16409.25	0.425	0.4241
Contribution to predicted toxicity	0.21%	0.00%	99.79%	

The tox per fraction is 200.6 for Rimsulfuron, 16409.25 for Nicosulfuron and 0.425 for Mesotrione. The NOEL for Mesotrione and surrogate NOEL are very similar this indicates that this active substance will contribute to ≥ 90 % to mixture toxicity, while the other components of the mixture will only have a marginal impact on the predicted risk. Consequently, the risk assessment will be driven by mesotrione, and hence the risk from combined exposure is covered by this active substance.

zRMS comments:

The presented risk assessment for mammals based on combined exposure is agreed by the zRMS.

The surrogate NOEL indicates that active substance mesotrione will contribute to ≥ 90 % to mixture toxicity, while the other components of the mixture will only have a marginal impact on the predicted risk. Consequently, the risk assessment can beperformed for the most toxic active substance – mesotrione alone.

9.3.2.2 Higher-tier risk assessment

Mesotrione
Higher tier risk assessment is required for risk resulting from exposure to mesotrione following application of product to crops for the uses and scenarios:

- Maize, BBCH 10-19, Small insectivorous mammal "shrew" (*Sorex araneus*)
- Maize, BBCH 10-29, Small herbivorous mammal "vole" (*Microtus arvalis*)
- Maize, BBCH 10-29, Small omnivorous mammal "mouse" (*Apodemus sylvaticus*)

Identification of relevant focal mammal species in early maize (BBCH 12-18)
First of all, there are many reasons why the risk assessment for vole is considered to be covered through the assessment of other small mammalian species:

- High fecundity and population recuperation of the vole.
- Primary source of food outside crops fields for the vole.
- Necessity of population control measures since the vole is considered a crop pest when high population levels are reached.
- Other agricultural techniques being also means of population control

Also, according to Pesticides Peer Review 111 Experts' Meeting on Ecotoxicology (3 -7 February 2014), it was concluded, based on literature data, that common voles do not inhabit maize fields at early growth stages. Therefore it is not a relevant species (refence: BENI, Bentazone 87% SG (SHA 0900 C)) and therefore acceptable risk can be conclude for common voles in BBCH 12-18.

Moreover, as PRIMARY MX is an herbicide, application will directly have an impact on the attractiveness and availability of the food for herbivores. (EFSA Journal 2015; 13(4):4077) This may be taken as a weight of evidence approach.

Consequently, voles are actively controlled by intense culturing, catching or by use of biocides/pesticides. In consideration of this, it is obvious that it is not possible to apply the same protection goal to the vole as to the other indicator species. Instead, it is more appropriate to use a lagomorph and an omnivore as representative generic focal species of herbivorous

Moreover, according to the Ctgb[3], it can be considered plausible that maize fields in an early stage are not a very attractive habitat to shrews. Therefore, the risk for insectivorous mammals is considered acceptable. Several field studies indicated that only wood mice are relevant in early stages of maize. Incidental feeding inside a maize field by lagomorphs, insectivores and small herbivores cannot be excluded, but this will not have an effect on population level.

In addition, based in generic field studies performed in Southern France and Austria and literature data, only the Wood mouse (*Apodemus sylvaticus*) and the European hare (*Lepus europaeus*) were identified as suitable focal species in maize fields. Same findings were reported in studies already evaluated by EFSA[4][5].

According to EFSA conclusions (2016), as well as other bibliography resources which display similar scenarios (i.e. Sulcotrione DAR (2006) and EFSA conclusions (2008)), the hare (*L. europaeus*) is a typical inhabitant of open areas, and may be found in cereal fields in the early developmental stages when the crop provides no shelter for small mammals. This might coincide with the application of PRIMARY MX early post-emergence of maize in late spring/early summer. The breeding season for *L. europaeus* is between midwinter (January/February) and mid-summer and coincides with the application time of PRI-

[3] https://pesticidesdatabase.ctgb.nl/authorisation/?id=15093&category=PPP
[4] http://www.efsa.europa.eu/en/efsajournal/pub/3540
[5] http://www.efsa.europa.eu/en/efsajournal/pub/4419

MARY MX. In June-August the hare's diet consists of 94% green plant material and 6% cereals (Zörner, H. 1989). However, in the Tier 2 risk assessment a worst case scenario is laid down in assuming a diet of 100% plant material.

Hence, the risk assessment for long-term exposure in maize fields will be focused in the small omnivorous mammal "mouse" (*A. sylvaticus*) and in large herbivorous mammal "hare" (*L. europaeus*). In order to refine the risk assessment, the below refined parameters were considered.

~~Toxicological endpoint~~

~~The TER calculation was based on the NOEL obtained in a multi-generations study in rats (specie chosen instead of mouse because more sensitive to mesotrione) based on reduced litter sizes. A NOAEL of 1.2 mg/kg bw/d corresponds to the reproduction and development endpoints of the first two generations F0 and F1 only. The continuous exposure of individuals for three generations can be considered unrepresentative of the exposure after one application of mesotrione.~~

~~Therefore, the NOAEL of 1.2 mg/kg bw/d considered as endpoint is more relevant for an ecotoxicological assessment and can be used for a refined long-term risk assessment to mammals.~~

~~Moreover, in the course of national evaluation of mesotrione containing product Elumis by the Competent Authority in Netherlands, the following findings were concluded:~~

~~An additional study was provided and summarized by the Ctgb (section toxicology). In order to address the potential long-term effects caused by a short exposure to mesotrione residues, a 28-day study was carried out with male rats (the most sensitive organisms to tyrosinemia effects). The study mimicked the potential exposure to mesotrione residues by altering the exposure concentrations provided in the food from a maximum of 13.03 mg/kg bw/day to 0.025 mg/kg bw/day. This study indicated that short-term exposure to rats will not cause tyrosinemia linked effects at concentrations up to an initial maximum exposure of 100 mg/kg diet (13.03 mg/kg bw/day). For the purpose of the risk assessment, the average two exposure value during the 28-day test period of 2.4 mg/kg bw/day has been used as the endpoint (Toelatingsnummer 13192 N, 12-06-2009, http://www.ctb.agro.nl/ctb_files/13192_01.html).~~

~~Therefore, the NOEL of 2.4 mg/kg bw/d will be used as an option in the risk assessment.~~

$\underline{DT_{50}}$

In the Tier I risk assessment, for the dissipation and degradation of residues from plant material a default DT_{50} value of 10 days was assumed. However, ~~four~~ decline trials on cereals are available for SEU and CEU (please refer to the DAR of mesotrione, Annex B.7, Residues data and the attached degradation kinetic assessments) and the results of those studies are detailed below.

Reference	**Crop**	**BBCH**	**Application rate (g a.s./ha)**	**Residues (mg/kg)**	**Time (days)**	**DT_{50} (days)**
France (S) Barnes, J.P. 1997a Trial Ref. DP59806	Maize (Cecilia)	18	150	2.57 (immature) <0.01 (immature) <0.01 (forage) <0.01 (silage)	0 15 60 91	1.87
France (S) Barnes, J.P.1997a Trial Ref. DP59806	Maize (Cecilia)	17-18	150	7.57 (immature) <0.01 (immature) <0.01 (forage) <0.01 (silage)	0 14 60 77	1.46
France (S) Barnes, J.P. 1997b Trial Ref. DP59808	Maize (Volga)	17	200	19 (immature) <0.01 (immature) <0.01 (forage) <0.01 (silage)	0 12 61 91	1.10

Reference	Crop	BBCH	Application rate (g a.s./ha)	Residues (mg/kg)	Time (days)	DT_{50} (days)
France (S) Barnes, J.P. 1997b Trial Ref. DP59808	Maize (Cecilia)	16	200	14.4 (immature) <0.01 (immature) <0.01 (forage) <0.01 (silage)	0 14 61 92	1.33
					Mean	**1.44**
				DT_{50}	**Geomean**	**1.41**
					90th percentile	**1.75**

Reference	Crop	BBCH	App. rate (g a.s./ha)	Residues (mg/kg)	Time (days)	DT_{50} (days)
Ile de France North France Barnes, J.P et al., 1997a DP 59806	Maize (Bangy)	6-8 leaves (16-18 BBCH)	150	4.58 (immature) <0.01 (immature) <0.01 (forage) <0.01 (silage)	0 14 63 80	1.58
Normandy North France Barnes, J.P et al., 1997b DP 59808	Maize (LG2243)	6-8 leaves (16-18 BBCH)	200	20.0 (immature) <0.01 (immature) <0.01 (forage) <0.01 (silage)	0 14 56 90	1.28
Sleswig-Holstein Germany Barnes, J.P et al., 1997c DP 59810	Maize (Diamant)	7 leaves (17 BBCH)	150	9.23 (immature) <0.01 (immature) <0.01 (forage) <0.01 (silage)	0 13 32 68	1.32
Bavaria Germany Barnes, J.P et al., 1997c DP 59810	Maize (General)	7 leaves (17 BBCH)	150	10.31 (immature) <0.01 (immature) <0.01 (forage) <0.01 (silage)	0 14 32 73	1.4
Bavaria Germany Barnes, J.P et al., 1997c DP 59810	Maize (Graf)	6-7 leaves (16-17 BBCH)	150	11.56 (immature) <0.01 (immature) <0.01 (forage) <0.01 (silage)	0 14 32 78	1.38
Saxe-Anhalt Germany Barnes, J.P et al., 1997c DP 59810	Maize (Anjou 207)	6-7 leaves (16-17 BBCH)	150	5.98 (immature) <0.01 (immature) <0.01 (forage) <0.01 (silage)	0 15 30 70	1.63
Sleswig-Holstein Germany Barnes, J.P et al., 1997d DP 59812	Maize (Janna)	6 leaves (16 BBCH)	200	23.2 (immature) 0.05 (immature) <0.01 (forage) <0.01 (silage)	0 14 44 86	1.58
Bavaria Germany Barnes, J.P et al., 1997d DP 59812	Maize (Ilias)	7 leaves (17 BBCH)	200	10.9 (immature) <0.01 (immature) <0.01 (forage) <0.01 (silage)	0 14 35 110	1.39

Reference	Crop	BBCH	App. rate (g a.s./ha)	Residues (mg/kg)	Time (days)	DT_{50} (days)
Saxe-Anhalt Germany Barnes, J.P et al., 1997d DP 59812	Maize (Helix)	8 leaves (18 BBCH)	200	9.21 (immature) <0.01 (immature) <0.01 (forage) <0.01 (silage)	0 14 36 88	1.42
					Mean	**1.44**
				DT_{50}	**Geomean**	**1.44**
					90th perc.	**1.59**

The calculated mean DT_{50} was 1.44, both for SEU and CEU, the geometric mean was 1.41 (SEU) and 1.44 (CEU), and the 90th percentile was 1.75 (SEU) and 1.59 (CEU). For the refinement of the long-term risk, the worst case 90th percentile DT_{50} value of 1.75 d was used. The degradation kinetic assessments are included in separate documents. In addition, as indicated in table above, the residues in immature maize were shown to have declined to <0.01 mg a.s./kg (below the limit of quantification) within 14 days when application was made at proposed rates.

TWA

In the Tier I assessment, a default TWA = 0.53 was used (estimates time-weighted exposure over 21 days, assuming a default DT_{50} of 10 days). However, the estimated decline of the residues of Mesotrione on plants is lower than the default value of 10 days. Considering the 90th percentile DT_{50} of 1.75 d, the TWA factor was re-calculated considering the formula of the EFSA/2009/1438, and the resulting TWA was 0.12. This value was used for the refinement.

PD values

For woodmouse, the modification of diet is proposed following literature data (Peltz, 1989), as already accepted by the EFSA (*Conclusion on the peer review of the pesticide risk assessment of the active substance topramezone*. EFSA Journal 2014;12(2):3540). In May, wood mouse diet consists of: 0.16 plant leaves, 0.34 weed seeds, 0.1 large arthropods and 0.4 earthworms. RUD in earthworms was calculated according to the bioaccumulation equation and the soil PEC value.

PT refinement

After radio tracking of wood mice performed in an ecological field study in Austria in pre-emergent and early post-emergent maize and sugar beet fields, the following PT information was obtained:

- Germinated maize: Five tracking session of 2 potential consumers, 1 of which was a consumer. The range of PT was 0.0 – 3.8% with a mean = 2.3% and a 90th percentile of 3.7%.
- Germinated sugar beet: Eight tracking session of 5 potential consumers, 3 of which were consumers. The range of PT was 0.0 – 100% with a mean = 31.5% and a 90th percentile of 93.1%.

Data set for maize was considered too small for calculation of a reliable PT value for risk assessment. 90th percentile PT value for consumers in maize and sugar beet combined = 98%.

In order to refine PT, applicant refers to the following field monitoring studies for which access is under negotiation:

After a generic field study performed in Germany on small mammals focal species and wood mouse (*Apodemus sylvaticus*) [xxx, T. 2013; P12225], a PT of 0.139 was obtained in maize fields, which was considered for risk assessment in woodmouse.

A generic monitoring study for European hare determined a PT of 0.62 in early maize fields in central Europe [xxx 2019; R1740045]. This PT was considered for risk assessment in hare.

Deposition factor

According to EFSA Guidance Document to obtain DegT50 values (EFSA Journal 2014;12(5):3662), the interception for maize in BBCH 12-18 would be 25%, which would remain in the plant material; hence the 75% of the total applied product would end up on earthworms, small seeds and ground arthropods.

Higher tier risk assessment for woodmouse

A FIR/bw corresponding to modified diet of woodmouse was calculated in accordance to the EFSA GD

Table 9.3-11: Calculation of FIR for woodmouse

Species	Body weight	Diet item	Daily energy expenditure, DEE [kJ/d]	Food energy, FE [kJ/d]	Moisture content, MC [%]	Assimilation efficiency, AE [%]	FIR	FIR/bw
Small omnivorous mammal "mouse"	21.70	Non-grass weeds & leafy crops	58.83	17.8	88.1	76	36.54	1.68
		Earthworms	58.83	19.3	84.6	85	23.28	1.07
		Small seeds	58.83	21.7	9.9	84	3.58	0.17
		Ground arthro-pods	58.83	22.7	68.8	87	9.55	0.44

As it was mentioned above, RUD in earthworms was calculated according to the bioaccumulation equation and the soil PEC value.

Bioconcentration factor for the earthworm ($BCF_{earthworm}$) was calculated using the equation:

$$BCF = (0.84 + 0.01K_{OW}) / (f_{OC} \times K_{OC})$$

With:
Koc = Organic carbon adsorption coefficient
foc = Organic carbon content of soil (take 0.02 as a default value)

Estimate residues in earthworms were then calculated using the equation:
$PEC_{earthworm} = PEC_{soil} \times BCF_{earthworm}$

Table 9.3-12: Calculation of RUD surrogate value in earthworms

Kow	Foc	Koc	$BCF_{earthworm}$	PEC_{soil}	PEC_{worm}
1.29	0.02	109	0.391	0.118	0.046

Table 9.3-13: Refined reproductive risk assessment for woodmouse following application of mesotrione to maize – refined parameters (*) are further described and justified in the text

Species	FIR/bw	Rate, kg as/ha	RUD unit	RUD	PD*	PT*	TWA*	MAF	AV	DF	ETE	∑ETE	NOEL*	TER
Small omnivorous mammal "mouse"	1.68	0.118	Non-grass weeds & leafy crops	28.7	0.16	0.139	0.12	1	1	1	0.015	0.03	0.3	9.2
	1.07		Earthworms	0.05	0.4	0.139	0.53	1	1	1/0.75	0.00			
	0.17		Small seeds	40.2	0.34	0.139	0.53	1	1	1/0.75	0.015			
	0.44		Ground arthropods	7.5	0.1	0.139	0.53	1	1	1/0.75	0.002			

Please refer to Toxicological endpoints

As the TER is above the trigger, it is concluded that no unacceptable risk to small herbivorous mammals is expected.

Higher tier risk assessment for brown hare

Table 9.3-14: Refined reproductive risk assessment for brown hare following application of mesotrione to maize – refined parameters (*) are further described and justified in the text

Species	bw (g)	FIR/bw	Rate, kg as/ha	RUD unit	RUD	PD	PT*	TWA*	MAF	AV	DF	ETE	NOEL	TER
Large herbivorous mammal brown hare	3230	0.334	0.118	Crop shoots	54.2	1	0.62	0.12	1	1	0.25	0.04	0.3	7.6

* Please refer to Toxicological endpoint

As the TER is above the trigger, it is concluded that no unacceptable risk to large herbivorous mammals is expected.

Refinement options for mammals

Focal species

The relevant focal species for maiz at early stages, as previously discussed, are the small omnivorous mammal "mouse" (*A. sylvaticus*) and the large herbivorous mammal "hare" (*L. europaeus*). This refinement option is in line with the conclusions at EU level.

PT refinement

In order to refine the PT value, applicant refers to the following field monitoring studies.

Wood mouse

xxx (2013): Generic field study on small mammals focal species and wood mouse (*Apodemus sylvaticus*) PT in maize fields in Germany, Report P12225 [Applicant has access to this study and LoA is provided].

From this study, conducted in Germany in early stages of maiz fields, a PT of 0.139 for the omnivorous wood mouse was agreed at EU level. Therefore the PT of 0.139 will be used in the higher tier risk assessment for wood mouse

Brown hare

xxx (2019): Generic monitoring of European hares to determine proportion of time spent foraging in early maize in Central Europe, Report R1740045 [Applicant has access to this study and LoA is provided] ~~is negotiating access to this study~~].

In this study, hares were monitoized in two Central Zone countries (Hungary and Germany) in maize field at early growth stages. From this study a mean PT of 0.36 and a 90th percentile PT of 0.62 were estimated from 21 radiotracked individuals that were observed to be crop consumers. For the higher tier risk assessment, the 90th percentile PT of 0.62 will be used.

PD refinement

Wood mouse

For the higher tier risk assessment, the default diet of wood mouse as indicated in EFSA (2009) will be considered: 25% weeds, 50% weed seeds and 25% ground arthropods.

Brown hare

In the higher tier risk assessment it will be considered that hares will feed exclusively on maize shoots available in the field as a worst case assumption.

In addition, additional calculations by applicant will be done for hare by considering a mixed diet based on comments received from the zRMS. For mixed diet, the brown hare diet relevant for maize is indicated in the Northern Zone Guidance Document. According to the Guidance Document in Norther Zone, the PD values for hare in maize in spring at growth stage BBCH 10-19 is 0.84 monocotyledons (cereals, grasses) and 0.16 dicotyledons (leafy crops, non-grass weeds). The PD values derived for hare are based on published data from studies of different authors (Frylestam 1980a, Tapper and Barnes 1986, Chappuis

1990, Olesen and Asferg 2006 and Hansen 1990).

DT_{50} and ftwa

Maize

Applicant refers to the residue decline study of North L (2016): Mesotrione – Foliage Decline study with A12739A on maize in Northern France and the United Kingdom in 2015, SYN File A12739A_11065. [Applicant has access to this study and LoA is provided] ~~is negotiating access to this study~~].

In this study five residue decline field trials on maize were conducted in Northern France and the United Kingdom in summer. Plant samples were collected at < 1 hour after application (HAA), 4 HAA, 10 HAA, 24 HAA, 34 HAA, 48-51 HAA, 72-78 HAA and 96-99 HAA. Samples were analysed for mesotrione and the results of the analysis for samples are given in the below table.

Application rate (g a.s./ha)	Crop part	Sampling	Mesotrione Residue (mg/kg)				
			Trial S15-02057-01 (UK)	Trial S15-02057-03 (UK)	Trial S15-02057-04 (UK)	Trial S15-02057-05 (FR)	Trial S15-02057-06 (UK)
1 x 150 g a.s./ha	Whole plant	< 1 HAA	7.09	13.96	4.24	14.99	3.09
		4 HAA	8.48	7.75	2.98	12.63	2.74
		10 HAA	4.11	6.25	3.33	8.61	2.05
		24 HAA	3.86	3.57	1.69	4.30	0.91
		34 HAA	2.79	2.95	0.50	2.19	0.80
		48-51 HAA	0.92	1.37	0.41	1.07	0.36
		72-78 HAA	0.16	0.63	0.14	0.31	<0.01
		96-99 HAA	0.12	0.11	0.06	0.13	0.10
Control	Whole plant	< 1HBA	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

The kinetic evaluation of the results of the study by North (2016) was performed by the softwere CAKE© version 3.4 by the applicant and calculations are provided in a separate report (Izquiero J.J., 2021) in Appendix 2. A summary of the derived DT_{50} and DT_{90} values

is presented in the table below.

Trial	DT_{50} (hours)	DT_{50} (d)	DT_{90} (hours)	χ^2 (%)	Model
Trial S15-02057-01(UK)	19.5	0.813	64.8	20.3	SFO
Trial S15-02057-03 (UK)	12.3	0.513	40.9	19.1	
Trial S15-02057-04 (UK)	16	0.679	53.3	16.3	
Trial S15-02057-05 (France)	12.8	0.533	42.5	2.93	
Trial S15-02057-06 (UK)	15.4	0.642	51.1	6.01	
Geomean (n = 5)	15.0	0.627			

Hence, the highest DT_{50} of 0.~~803~~ 0.813 days obtained from the study is used for the refinement of the ftwa, which gives a value of 0.055 that will be used in the higher tier risk assessment.

Weeds

Applicant refers to the residue decline study of Allen L (2019: Mesotrione – Foliage Decline study on clover in Hungary, Germany, United Kingdom, Northern France and Belgium in 2018, SYN File A12738A_10535. Applicant ~~is negotiating~~ has access to this study and LoA was provided].

In this study, eleven residue decline trials were conducted on clover in Hungary, Germany, United Kingdom, Northern France and Belgium. Following the application, treated clover plant samples were collected at < 1hour after application (HAA), 8 HAA, 24 HAA, 32 HAA, 48 HAA, 3 days after application (DAA), 4 DAA and 7 DAA and untreated clover whole plant samples were collected < 1 hour before

application (HBA). Samples were analysed for Mesotrione and results of analyses are given in the below table.

Time	SRUK 18-001-037HR	SRUK 18-002-037HR	SRHU 18-053-037HR	SRHU 18-054-037HR	FRFR 18-010-037HR	SRFR 18-011-037HR	SRDE 18-001-037HR	SRDE 18-002-037HR	SRPL 18-014-037HR	SRPL 18-015-037HR	G006-18H
0	6.10	3.63	11.97	11.69	11.51	8.75	4.46	9.11	6.15	6.50	8.58
8 HAA	6.03	4.20	11.41	8.99	8.78	9.98	5.66	2.71	4.34	4.58	8.48
24 HAA	4.58	3.39	11.02	8.76	9.86	8.73	4.59	2.59	2.28	4.72	8.17
32 HAA	2.69	4.09	9.02	8.80	6.72	4.77	3.98	3.29	2.06	6.37	5.65
48 HAA	2.73	2.61	7.14	6.89	5.47	4.66	4.21	2.54	1.78	5.78	5.54
72 HAA	1.95	2.17	6.06	5.51	2.00	4.77	0.22	2.61	3.82	1.8	3.26
96 HAA	0.58	2.76	0.14	0.12	0.58	4.37	0.08	0.43	1.67	1.66	3.43
168 HAA	0.27	0.19	0.11	0.07	0.09	0.70	0.09	0.05	1.23	0.27	1.70
0 DBA	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

DBA: days before application, HAA: hours after application

The kinetic evaluation of the results of the study by Allen (2019) was performed with the software CAKE© version 3.4 by the applicant and calculations are provided in a separate report (Izquierdo J.J. 2021). A summary of the derived DT_{50} and DT_{90} values is presented in the table below.

Trial	DT_{50} (hours)	DT_{50} (d)	DT_{90} (hours)	χ^2 (%)	Model
SRUK18-001-037HR	35.9	1.50	119	11.4	SFO
SRUK18-002-037HR	85.8	3.58	285	16	
SRHU18-053-037HR	47.7	1.99	159	17	
SRHU18-054-037HR	48.3	2.01	160	17.8	
SRFR18-010-037HR	37.1	1.55	123	16.7	
SRFR18-011-037HR	61.7	2.57	205	15.8	
SRDE18-001-037HR	42.5	1.77	141	29.2	
SRDE17-002-037HR*	25.3	1.05	83.9	40.1	Unreliable
SRPL18-014-037HR	57.6	2.40	192	29.7	SFO
SRPL18-015-037HR	63.2	2.63	210	23.8	
G006-18H	63.6	2.65	211	8.94	
Geometric mean	52.53	**2.19**			

* Not used for geomean calculations

For the refinement, is is proposed to use the geometric mean DT_{50} value of 2.19 days.

The ftwa factor was recalculated considering the formula of the EFSA/2009/1438 and the DT_{50} of 2.19 d. The obtained ftwa value is 0.150 that will be used in the higher tier risk assessment.

Food intake rate

Wood mouse

According to the EFSA (2009), the FIR/bw for wood mouse considering the default mixed diet as will be used in the higher tier risk assessment is 0.27. Therefore, this value will be considered in the refinement for wood mouse

Brown hare

The FIR/bw for hare was calculated by the applicant considering a body weight of 3800 g as indicated in the EFSA (2009) and considering that hare will feed exclusively on maize shoots (monocots). In addition, Applicant has also calculated the FIR/bw considering the mixed diet as proposed above. Calculation is presented below. The FIR/bw was calculated using the equation provided in Appendix G of the Guidance Document.

$$FIR = \left(\frac{DEE}{FE * \left(1 - \frac{MC}{100}\right) * \left(\frac{AE}{100}\right)} \right) \quad [\text{g fresh weight/d}]$$

In which

$$\log DEE = \log a + b \times \log bw$$

Table 9.3-15: Calculation of FIR/bw for hare (monocotyledons)

Food type	Daily energy expenditure (DEE)	bw (g)	Food energy (kJ/dry g)	Moisture content (%)	Assimilation efficiency (%)	FIR (g/day)	FIR/bw
Monocots (maize shoots)	2363.444	3800	17.6[a]	76.4[a]	47[b]	1210.66	0.32

[a] From table 3 of Appendix G in EFSA (2009)
[b] From table 4 of Appendix G in EFSA (2009)

Table 9.3-16: Calculation of FIR/bw for hare (mixed diet)

Food type	Daily energy expenditure (DEE)	bw (g)	Food energy (kJ/dry g)	Moisture content (%)	Assimilation efficiency (%)	FIR (g/day)	FIR/bw
Monocots (maize shoots)	2363.444	3800	17.6[a]	76.4[a]	47[b]	1245.61	0.328
Non-grass herbs			17.8[a]	88.1 [a]	76 [b]		

[a] From table 3 of Appendix G in EFSA (2009)
[b] From table 4 of Appendix G in EFSA (2009)

Application rate
In the proposed GAP the max appl rate of mesotrione is 118 g a.s./ha. For the refinement of the risk to mammals, the maximum application rate of mesotrione will be reduced and 90 g a.s./ha will be considered.

Refined TER calculations for wood mouse and hare are presented in the tables below.

Table 9.3-~~17~~16: Refined reproductive risk assessment for woodmouse following application of mesotrione to maize – refined parameters (*) are further described and justified in the text

Intended use	Maize
Active substance/product	Mesotrione
Application rate (g/ha)	1 x 90*
Reprod. Toxicity (mg/kg bw/d)	0.3

TER criterion		5						
Crop scenario Growth stage	**Generic focal species**	**PD/diet type**	**FIR/bw**	**RUD**	**MAFm x f_{twa}**	**PT**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
BBCH 10-29	Small omnivorous mammal "mouse"	0.25 (plant material - maize)	0.27	54.2	1 x 0.055*	0.139*	0.003	
		0.25 (ground arthropods)		3.5[a]	1 x 0.53	0.139*	0.002	
		0.50 (weed seeds)		40.2	1 x 0.53	0.139*	0.036	
						Total	0.040	7.49

[a] According to Appendix A from EFSA (2009), RUD values for arthropods with interception are relevant to maize at BBCH 10-29

According to the results, an acceptable risk is obtained for wood mouse following the application of Primary MX when the application rate of 90 g mesotrine/ha is considered.

Table 9.3-~~18~~17: Refined reproductive risk assessment for hare following application of mesotrione to maize – refined parameters (*) are further described and justified in the text

Intended use		Maize						
Active substance/product		Mesotrione						
Application rate (g/ha)		1 x 90*						
Reprod. Toxicity (mg/kg bw/d)		0.3						
TER criterion		5						
Crop scenario Growth stage	**Generic focal species**	**PD/diet type**	**FIR/bw**	**RUD**	**MAFm x f_{twa}**	**PT**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
BBCH 10-29	Large herbivorous mammal "hare"	1 / Maize shoots	0.32*	54.2	1 x 0.055*	0.62*	0.05	5.64

Table 9.3-19: Refined reproductive risk assessment for hare following application of mesotrione to maize – refined parameters (*) are further described and justified in the text

Intended use		Maize						
Active substance/product		Mesotrione						
Application rate (g/ha)		1 x 90*						
Reprod. Toxicity (mg/kg bw/d)		0.3						
TER criterion		5						
Crop scenario Growth stage	**Generic focal species**	**PD/diet type**	**FIR/bw**	**RUD**	**MAFm x f_{twa}**	**PT**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
BBCH 10-29	Large herbivorous mammal "hare"	0.84 (Maize shoots)	0.328*	54.2	1 x 0.055*	0.62*	0.05	
		0.16		28.7	1 x 0.150*	0.62*	0.01	

		(dicot weeds)						
						Total	0.06	5.13

According to the results, the TER is above the trigger and an acceptable risk is observed for hare following the application of Primary MX when the application rate of 90 g mesotrione/ha is considered.

Further refinement - RUD

Even although in the higher tier risk assessment presented above the TERlt values for wood mouse and hare were above the trigger, it has to be noted the default mean RUD value as given in EFSA (2009) for maize was used (54.2). In order to go further in the refined risk assessment, applicant would like to refer to the analysis done by Murfitt *et al.*, 2015 of measured residues on maize foliage (a copy of the document is presented). Murfitt *et al.* analysed a large dataset of industry residue trials (436 trials, 255 in North EU and 181 in South EU) with initial (0 days after application) foliar residue values for spray applications of pesticides to maize plants in growth stage BBCH 10-19 which were converted to RUD values using the application rate. The summary of the measured maize foliage RUD values is given in the table below.

Crop	Residue region	No. of field trials	90th %ile RUD (mg a.s/kg fw)	Mean RUD (mg a.s/kg fw)
Maize	Europe	436	80.3	46.8
	North	255	80.4	46.1
	South	181	79.1	47.6
Grasses and cereals (EFSA, 2009)	Europe	132	102.3	54.2

According to the results, a mean RUD value of 46.8 mg/kg for maize is obtained. Applicant considers this mean RUD value of 46.8 mg/kg as a more realistic RUD value for maize and presents calculations in addition to the calculations based on the default mean RUD value from EFSA (2009) which comes from grass-cerals trials.

Table 9.3-~~20~~19: Refined reproductive risk assessment for woodmouse following application of mesotrione to maize – refined parameters (*) are further described and justified in the text

Intended use		Maize						
Active substance/product		Mesotrione						
Application rate (g/ha)		1 x 90*						
Reprod. Toxicity (mg/kg bw/d)		0.3						
TER criterion		5						
Crop scenario Growth stage	**Generic focal species**	**PD/diet type**	**FIR/bw**	**RUD**	**MAFm x f_{twa}**	**PT**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
BBCH 10-29	Small omnivorous mammal	0.25 (plant material - maize)	0.27	46.8*	1 x 0.055*	0.139*	0.002	
		0.25 (ground		3.5[a]	1 x 0.53	0.139*	0.002	

	"mouse"	arthropods)						
		0.50 (weed seeds)		40.2	1 x 0.53	0.139*	0.036	
						Total	0.040	7.55

[a] According to Appendix A from EFSA (2009), RUD values for arthropods with interception are relevant to maize at BBCH 10-29

Table 9.3-~~21~~20: Refined reproductive risk assessment for hare following application of mesotrione to maize – refined parameters (*) are further described and justified in the text

Intended use		Maize						
Active substance/product		Mesotrione						
Application rate (g/ha)		1 x 90*						
Reprod. Toxicity (mg/kg bw/d)		0.3						
TER criterion		5						
Crop scenario Growth stage	**Generic focal species**	**PD/diet type**	**FIR/bw**	**RUD**	**MAFm x f_{twa}**	**PT**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
BBCH 10-29	Large herbivorous mammal "hare"	1 / Maize shoots	0.32*	46.8*	1 x 0.055*	0.62*	0.05	6.53

Table 9.3-21: Refined reproductive risk assessment for hare following application of mesotrione to maize – refined parameters (*) are further described and justified in the text

Intended use		Maize						
Active substance/product		Mesotrione						
Application rate (g/ha)		1 x 90*						
Reprod. Toxicity (mg/kg bw/d)		0.3						
TER criterion		5						
Crop scenario Growth stage	**Generic focal species**	**PD/diet type**	**FIR/bw**	**RUD**	**MAFm x f_{twa}**	**PT**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
BBCH 10-29	Large herbivorous mammal "hare"	0.84 (Maize shoots)	0.328*	46.8*	1 x 0.055*	0.62*	0.04	
		0.16 (dicot weeds)		28.7	1 x 0.150*	0.62*	0.01	
						Total	0.05	5.75

TERlt values for wood mouse and hare were above the trigger showing no risk.

zRMS comments:

In order to refine the risk assessment, the below refined parameters were considered by zRMS.

1. Ecologically relevant endpoints for wild mammals:

For Tier 1 risk assessment the reproductive risk assessment for mammals was evaluated by taking into consideration the EU agreed reproductive endpoint of 0.3 mg kKg bw/d following the experts' decision (Pesticides Peer Review meeting of December 2015).

2. Consideration of residue dissipation:

The applicant provided the LoA to study by North 2016 for residue decline for mesotrione in maize.
The kinetic analysis of DT_{50} value in maize based on the data from this study was provided in Appendix 2.
The current refined ftwa of 0.055 parameter based on the $DT_{50\ of}$ 0.813 d for maize was accepted by zRMS.
In addition, the refined parameter of ftwa 0.150 d for dicot plants were estimated based on the DT_{50}=2.19 d from study by Allen, 2019. The kinetic analysis of DT_{50} value in maize based on the data from this study was provided by the applicant in Appendix 2.

3. Identification of relevant focal species: zRMS agrees with the applicant approach that wood mouse nad hare are the most relevant focal species for post-emergene application in maize based on field studies and all availlable information.

4. PT value

Wood mouse

xxx (2013): Generic field study on small mammals' focal species and wood mouse (*Apodemus sylvaticus*) PT in maize fields in Germany, Report P12225. Applicant has access to this study and LoA is provided to zRMS-PL.
From this study, conducted in Germany in early stages of maiz fields, a PT of 0.139 for the omnivorous wood mouse was agreed at EU level. Therefore, the PT of 0.139 will be used in the higher tier risk assessment for wood mouse.

Brown hare

xxx (2019): Generic monitoring of European hares to determine proportion of time spent foraging in early maize in Central Europe, Report R1740045.
In this study, hares were monitoized in two Central Zone countries (Hungary and Germany) in maize field at early growth stages. From this study a mean PT of 0.36 and a 90th percentile PT of 0.62 were estimated from 21 radio-tracked individuals that were observed to be crop consumers. For the higher tier risk assessment, the 90th percentile PT of 0.62 will be used. Applicant has access to this study and LoA is provided to zRMS-PL.

5. PD

PD value for wood mouse was considered in the risk assessment by zRMS according to Appendix A of EFSA (2009). The brown hare diet relevant for maize is indicated in the Northern Zone Guidance Document.

			PD (fresh weight)

Crop	Growth stage	Season	Monocotyledons (Cereals, grasses)	Dicotyledons (Leafy crops, non-grass weeds)	Bush berry plants (buds, leaves)
Maize	BBCH 10-19	Spring	0.84	0.16	-

The FIR/bw for the brown hare with consideration of the bodyweight of 3800 g, as indicated in EFSA (2009) and the mixed diet (PD of 0.84 and 0.16 for monocots and dicots, respectively). Calculation was performed in line with indications of Appendix G of EFSA (2009) and is presented below.

Maize		April-May	
		Maize shoots [f]	Non-grass herbs
Fraction of food item in mixed diet [a]	PD_i fresh (%)	84%	16%
Food energy of food item [i] in mixed diet [b]	FE (kJ/dry g)	17.6	17.8
Moisture content of food item [i] in mixed diet [b]	MC (%)	76.4	88.1
Assimilation efficiency of food item [i] in mixed diet [c]	AE (%)	47	76
Food energy of food item in diet [d]	FE_{item},fresh (kJ/g fresh weight)	1.640	0.258
Food energy of total mixed diet [d]	FE_{total},fresh (kJ/g fresh weight)	1.897	
Daily energy expenditure [d]	DEE (kJ/day)	2363.4	
Food intake rate of total mixed diet [d]	FIR_{total}, fresh (g fresh weight/day)	1245.61	
b.w. [e]	(g)	3800	
FIR/b.w.	(g fresh weight/b.w./day)	0.328	

[a] PD for the hare according to published papers, as discussed above
[b] From Table 3 of Appendix G in EFSA (2009)
[c] From Table 4 of Appendix G in EFSA (2009)
[d] Calculated according to EFSA (2009; Appendix G)
[e] Body weight of brown hare from either the EFSA Guidance (3800g)
[f] Monocot plant material is assumed to be = maize shoots (using the default values for grasses and cereal shoots)

As all parameters are agreed by the zRMS, the FIR/be of 0.328 will be used in the risk refinement.
For brown hare feeding exclusively with maize shoots, the FIR/bw of 0.32 as calculated with consideration of the bodyweight of 3800 g will be used.

In which

$$\log DEE = \log a + b \times \log bw$$

Calculation of food intake rate for the hare considering a single item diet in post-emergence maize

Food item	Hare bodyweight	DEE	FIR	FIR/bw
Maize shoots (grasses/ cereals)	3800 g	2363	1210.7	0.32 [a]

6. Risk assessment for focal species provided by zRMS:

~~Wood mouse~~

~~Intended use~~	~~Maize~~
~~Active substance/product~~	~~mesotrione~~
~~Application rate (g a.s./ha)~~	~~1 × 90~~
~~Reprod. toxicity (mg/kg bw/d)~~	~~0.3~~
~~TER criterion~~	~~5~~

~~Crop scenario Growth stage~~	~~Generic focal species~~	~~PD/diet type~~	~~FIR/bw~~	~~RUD_m~~	~~MAF_m × TWA~~	~~PT~~	~~DDD_m (mg/kg bw/d)~~	~~TER_{lt}~~
~~BBCH 10–29~~	~~Small omnivorous mammal "mouse"~~	~~0.25 (maize)~~	~~0.27~~	~~54.2~~	~~1.0 × 0.055~~	~~0.139~~	~~0.0025~~	
		~~0.5 (seeds)~~		~~40.2~~	~~1.0 × 0.53~~		~~0.036~~	
		~~0.25 (arthropods)~~		~~3.5~~ [1]	~~1.0 × 0.53~~		~~0.0016~~	
						~~Sum of DDD_m~~	~~0.04~~	~~7.5~~

[1] according to Appendix A of EFSA (2009) RUD values for arthropods with interception are relevant for scenario maize at BBCH 10-29

Wood mouse

Intended use	**Maize**
Active substance/product	mesotrione
Application rate (g a.s./ha)	1 × 90
Reprod. toxicity (mg/kg bw/d)	0.3
TER criterion	5

Crop scenario Growth stage	**Generic focal species**	**PD/diet type**	**FIR/bw**	**RUD$_m$**	**MAF$_m$ × TWA**	**PT**	**DDD$_m$ (mg/kg bw/d)**	**TER$_{lt}$**
BBCH 10 -29	Small omnivorous mammal "mouse"	0.25 (weeds)	0.27	28.7	1.0 × 0.15	0.139	0.0036	
		0.5 (seeds)		40.2	1.0 × 0.53		0.036	
		0.25 (arthropods)		3.5 [1]	1.0 × 0.53		0.0016	
						Sum of DDD$_m$	0.0412	7.28

[1] according to Appendix A of EFSA (2009) RUD values for arthropods with interception are relevant for scenario maize at BBCH 10-29

TER$_{LT}$ value is above 5 indicating acceptable risk for wood mouse.

Brown hare

Intended use	**Maize,**
Active substance/product	mesotrione
Application rate (g a.s./ha)	1 × 90
Reprod. toxicity (mg/kg bw/d)	0.3
TER criterion	5

Crop scenario Growth stage	**Focal species**	**PD/diet type**	**FIR/bw**	**RUD$_m$**	**MAF$_m$ × TWA**	**PT**	**DDD$_m$ (mg/kg bw/d)**	**TER$_{lt}$**
BBCH 10 -29	Brown hare	0.84 (maize)	0.328	54.2	1.0 × 0.055	0.62	0.046	
		0.16 (dicot weeds)		28.7	1.0 × 0.150		0.012	
						Sum of DDD$_m$	0/058	5.17
BBCH 10 -29	Brown hare	1.0 (maize)	0.32	54.2	1.0 × 0.055	0.62	0.053	5.66

The risk assessment for hare is considered acceptable as TER$_{LT}$ values are abobe trigger of 5.

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 of 42.4 (geometric mean, n = 4 (EFSA Scientific Report (2005) 45, 1-61)), Rimsulfuron belongs to the group of less sorptive substances.

Effective application rate (g/ha)	=	9.9		
Acute toxicity (mg/kg bw)	=	5000	quotient	= 0.002
Reprod. toxicity (mg/kg bw/d)	=	11.8	quotient	= 0.84

As the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) do not exceed the value of 50 for Rimsulfuron, it is not necessary to conduct a drinking water risk assessment for mammals.

With a K(f)oc of 20.7 (EFSA Scientific Report (2007) 120, 1-91), Nicosulfuron belongs to the group of less sorptive substances.

Effective application rate (g/ha)	=	39.6		
Acute toxicity (mg/kg bw)	=	> 5000	quotient	= 0.008
Reprod. toxicity (mg/kg bw/d)	=	3861	quotient	= 0.01

As the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) do not exceed the value of 50 for Nicosulfuron, it is not necessary to conduct a drinking water risk assessment for mammals.

With a K(f)oc of 14 (EFSA Journal 2016;14(3):4419), Mesotrione belongs to the group of less sorptive substances.

Effective application rate (g/ha)	=	~~118~~ 90		
Acute toxicity (mg/kg bw)	=	> 5000	quotient	= 0.02
Reprod. toxicity (mg/kg bw/d)	=	0.3	quotient	= ~~393.3~~ **300**

Since the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) exceeds the critical value of 50 for maize, a quantitative risk assessment (calculation of TER values) is necessary.

For such purpose, PEC_{puddle} is calculated following the formula described in EFSA/2009/1438, which is presented below:

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

Where:

AR = application rate [g/ha]; divisor of 10 to achieve rate in mg/m^2
w = 0.02 (pore water term: volume)
s = 0.0015 (soil term: volume, density, organic carbon content)

The resulting PEC_{puddle} value is 0.22 ~~0.288~~ mg/L.

Table 9.3-22: Assessment of the risk for mammals due to exposure to Mesotrione via contaminated drinking water in puddles

Intended use	Maize				
Active substance	Mesotrione				
Application rate (g/ha)	1 x 90 ~~118~~				
Reprod. toxicity (mg/kg bw/d)	0.3				
TER criterion	5				
Soil-relevant applic. rate (g/ha)	**Koc (L/kg)**	**PEC_{puddle} (mg/L)**	**DW uptake (L/kg bw/d)**	**Daily dose (mg/kg bw/d)**	**TER_{lt}**
118	14	~~0.288~~ 0.22	0.24	~~0.07~~ 0.05	~~4.3~~ 6

PEC_{puddle}: concentration in puddles; DW: drinking water; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Considering an application rate of 90 g mesotrione/ha the TER_{lt} exceeds the trigger value of 5, hence no risk for mammals is expected due to exposure to Mesotrione via contaminated drinking water in puddles.

zRMS comments:

The risk resulting from exposure to mesotrione, rimsulfuron and nicosulfuron via drinking water is agreed by the zRMS.

~~Considering the worst case Koc of 14 L/kg (pH >7), the TER_{lt} is lower than the long term trigger of 5 indicating an unacceptable long term risk to mammals from drinking water. A refinement of the toxicological endpoint with a NOAEL of 1.2 mg/kg bw/d was performed. The risk assessment was presented below.~~

~~Table 9.3-23: Refinement of assessment of the long-term risk for mammals due to exposure to Mesotrione via contaminated drinking water in puddles~~

~~Intended use~~	~~Maize~~				
~~Active substance~~	~~Mesotrione~~				
~~Application rate (g/ha)~~	~~1 x 118~~				
~~Reprod. toxicity (mg/kg bw/d)~~	~~1.2~~				
~~TER criterion~~	~~5~~				
~~Soil-relevant applic. rate (g/ha)~~	~~Koc (L/kg)~~	~~PEC_{puddle} (mg/L)~~	~~DW uptake (L/kg bw/d)~~	~~Daily dose (mg/kg bw/d)~~	~~TER_{lt}~~
~~90.5~~	~~14~~	~~0.288~~	~~0.24~~	~~0.07~~	~~17.4~~

~~PEC_{puddle}: concentration in puddles; DW: drinking water; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.~~

~~Considering the worst case Koc of 14 L/kg (pH >7), the TER_{lt} is lower than the long term trigger of 5 indicating an unacceptable long term risk to mammals from drinking water.~~

~~According to EFSA/2009/1438, in case of PEC_{puddle} calculation, crop interception may be considered in the same way as for calculation of PEC_{soil}, PEC_{sw} and PEC_{sw} in order to increase realism. The calculation above was performed considering 0% interception as worst case. The Applicant wishes to consider an interception factor of 25% for maize at BBCH 12-18 according to the interception values of FOCUS~~

(2014)[6]. Therefore, for the refinement of the risk a deposition factor of 0.75 should be applied in calculations.

Also, for mesotrione the adsorption is pH dependent. A first tier risk assessment of via contaminated drinking water in puddles was performed considering the worst case of koc of 14 L/kg (pH >7). A new refinement is proposed by the Applicant considering the mean of 83.3 L/kg. The refinement is performed below:

Table 9.3.24: Assessment of the risk for mammals due to exposure to Mesotrione via contaminated drinking water in puddles – * refined parameters

Intended use	Maize
Active substance	Mesotrione
Application rate (g/ha)*	1 x 88.5
Reprod. toxicity (mg/kg bw/d)	0.5
TER criterion	5

Soil relevant applic. rate (g/ha)	Koc* (L/kg)	PEC_{puddle} (mg/L)	DW uptake (L/kg bw/d)	Daily dose (mg/kg bw/d)	TER_{lt}
66.3	83.3	0.064	0.24	0.01	20.5

PEC_{puddle}: concentration in puddles; DW: drinking water; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.
*Application rate considering an interception factor of 25%.

The TER_{lt} exceeds the trigger value of 5, hence no risk for mammals is expected due to exposure to Mesotrione via contaminated drinking water in puddles.

9.3.2.4 Effects of secondary poisoning

The log P_{ow} of Rimsulfuron amounts to -1.46 at pH7 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

The log P_{ow} of Nicosulfuron amounts to 0.61 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

The log P_{ow} of Mesotrione amounts to < -1 at pH 7 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

Risk assessment for earthworm-eating mammals via secondary poisoning

Not required.

zRMS comments:

The evaluation of the risk of secondary poisoning for earthworm-eating mammals for mesotrione, rimsulfuron and nicosulfuron is not triggered due to log Pow being <3.

[6] FOCUS (2014) "Focus groundwater scenarios in the EU review of active substances" Report of the FOCUS Groundwater Scenarios Workgroup, EC Document Reference Sanco/321/2000 rev.2.2, 202 pp.

Risk assessment for fish-eating mammals via secondary poisoning

Not required.

zRMS comments:

The evaluation of the risk of secondary poisoning for fish-eating-mammals for mesotrione, rimsulfuron and nicosulfuron is not triggered due to log Pow being <3.

9.3.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.3.4 Overall conclusions

According to the screening assessment for maize, the TER_a and TER_{lt} values for the active substances Rimsulfuron and Nicosulfuron are greater than the Annex VI trigger of 10 and 5, respectively. After screening and first-tier assessment for active substance Mesotrione, the TER_a value is greater than the Annex VI trigger of 10 whereas TER_{lt} values are lower than the Annex VI trigger of 5 for the use on maize, indicating that PRIMARY MX presents an unacceptable long-term risk to mammals. A refinement of the risk was done by selecting the two focal species european brown hare and wood mouse, using a PT value of ~~0.98~~ 0.139 for wood mouse and 0.62 for hare, ~~a refined endpoint, the specific deposition factor of the crop and~~ a refined TWA for maize and also a reduction in rate to 90 g mesotrione/ha. In addition a refinement of RUD for maize was presented but was not considered by zRMS in the risk assessment. Therefore, there is no unacceptable acute and long-term risk for mammals as well as for drinking water exposures and secondary poisoning. According to results, no unacceptable acute and long-term risk due to combined exposure are obtained according to the proposed GAP.

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

No data available.

9.5 Effects on aquatic organisms (KCP 10.2)

9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with Rimsulfuron, Nicosulfuron, Mesotrione and their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.
Effects on aquatic organisms of PRIMARY MX were not evaluated as part of the EU assessment of Rimsulfuron, Nicosulfuron and Mesotrione. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

process.

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

Species	Substance	Exposure System	Results	Reference
Fish				EFSA Scientific Report (2005) 45, 1-61
L. macrochirus	Rimsulfuron	96 h	LC_{50} > 390 mg a.s./L_{mm}	
O. mykiss	Rimsulfuron	96 h	**LC_{50} > 390 mg a.s./L_{mm}**	
O. mykiss	Rimsulfuron	90 d	NOEC = 125 mg a.s./L_{nom}	
O. mykiss	Rimsulfuron	21 d	**NOEC = 125 mg a.s./L_{nom}**	
O. mykiss	IN-70941	96 h	**LC_{50} > 110 mg/L_{nom}**	
O. mykiss	IN-70942	96 h	**LC_{50} = 180 mg/L_{nmm}**	
O. mykiss	IN-E9260	96 h	**LC_{50} > 314 mg/L_{mm}**	
Aquatic invertebrates				
D. magna	Rimsulfuron	48 h	**EC_{50} > 360 mg a.s./L_{mm}**	
D. magna	Rimsulfuron	21 d	**NOEC = 1 mg a.s./L_{nom}**	
D. magna	IN-70941	48 h	**EC_{50} = 95 mg/L_{nom}**	
D. magna	IN-70942	48 h	**EC_{50} = 178 mg/L_{mm}**	
D. magna	IN-E9260	48 h	**EC_{50} = 184 mg/L_{nom}**	
Sediment-dwelling organisms				
C. riparius	IN-70942	28 d	NOEC ≥ 0.2 mg/kg sed_{nom}	
Algae				
P. subcapitata	Rimsulfuron	72 h, s	E_bC_{50} = 1.2 mg/L_{mm}	
P. subcapitata	IN-70941	72 h, s	E_bC_{50} > 8.9 mg/L_{mm}	
P. subcapitata	IN-70942	72 h, s	E_bC_{50} > 10 mg/L_{nom}	
S. subspicatus	IN-E9260	72 h, s	E_bC_{50} > 100 mg/L_{nom}	
Higher plant				
L. minor	Rimsulfuron	14 d	Frond count: **E_rC_{50} = 0.0046 mg/L_{mm}**	
L. gibba	IN-70942	14 d, s	Frond count: E_rC_{50} > 0.02 mg/L_{nom}	
L. gibba	Rimsulfuron 25 WG	14 d, s	Frond count: E_rC_{50} = 0.03 mg/L	
L. gibba	Rimsulfuron 25 WG + IN-KG691	14 d, s	Frond count: E_rC_{50} = 0.16 mg/L	
Higher-tier studies (micro- or mesocosm studies)				
Not relevant.				

Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – Nicosulfuron and relevant metabolites

Species	Substance	Exposure System	Results	Reference
Fish				
O. mykiss	Nicosulfuron	96 h	LC_{50} = 65.7 mg a.s./L	EFSA Scientific Report (2007) 120, 1-91
O. mykiss	Nicosulfuron	28 d	NOEC = 10 mg a.s./L	
O. mykiss	SL-950 4% SC	96 h	LC_{50} = 2.2 – 4.0 mg a.s./L	
L. macrochirus	ASDM###	96 h	LC_{50} > 100 mg/L	
B. rerio (zebra fish)	AUSN	96 h	LC_{50} > 100 mg/L	
O. mykiss	MU-466	96h	LC_{50} > 100 mg/L	
O. mykiss	HMUD	96h	LC_{50} > 100 mg/L	
O. mykiss	ADMP	96h	LC_{50} > 100 mg/L	
Aquatic invertebrate				
D. magna	Nicosulfuron	48 h	EC_{50} = 90 mg a.s./L	EFSA Scientific Report (2007) 120, 1-91
D. magna	Nicosulfuron	21 d	NOEC = 5.2 mg a.s./L	
D. magna	SL-950 4% SC	48 h	EC_{50} = 3.3 mg a.s./L	
D. magna	ASDM###	48 h	EC_{50} > 954 mg/L	
D. magna	AUSN	48 h	EC_{50} > 100 mg/L	
D. magna	MU-466	48 h	EC_{50} > 100 mg/L	
D. magna	HMUD	48 h	EC_{50} > 100 mg/L	
D. magna	UCSN	48 h	EC_{50} > 100 mg/L	
D. magna	ADMP	48 h	EC_{50} > 100 mg/L	
Algae				
A. flos-aquae	Nicosulfuron	72 h	E_bC_{50} = 7.8 mg a.s./L	EFSA Scientific Report (2007) 120, 1-91
S. subspicatus	SL-950 4% SC	72 h	E_rC_{50} > 4.0 mg a.s./L	
P. subcapitata	ASDM###	72 h	E_rC_{50} > 336 mg/L E_bC_{50} > 54 mg/L	
S. subspicatus	AUSN	72 h	E_rC_{50} & E_bC_{50} > 100 mg/L	
S. subspicatus	MU-466	72 h	E_rC_{50} > 100 mg/L E_bC_{50} = 84.4 mg/L	
S. subspicatus	HMUD	72 h	E_rC_{50} & E_bC_{50} > 100 mg/L	
S. subspicatus	UCSN	72 h	E_rC_{50} & E_bC_{50} > 100 mg/L	
S. subspicatus	ADMP	72 h	E_rC_{50} & E_bC_{50} > 100 mg/L	
Higher plant				
L. gibba	Nicosulfuron	7 d front count Growth rate	EC_{50} = 0.0017 mg/L E_rC_{50} = 0.0027 mg/L	EFSA Scientific Report (2007) 120, 1-91
L. gibba	SL-950 4% SC	7 day frond count Spec. growth rate Biomass (dry wt.)	EC_{50} = 0.0024 mg a.s./L E_rC_{50} = 0.0042 mg a.s./L E_bC_{50} > 0.0092 mg a.s./L	

Species	Substance	Exposure System	Results	Reference
L. gibba	ASDM###	7 d front count, growth rate & biomass	EC_{50}, E_rC_{50} & E_bC_{50} > 100 mg/L	
L. gibba	AUSN	7 d front count, growth rate & biomass	EC_{50}, E_rC_{50} & E_bC_{50} > 100 mg/L	
L. gibba	HMUD	7 d front count, growth rate & biomass	EC_{50}, E_rC_{50} & E_bC_{50} > 1 mg/L	
L. gibba	UCSN	7 d front count, growth rate & biomass	EC_{50}, E_rC_{50} & E_bC_{50} > 100 mg/L	
Higher-tier studies (micro- or mesocosm studies)				
L. gibba	Nicosulfuron tech.	7 d, s	7-day E_yC_{50} = 1.2 µg a.s/L (frond number) 7-day E_rC_{50} = 2.1 µg a.s/L (frond number) NOEC= 0.28 µg a.s/L LOEC=0.74 µg a.s/L	KCP 10.2.1-05 Bätscher, R. 2008 B75341

###: ASDM is code named 'DAM 520' in some of the submitted toxicity reports

Table 9.5-3: Endpoints and effect values relevant for the risk assessment for aquatic organisms – Mesotrione and relevant metabolites

Species	Substance	Exposure System	Results	Reference
Fish				
O. mykiss	Mesotrione	96 h, s	**LC_{50} > 120 mg a.s./L $_{nom}$**	EFSA Journal 2016 ;14(3) :4419
L. macrochirus	Mesotrione	96 h, s	LC_{50} > 120 mg a.s./L $_{nom}$	
P. promelas	Mesotrione	36 d, f	**NOEC = 12.5 mg a.s./L_{nom}***	
C. carpio	Preparation	96 h, s	LC_{50} = 71 mg a.s./L $_{nom}$	
O. mykiss	MNBA	96 h, s	**LC_{50} > 120 mg /L $_{nom}$**	
O. mykiss	AMBA	96 h, s	**LC_{50} = 150 mg/L_{nom}**	
Aquatic invertebrates				
D. magna	Mesotrione	48 h, s	**EC_{50} > 622 mg a.s./L_{mm}****	EFSA Journal 2016 ;14(3) :4419
D. magna	Preparation	48 h, s	EC_{50} = 49 mg a.s./L_{mm}	
D. magna	Mesotrione	21 d, ss	**NOEC = 180 mg a.s./L_{npm}**	
D. magna	MNBA	48 h, s	**EC_{50} = 130 mg/L_{nom}**	
D. magna	AMBA	48 h, s	**EC_{50} = 160 mg/L_{nom}**	
Algae				
P. subcapitata	Mesotrione	96 h, s	E_bC_{50} = 3.5 mg a.s./L_{nom} **E_rC_{50} = 13 mg a.s./L_{nom}**	EFSA Journal 2016 ;14(3) :4419
P. subcapitata	Callisto 100 SC	96 h, s	E_bC_{50} = 72 mg/L_{nom} E_rC_{50} > 100 mg/L_{nom}	
P. subcapitata	MNBA	96 h, s	E_bC_{50} = 38 mg/L_{nom} **E_rC_{50} = 42 mg/L_{nom}**	

Species	Substance	Exposure System	Results	Reference
P. subcapitata	AMBA	96 h, s	E_bC_{50} = 9.4 mg/L_{nom} **E_rC_{50} = 14 mg/L_{nom}**	
Higher plant				
L. gibba	Mesotrione	14 d, ss	E_bC_{50} = 0.022 mg a.s./L_{nom} **E_bC_{50} = 0.0077 mg a.s./L_{nom}**	EFSA Journal 2016 ;14(3) :4419
L. gibba	Callisto 100 SC	7 d, ss	E_rC_{50} = 0.117 mg a.s./L_{nom} E_yC_{50} = 0.0269 mg a.s./L_{nom}	
L. gibba	MNBA	7 d, ss	**E_rC_{50} & E_yC_{50} > 97 mg/L_{mm}**	
L. gibba	AMBA	7 d, ss	**E_rC_{50} & E_yC_{50} > 90 mg/L_{mm}**	
L. gibba	SYN546974	7 d, ss	**E_rC_{50} > 95 mg/L_{mm}** E_yC_{50} = 93 mg/L_{mm}	
Higher-tier studies (micro- or mesocosm studies)				
Not needed.				

*: physical symptoms: loss of balance, less activity, spinal deformity, skin lesions and internal bleeding
**: in the LoEP of Review Report of Mesotrione (January 2013, SANCO/1416/2001 – Final); the value of > 900 mg mesotrione/L was misreported for acture invertebrate toxicity.
s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations; im: based on initial measured concentrations

Table 9.5-4: Endpoints and effect values relevant for the risk assessment for aquatic organisms – PRIMARY MX

Species	Substance	Exposure System	Results	Reference
O. mykiss	PRIMARY MX	96 h, s	LC_{50} > 100 mg/L $_{nom}$	KCP 10.2.1-01 xxx., 2018 W/204/17
P. subcapitata	PRIMARY MX	72 h, s	E_rC_{50} = 20.211 mg/L $_{nom}$ E_yC_{50} = 2.657 mg/L $_{nom}$	KCP 10.2.1-02 Bak, P., 2018 W/205/17
D. magna	PRIMARY MX	48 h, s	EC_{50} > 100 mg/L $_{nom}$	KCP 10.2.1-03 Bak, P., 2018 W/206/17
L. gibba	PRIMARY MX	7 d, ss	Frond: E_rC_{50} = 0.0166 mg/L_{nom} E_yC_{50} = 0.0112 mg/L_{nom} Dry weight: E_rC_{50} = 10.9479 mg/L_{nom} E_yC_{50} = 0.0264 mg/L_{nom} Frond: E_rC_{50} = 0.0149 mg/L* E_yC_{50} = 0.0100 mg/L* Dry weight: E_rC_{50} = 9.796 mg/L* E_yC_{50} = 0.0236 mg/L*	KCP 10.2.1-04 Bak, P., 2018 W/207/17

Species	Substance	Exposure System	Results	Reference
Higher-tier studies (micro- or mesocosm studies)				
No data submitted				

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations
* Product endpoints recalculated by cMS suggestion following the specified procedure for formulation tests with more than one active substance, when all active substances have been analytically measured; agreed and detailed in the "*Outcome of pesticides peer review meeting on recurring issues in ecotoxicology*" (EFSA Supporting publication 2019:EN-1673).

9.5.1.1 Justification for new endpoints

The used endpoints are the EU agreed ones, except for formulation, corresponding to data proper to PRIMARY MX formulation.

9.5.2 Risk assessment

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

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

Rimsulfuron

Table 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Rimsulfuron for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of PRIMARY MX in maize

~~Group~~		~~Fish acute~~	~~Fish pro-longed~~	~~Inverteb. acute~~	~~Inverteb. prolonged~~	~~Algae~~	~~Higher-tier information~~
~~Test species~~		~~*O. mykiss*~~	~~*O. mykiss*~~	~~*D. magna*~~	~~*D. magna*~~	~~*S. capricornutum*~~	~~*L. gibba*~~
~~Endpoint~~		~~LC_{50}~~	~~NOEC~~	~~EC_{50}~~	~~NOEC~~	~~EC_{50}~~	~~E_rC_{50}~~
~~(µg/L)~~		~~390000~~	~~125000~~	~~360000~~	~~1000~~	~~1200~~	~~4.6~~
~~AF~~		~~100~~	~~10~~	~~100~~	~~10~~	~~10~~	~~10~~
~~RAC (µg/L)~~		~~3900~~	~~12500~~	~~3600~~	~~100~~	~~120~~	~~0.46~~
~~FOCUS Scenario~~	~~$PEC_{gl\ max}$ (µg/L)~~						
~~Step 1~~							
~~-~~	~~3.21~~	~~0.001~~	~~< 0.001~~	~~0.001~~	~~0.032~~	~~0.027~~	~~**6.978**~~
~~Step 2~~	~~-~~	~~-~~	~~-~~	~~-~~	~~-~~	~~-~~	~~-~~
~~S Europe~~	~~0.87~~	~~< 0.001~~	~~< 0.001~~	~~< 0.001~~	~~0.009~~	~~0.007~~	~~**1.891**~~
~~N Europe~~	~~0.47~~	~~< 0.001~~	~~< 0.001~~	~~< 0.001~~	~~0.005~~	~~0.004~~	~~**1.022**~~
~~Step 3~~	~~-~~	~~-~~	~~-~~	~~-~~	~~-~~	~~-~~	~~-~~
~~D3/ditch~~	~~0.055~~	~~< 0.001~~	~~< 0.001~~	~~< 0.001~~	~~0.001~~	~~< 0.001~~	~~0.120~~

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Higher-tier information
D4/pond	0.009	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.020
D4/stream	0.046	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.100
D5/pond	0.004	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.009
D5/stream	0.048	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.104
D6/ditch	0.052	< 0.001	< 0.001	< 0.001	0.001	< 0.001	0.113
R1/pond	0.005	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.011
R1/stream	0.115	< 0.001	< 0.001	< 0.001	0.001	0.001	0.250
R2/stream	0.276	< 0.001	< 0.001	< 0.001	0.003	0.002	0.600
R3/stream	0.409	< 0.001	< 0.001	< 0.001	0.004	0.003	0.889
R4/stream	0.413	< 0.001	< 0.001	< 0.001	0.004	0.003	0.898

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher-tier information**
Test species		***O. mykiss***	***O. mykiss***	***D. magna***	***D. magna***	***S. capricornutum***	***L. gibba***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	EC_{50}	E_rC_{50}
(µg/L)		390000	125000	360000	1000	1200	4.6
AF		100	10	100	10	10	10
RAC (µg/L)		3900	12500	3600	100	120	0.46
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	2.44	0.001	<0.001	0.001	0.024	0.020	**5.304**
Step 2							
S-Europe	0.66	<0.001	<0.001	<0.001	0.007	0.006	**1.435**
N-Europe	0.36	<0.001	<0.001	<0.001	0.004	0.003	0.783
Step 3							
D3/ditch	0.042	<0.001	<0.001	<0.001	<0.001	<0.001	0.091
D4/pond	0.007	<0.001	<0.001	<0.001	<0.001	<0.001	0.015
D4/stream	0.035	<0.001	<0.001	<0.001	<0.001	<0.001	0.076
D5/pond	0.003	<0.001	<0.001	<0.001	<0.001	<0.001	0.007
D5/stream	0.036	<0.001	<0.001	<0.001	<0.001	<0.001	0.078
D6/ditch	0.040	<0.001	<0.001	<0.001	<0.001	<0.001	0.087
R1/pond	0.003	<0.001	<0.001	<0.001	<0.001	<0.001	0.007
R1/stream	0.087	<0.001	<0.001	<0.001	0.001	0.001	0.189
R2/stream	0.209	<0.001	<0.001	<0.001	0.002	0.002	0.454

Group		Fish acute	Fish pro-longed	Inverteb. acute	Inverteb. prolonged	Algae	Higher-tier information
R3/stream	0.310	<0.001	<0.001	<0.001	0.003	0.003	0.674
R4/stream	0.313	<0.001	<0.001	<0.001	0.003	0.003	0.680

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

In order to obtain an acceptable risk in the risk assessment for the combinations of active substances, step 4 calculations have been done for the most sensitive group of aquatic organisms (risk for higher plant as characterised by an EC_{50} for *Lemna gibba* of 4.6 in connection with an assessment factor of 10) considering reduced exposure of surface water bodies. Furthermore, VFSMOD calculations have been done as refinement for all R scenarios, with the exception of R1 pond scenario.

Table 9.5-5(2): Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for Rimsulfuron based on FOCUS Step 4 calculations and toxicity data for higher plant with mitigation of spray drift and run-off for the use of PRIMARY MX in maize

Intended use		Maize			
Active substance		Rimsulfuron			
Application rate (g/ha)		1 x 9.9			
Nozzle reduction	Vegetated filter strip (m)	5	10	15	20
	No-spray buffer (m)	5	10	15	20
None	R1 stream	0.053	0.036	-	-
	R2 stream	0.134	0.092	0.071	0.048
	R3 stream	0.201	0.140	0.107	0.073
	R4 stream	0.204	0.142	0.109	0.075
RAC (µg/L)		PEC/RAC ratio			
0.46					
None	R1 stream	0.115	0.078	-	-
	R2 stream	0.291	0.200	0.154	0.104
	R3 stream	0.437	0.304	0.233	0.159
	R4 stream	0.443	0.309	0.237	0.163

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

Table 9.5-5(3): Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for Rimsulfuron based on VFSMOD Step 4 calculations and toxicity data for higher plant with mitigation of spray drift and run-off for the use of PRIMARY MX in maize

Intended use		Maize
Active substance		Rimsulfuron
Application rate (g/ha)		1 x 9.9
Nozzle reduction	Vegetated filter strip (m)	5

	No-spray buffer (m)	5
None	R1 stream	0.011
	R2 stream	0.015
	R3 stream	0.016
	R4 stream	0.011
RAC (µg/L)		
0.46		**PEC/RAC ratio**
None	R1 stream	0.024
	R2 stream	0.033
	R3 stream	0.035
	R4 stream	0.024

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

Metabolites of Rimsulfuron

Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for IN-70941 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of PRIMARY MX in maize

Group		Fish acute	Inverteb. acute	Algae
Test species		*O. mykiss*	*D. magna*	*S. subcapitata*
Endpoint (µg/L)		LC_{50} 110000	EC_{50} 95000	EC_{50} 8900
AF		100	100	10
RAC (µg/L)		1100	950	890
FOCUS Scenario	PEC_{gl-max} (µg/L)			
Step 1				
-	3.72	0.003	0.004	0.004
Step 2	-			
S-Europe	1.05	0.001	0.001	0.001
N-Europe	0.55	0.001	0.001	0.001

Group		**Fish acute**	**Inverteb. acute**	**Algae**
Test species		***O. mykiss***	***D. magna***	***S. subcapitata***
Endpoint (µg/L)		LC_{50} 110000	EC_{50} 95000	EC_{50} 8900
AF		100	100	10
RAC (µg/L)		1100	950	890
FOCUS Scenario	**PEC_{gl-max} (µg/L)**			
Step 1				
	2.82	0.003	0.003	0.003
Step 2				
S-Europe	0.80	0.001	0.001	0.001
N-Europe	0.42	<0.001	<0.001	<0.001

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

Table 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for IN-70942 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of PRIMARY MX in maize

Group		Fish acute	Inverteb. acute	Algae	Higher-tier information		Sed. dwell. prolonged
Test species		*O. mykiss*	*D. magna*	*S.capricornutum*	*L. gibba*		*C. riparius*
Endpoint (µg/L)		LC_{50} 180000	EC_{50} 178000	EC_{50} 10000	EC_{50} 20		NOEC 200
AF		100	100	10	10		10
RAC (µg/L)		1800	1780	1000	2		20
FOCUS Scenario	PEC_{gl-max} (µg/L)					PEC_{sed} (µg/L)	
Step 1							
-	2.18	0.001	0.001	0.002	**1.090**	4.13	0.207
Step 2	-	-	-	-	-	-	-
S-Europe	0.61	< 0.001	< 0.001	0.001	0.305	1.16	0.031
N-Europe	0.33	< 0.001	< 0.001	< 0.001	0.165	0.62	0.017

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Higher-tier information**		**Sed. dwell. prolonged**
Test species		***O. mykiss***	***D. magna***	***S.capricornutum***	***L. gibba***		***C. riparius***
Endpoint (µg/L)		LC_{50} 180000	EC_{50} 178000	EC_{50} 10000	EC_{50} 20		NOEC 200
AF		100	100	10	10		10
RAC (µg/L)		1800	1780	1000	2		20
FOCUS Scenario	**PEC_{gl-max} (µg/L)**					**PEC_{sed} (µg/L)**	
Step 1							
	1.65	0.001	0.001	0.002	0.825	3.13	0.157
Step 2							
S-Europe	0.46	0.000	0.000	0.000	0.230	0.88	0.044
N-Europe	0.25	0.000	0.000	0.000	0.125	0.47	0.024

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 IN-E9260 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of PRIMARY MX in maize

Group		Fish acute	Inverteb. acute	Algae
Test species		*O. mykiss*	*D. magna*	*P. subcapitata*
Endpoint (µg/L)		LC_{50} 314000	EC_{50} 184000	EC_{50} 100000
AF		100	100	10
RAC (µg/L)		3140	1840	10000
FOCUS Scenario	PEC_{gl-max} (µg/L)			
Step 1				
-	0.66	< 0.001	< 0.001	< 0.001
Step 2	-	-	-	-
S-Europe	0.19	< 0.001	< 0.001	< 0.001
N-Europe	0.10	< 0.001	< 0.001	< 0.001

Group		**Fish acute**	**Inverteb. acute**	**Algae**
Test species		***O. mykiss***	***D. magna***	***P. subcapitata***
Endpoint (µg/L)		LC_{50} 314000	EC_{50} 184000	EC_{50} 100000
AF		100	100	10
RAC (µg/L)		3140	1840	10000
FOCUS Scenario	**PEC_{gl-max} (µg/L)**			
Step 1				
-	0.50	< 0.001	< 0.001	< 0.001
Step 2	-			
S-Europe	0.14	< 0.001	< 0.001	< 0.001
N-Europe	0.08	< 0.001	< 0.001	< 0.001

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

Nicosulfuron

Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Nicosulfuron for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of PRIMARY MX in maize

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Higher-tier information
Test species		*O. mykiss*	*O. mykiss*	*D. magna*	*D. magna*	*A. flos-aquae*	*L. gibba*
Endpoint (µg/L)		LC_{50} 2200	NOEC 10000	EC_{50} 3300	NOEC 5200	E_bC_{50} >4000	EC_{50} 1.7
AF		100	10	100	10	10	10
RAC (µg/L)		22	1000	33	520	400	0.17
FOCUS Scenario	$PEC_{gl\ max}$ (µg/L)						
Step 1							
-	13.30	0.605	0.013	0.403	0.026	0.033	**78.235**
Step 2	-	-	-	-	-	-	-
S-Europe	1.43	0.065	0.001	0.043	0.003	0.004	**8.412**
N-Europe	2.52	0.115	0.003	0.076	0.005	0.006	**14.824**
Step 3	-	-	-	-	-	-	-
D3/ditch	0.226	0.010	< 0.001	0.007	< 0.001	0.001	**1.329**
D4/pond	0.044	0.002	< 0.001	0.001	< 0.001	< 0.001	0.259
D4/stream	0.189	0.009	< 0.001	0.006	< 0.001	< 0.001	**1.112**
D5/pond	0.018	0.001	< 0.001	0.001	< 0.001	< 0.001	0.106
D5/stream	0.190	0.009	< 0.001	0.006	< 0.001	< 0.001	**1.118**
D6/ditch	0.209	0.010	< 0.001	0.006	< 0.001	0.001	**1.229**
R1/pond	0.014	0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.082
R1/stream	0.440	0.020	< 0.001	0.013	0.001	0.001	**2.588**
R2/stream	1.344	0.061	0.001	0.041	0.003	0.003	**7.906**
R3/stream	1.603	0.073	0.002	0.049	0.003	0.004	**9.429**
R4/stream	1.713	0.078	0.002	0.052	0.003	0.004	**10.076**

Group		Fish acute	Fish pro-longed	Inverteb. acute	Inverteb. prolonged	Algae	Higher-tier information	
Test species		*O. mykiss*	*O. mykiss*	*D. magna*	*D. magna*	*A. flos-aquae*	*L. gibba*	
Endpoint (µg/L)		LC_{50} 2200	NOEC 10000	EC_{50} 3300	NOEC 5200	E_bC_{50} >4000	EC_{50} 1.7	ErC_{50}* 2.7
AF		100	10	100	10	10	10	10
RAC (µg/L)		22	1000	33	520	400	0.17	0.27

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher-tier information**	
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**							
Step 1								
	10.07	0.458	0.010	0.305	0.019	0.025	**59.235**	**37.296**
Step 2								
S-Europe	2.74	0.125	0.003	0.083	0.005	0.007	**16.118**	**10.148**
N-Europe	1.50	0.068	0.002	0.045	0.003	0.004	**8.824**	**5.556**
Step 3								
D3/ditch	0.170	0.008	<0.001	0.005	<0.001	<0.001	**1.000**	0.630
D4/pond	0.032	0.001	<0.001	0.001	<0.001	<0.001	0.188	0.119
D4/stream	0.143	0.007	<0.001	0.004	<0.001	<0.001	0.841	0.530
D5/pond	0.014	0.001	<0.001	<0.001	<0.001	<0.001	0.082	0.052
D5/stream	0.144	0.007	<0.001	0.004	<0.001	<0.001	0.847	0.533
D6/ditch	0.159	0.007	<0.001	0.005	<0.001	<0.001	0.935	0.589
R1/pond	0.011	0.001	<0.001	<0.001	<0.001	<0.001	0.065	0.041
R1/stream	0.334	0.015	<0.001	0.010	0.001	0.001	**1.965**	**1.237**
R2/stream	1.015	0.046	0.001	0.031	0.002	0.003	**5.971**	**3.759**
R3/stream	1.215	0.055	0.001	0.037	0.002	0.003	**7.147**	**4.500**
R4/stream	1.296	0.059	0.001	0.039	0.002	0.003	**7.624**	**4.800**

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* the value agreed at EU level.

Metabolites of Nicosulfuron

Table 9.5-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for ASDM for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of PRIMARY MX in maize

Group		Fish acute	Inverteb. acute	Algae	Higher-tier information
Test species		*L. macrochirus*	*D. magna*	*P. subcapitata*	*L. gibba*
Endpoint (µg/L)		LC_{50} 100000	EC_{50} 954000	E_bC_{50} 336000	EC_{50} 100000
AF		100	100	10	10
RAC (µg/L)		1000	9540	33600	10000
FOCUS Scenario	PEC $_{gl-max}$ (µg/L)				
Step 1					
-	9.24	0.009	0.001	< 0.001	0.001
Step 2	-	-	-	-	-
S-Europe	2.61	0.003	< 0.001	< 0.001	< 0.001
N-Europe	1.37	0.001	< 0.001	< 0.001	< 0.001

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Higher-tier information**
Test species		***L. macrochirus***	***D. magna***	***P. subcapitata***	***L. gibba***
Endpoint (µg/L)		LC_{50} 100000	EC_{50} 954000	E_bC_{50} 336000	EC_{50} 100000
AF		100	100	10	10
RAC (µg/L)		1000	9540	33600	10000
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**				
Step 1					
-	7.00	0.007	0.001	<0.001	0.001
Step 2					
S-Europe	1.98	0.002	<0.001	<0.001	<0.001
N-Europe	1.04	0.001	<0.001	<0.001	<0.001

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

Table 9.5-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AUSN for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of PRIMARY MX in maize

Group		Fish acute	Inverteb. acute	Algae	Higher-tier information
Test species		*Oncorhynchus mykiss*	*Daphnia magna*	*Scenedesmus subspicatus*	*Lemna gibba*
Endpoint (µg/L)		LC_{50} 100000	EC_{50} 100000	E_rC_{50} 100000	EC_{50} 100000
AF		100	100	10	10
RAC (µg/L)		1000	1000	10000	10000
FOCUS Scenario	PEC_{gl-max} (µg/L)				
Step 1					
-	3.74	0.004	0.004	< 0.001	< 0.001
Step 2	-				
S-Europe	0.72	0.001	0.001	< 0.001	< 0.001
N-Europe	0.38	< 0.001	< 0.001	< 0.001	< 0.001

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Higher-tier information**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Scenedesmus subspicatus***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} 100000	EC_{50} 100000	E_rC_{50} 100000	EC_{50} 100000
AF		100	100	10	10
RAC (µg/L)		1000	1000	10000	10000
FOCUS Scenario	**PEC_{gl-max} (µg/L)**				
Step 1					
	2.83	0.003	0.003	<0.001	<0.001
Step 2					
S-Europe	0.81	0.001	0.001	<0.001	<0.001
N-Europe	0.42	<0.001	<0.001	<0.001	<0.001

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

Table 9.5-12: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for HMUD for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of PRIMARY MX in maize

Group		Fish acute	Inverteb. acute	Algae	Aquatic plants
Test species		*O. mykiss*	*D. magna*	*S. subcapitata*	*L. gibba*
Endpoint		LC_{50}	EC_{50}	E_rC_{50}	EC_{50}
(µg/L)		100000	100000	100000	1000
AF		100	100	10	10
RAC (µg/L)		1000	1000	10000	100
FOCUS Scenario	PEC $_{gl\text{-}max}$ (µg/L)				
Step 1					
-	4.34	0.004	0.004	< 0.001	0.043
Step 2	-	-	-		
S-Europe	0.81	0.001	0.001	< 0.001	0.008
N-Europe	0.44	< 0.001	< 0.001	< 0.001	0.004

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Aquatic plants**
Test species		***O. mykiss***	***D. magna***	***S. subcapitata***	***L. gibba***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}	EC_{50}
(µg/L)		100000	100000	100000	1000
AF		100	100	10	10
RAC (µg/L)		1000	1000	10000	100
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**				
Step 1					
	3.29	0.003	0.003	<0.001	0.033
Step 2					
S-Europe	0.89	0.001	0.001	<0.001	0.009
N-Europe	0.47	<0.001	<0.001	<0.001	0.005

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 9.5-13: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for ADMP for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of PRIMARY MX in maize

Group		Fish acute	Inverteb. acute	Algae
Test species		*O. mykiss*	*D. magna*	*S. subspicatus*
Endpoint (µg/L)		LC_{50} 100000	EC_{50} 100000	E_rC_{50} 100000
AF		100	100	10
RAC (µg/L)		1000	1000	10000
FOCUS Scenario	PEC $_{gl\text{-}max}$ (µg/L)			
Step 1				
-	1.57	0.002	0.002	< 0.001
Step 2	-			
S-Europe	0.26	< 0.001	< 0.001	< 0.001
N-Europe	0.15	< 0.001	< 0.001	< 0.001

Group		**Fish acute**	**Inverteb. acute**	**Algae**
Test species		***O. mykiss***	***D. magna***	***S. subspicatus***
Endpoint (µg/L)		LC_{50} 100000	EC_{50} 100000	E_rC_{50} 100000
AF		100	100	10
RAC (µg/L)		1000	1000	10000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
-	1.19	0.001	0.001	<0.001
Step 2				
S-Europe	0.29	<0.001	<0.001	<0.001
N-Europe	0.15	<0.001	<0.001	<0.001

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

Table 9.5-14: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for UCSN for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of PRIMARY MX in maize

Group		Inverteb. acute	Algae	Aquatic plants
Test species		*Daphnia magna*	*S. subspicatus*	*L. gibba*
Endpoint (µg/L)		EC_{50} 100000	E_rC_{50} 100000	EC_{50} 100000
AF		100	10	10
RAC (µg/L)		1000	10000	10000
FOCUS Scenario	PEC_{gl-max} (µg/L)			
Step 1				
-	1.79	0.002	< 0.001	< 0.001
Step 2	-			
S-Europe	0.35	< 0.001	< 0.001	< 0.001
N-Europe	0.35	< 0.001	< 0.001	< 0.001

Group		**Inverteb. acute**	**Algae**	**Aquatic plants**
Test species		***Daphnia magna***	***S. subspicatus***	***L. gibba***
Endpoint (µg/L)		EC_{50} 100000	E_rC_{50} 100000	EC_{50} 100000
AF		100	10	10
RAC (µg/L)		1000	10000	10000
FOCUS Scenario	**PEC_{gl-max} (µg/L)**			
Step 1				
-	1.35	0.001	<0.001	<0.001
Step 2	-			
S-Europe	0.39	<0.001	<0.001	<0.001
N-Europe	0.20	<0.001	<0.001	<0.001

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

For the intended uses on maize, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for higher plant as characterised by an EC_{50} for *Lemna gibba* of 1.7 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.

Table 9.5-15: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for Nicosulfuron based on FOCUS Step 4 calculations and toxicity data for higher plant with mitigation of spray drift and run-off for the use of PRIMARY MX in maize

Intended use	Maize

Active substance **Application rate (g/ha)**		Nicosulfuron 1 x 39.6				
Nozzle reduction	**Vegetated filter strip (m)**	None	5	10	15	20
	No-spray buffer (m)	5	5	10	15	20
None	D3 ditch	0.086	-	-	-	-
	D4 stream	0.086	-	-	-	-
	D5 stream	0.082	-	-	-	-
	D6 ditch	0.069	-	-	-	-
	R1 stream	-	0.269	0.181	0.136	-
	R2 stream	-	0.860	0.593	0.453	0.307
	R3 stream	-	1.041	0.725	0.556	0.379
	R4 stream	-	1.117	0.779	0.598	0.408
RAC (µg/L) 0.17		**PEC/RAC ratio**				
None	D3 ditch	0.506	-	-	-	-
	D4 stream	0.506	-	-	-	-
	D5 stream	0.482	-	-	-	-
	D6 ditch	0.406	-	-	-	-
	R1 stream	-	**1.582**	**1.065**	0.800	-
	R2 stream	-	**5.059**	**3.488**	**2.665**	**1.806**
	R3 stream	-	**6.124**	**4.265**	**3.271**	**2.229**
	R4 stream	-	**6.571**	**4.582**	**3.518**	**2.400**

Intended use **Active substance** **Application rate (g/ha)**		**Maize** Nicosulfuron 1 x 39.6				
Nozzle reduction	**Vegetated filter strip (m)**	None	5	10	15	20
	No-spray buffer (m)	5	5	10	15	20
None	D3 ditch	0.065	-	-	-	-
	R1 stream	-	0.204	0.137	-	-
	R2 stream	-	0.649	0.448	0.341	0.232
	R3 stream	-	0.789	0.550	0.421	0.287
	R4 stream	-	0.846	0.590	0.452	0.309
RAC (µg/L) 0.17		**PEC/RAC ratio**				
None	D3 ditch	0.382	-	-	-	-

	R1 stream	-	**1.200**	0.806	-	-
	R2 stream	-	**3.818**	**2.635**	**2.006**	**1.365**
	R3 stream	-	**4.641**	**3.235**	**2.476**	**1.688**
	R4 stream	-	**4.976**	**3.471**	**2.659**	**1.818**
RAC (µg/L) 0.27*		**PEC/RAC ratio**				
None	R1 stream	-	0.756	0.507	-	-
	R2 stream	-	**2.404**	**1.659**	**1.263**	0.859
	R3 stream	-	**2.922**	**2.037**	**1.559**	**1.063**
	R4 stream	-	**3.133**	**2.185**	**1.674**	**1.144**

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* value based on 7 d ErC_{50} of 2.7 µg/L.

FOCUS Step 4 modelling PEC_{sw} values assuming a 5 meter no spray buffer zone for the remaining surface water resulted in an acceptable PEC/RAC values for scenarios D3 ditch, D4 stream, D5 stream and D6 ditch. In addition, a 15 5 meter no spray buffer zone including 15 5 m vegetative buffer strip, resulted in an acceptable PEC/RAC values for the remaining surface water scenario R1 stream, and a 20 meter no spray buffer zone including 20 m vegetative buffer strip for the R2 stream scenario. However, unacceptable PEC/RAC values were obtained for R2, R3 and R4 stream scenarios even with a 20 meter no spray buffer zone including 20 m vegetative buffer strip.

A refinement was performed considering the results of recovery from a new study submitted with this application.

Based on the results of the recovery phase of the study on *Lemna* conducted with nicosulfuron (new report KCP 10.2.1-05 submitted with this application are listed in Appendix 1 and summarised in Appendix 2) the effects of nicosulfuron on it are expected to be reversible at concentrations lower than or equal to 0.74 µg nicosulfuron/L. This value is above initial PECsw for all scenarios, therefore the risk is considered acceptable with an unsprayed vegetated buffer zone of 15 10 m.

Table 9.5-16: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for Nicosulfuron based on FOCUS Step 4 calculations and toxicity data for higher plant with mitigation of spray drift and run-off for the use of PRIMARY MX in maize – refined endpoint

Intended use Active substance Application rate (g/ha)		Maize Nicosulfuron 1 x 39.6				
Nozzle reduction	Vegetated filter strip (m)	None	5	10	15	20
	No-spray buffer (m)	5	5	10	15	20
None	D3 ditch	0.086	-	-	-	-
	D4 stream	0.086	-	-	-	-
	D5 stream	0.082	-	-	-	-
	D6 ditch	0.069	-	-	-	-
	R1 stream	-	0.269	0.181	0.136	-
	R2 stream	-	0.860	0.593	0.453	0.307
	R3 stream	-	1.041	0.725	0.556	0.379
	R4 stream	-	1.117	0.779	0.598	0.408
RAC (µg/L) 0.74		PEC/RAC ratio				
None	D3 ditch	0.116	-	-	-	-
	D4 stream	0.116	-	-	-	-
	D5 stream	0.111	-	-	-	-
	D6 ditch	0.093	-	-	-	-
	R1 stream	-	0.364	0.245	0.184	-
	R2 stream	-	**1.162**	0.801	0.612	0.415
	R3 stream	-	**1.407**	0.980	0.751	0.512
	R4 stream	-	**1.509**	**1.053**	0.808	0.551

Intended use **Active substance** **Application rate (g/ha)**		**Maize** Nicosulfuron 1 x 39.6				
Nozzle reduction	**Vegetated filter strip (m)**	None	5	10	15	20
	No-spray buffer (m)	5	5	10	15	20
None	D3 ditch	0.065	-	-	-	-
	R1 stream	-	0.204	0.137	-	-
	R2 stream	-	0.649	0.448	0.341	0.232
	R3 stream	-	0.789	0.550	0.421	0.287

	R4 stream	-	0.846	0.590	0.452	0.309
RAC (µg/L) 0.74		**PEC/RAC ratio**				
None	D3 ditch	0.088	-	-	-	-
	R1 stream	-	0.276	0.185	-	-
	R2 stream	-	0.877	0.605	0.461	0.314
	R3 stream	-	**1.066**	0.743	0.569	0.388
	R4 stream	-	**1.143**	0.797	0.611	0.418

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

Further refinement was done for those scenarios at which unacceptable risk is obtained considering the proposed endpoint E_rC_{50} of 2.7 µg s.a./L. VFSMOD calculations have been done for all R scenarios, except for R1 pond. The PEC/RAC calculations are given below:

Table 9.5-16(2): Aquatic organisms: Global maximum PECsw calculation and acceptability of risk (PEC/RAC < 1) for Nicosulfuron based on VFSMOD Step 4 calculations and toxicity data for higher plant with mitigation of spray drift and run-off for the use of PRIMARY MX in maize

Intended use		**Maize**
Active substance		Nicosulfuron
Application rate (g/ha)		1 x 39.6
Nozzle reduction	**Vegetated filter strip (m)**	5
	No-spray buffer (m)	5
None	R1 stream	0.045
None	R2 stream	0.061
None	R3 stream	0.064
None	R4 stream	0.046
RAC (µg/L) 0.27		**PEC/RAC ratio**
None	R1 stream	0.167
None	R2 stream	0.226
None	R3 stream	0.237
None	R4 stream	0.170

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

Based on the results, acceptable PEC/RAC values were obtained for R stream scenarios with a 5 meter no spray buffer zone including 5 m vegetative buffer strip when VFSMOD is considered.

zRMS comments:

<u>Nicosulfuron:</u>

The risk assessment presented in Tables 9.5-9 to 9.5-16 above is agreed by the zRMS.

For fish, aquatic invertebrates and algae acceptable acute and chronic risk for a.s.- nicosulfuron and its metabolites could be concluded already for Step 1 PEC_{sw} values.

For aquatic macrophytes – Lemna sp. two approaches in the risk assessment for the a.s.- **nicosulfuron** were considered by the Applicant:

- PEC/RAC calculated on the basis of the lowest E_yC_{50} with 1.7 µg a.s./L
- PEC/RAC calculated on the basis on RAC≤ 0.74 µg s.a/L

At the zonal level the standard approach in line with EFSA AGD (2013) is required.

When the risk assessment is based on EyC_{50} value, unacceptable risk is identified for D3, R1 (stream), R (stream), R4 (stream) and R3 (stream) scenarios.

FOCUS Step 4 modelling PEC_{sw} value assuming a 5 meter no spray buffer zone for the remaining surface water resulted in an acceptable PEC/RAC value for D3 (ditch) scenario.

In addition, a 10 meter no spray buffer zone including 10 m vegetative buffer strip, resulted in an acceptable PEC/RAC value for the remaining surface water scenario R1 stream.

However, unacceptable PEC/RAC values were obtained for **R2, R3 and R4** stream scenarios even with a 20 meter no spray buffer zone including 20 m vegetative buffer strip.

However, as consideration of E_yC_{50} value is not in line with recommendations of EFSA (2013), further evaluation was not performed at the zonal level and is deemed necessary in concerned Member States that prefer to use this approach in the aquatic risk assessment.

For this reason PEC/RAC calculations based on E_rC_{50} of 2.7 µg s.a/L (RAC-0.27 µg s.a/L) for aquatic macrophytes, agreed at EU level was provided.

It should be noted that zRMS did not accept the risk assessment based on RAC of 0.74 µg s.a./L value proposed by the applicant.

In zRMS opinion this value is not appropriate to replace the agreed E_rC_{50} of 2.7 µg s.a./L value included in the LoEP for nicosulfuron.

On the basis of the standard risk assessment performed in line with EFSA aquatic guidance (2013) and RAC=0.27 µg s.a./L following conclusions could be derived:

- Acceptable risk to aquatic macrophytes with no need for risk mitigation measures based on Step 3 calculations was demonstrated in scenarios D3, D4, D5, D6, R1 (pond).
- Acceptable risk to aquatic macrophytes with consideration of 5 m vegetative filter strip was demonstrated in scenarios R1 stream scenario and 20 m vegetative filter strip for R2 scenarios.

After commenting II following zRMS comment was added:

An unacceptable risk to aquatic macrophytes with consideration of 20 m vegetated filter strip was demonstrated in scenarios R3 and R4. Therefore, the applicant provided further refinement of the

risk assessment using PEC_{sw} VFSmod calculations. Due to the fact that the applicant has not submitted NA risk assessment using PEC_{sw} VFSmod calculations have been marked and are for use by MS that accept VFSmod.

For the intended uses on maize, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for higher plant as characterised by an EC_{50} for *Lemna gibba* of 2.7 µg/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. As R3 and R4 scenarios showed an unacceptable risk, an alternative approach was followed using VFS_{MOD} Global maximum PECsw values.

Hence, based on the results of the risk assessment using VFSmod calculations, the following conclusions regarding buffer zones and vegetative buffer strips may be drawn for maize use:

R1 stream, R2 stream, R3 stream and R4 stream scenarios:

- 5 m no-spray buffer zone and a 5 m vegetative buffer strip are required

For ASDM, AUSN, HMUD, ADMP and UCSN metabolites, all PEC/RAC values are below the trigger value of 1 at step 1-2. Therefore, no further assessment is necessary.

Mesotrione

Table 9.5-17: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Mesotrione for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of PRIMARY MX in maize

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic plants
Test species		*O. mykiss*	*P. promelas*	*D. magna*	*D. magna*	*P. subcapitata*	*L. gibba*
Endpoint (µg/L)		LC_{50} 120000	NOEC 12500	EC_{50} 622000	NOEC 180000	E_rC_{50} 13000	EC_{50} 7.7
AF		100	10	100	10	10	10
RAC (µg/L)		1200	1250	6220	18000	1300	0.77
FOCUS Scenario	$PEC_{gl\text{-}max}$ (µg/L)*						
Step 1							
-	40.42	0.034	0.032	0.006	0.002	0.031	**52.494**
Step 2	-						
S Europe	6.69	0.006	0.005	0.001	< 0.001	0.005	**8.688**
N Europe	3.67	0.003	0.003	0.001	< 0.001	0.003	**4.766**
Step 3	-						
D3/ditch	0.619	0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.804
D4/pond	0.026	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.034
D4/stream	0.532	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.691

Group		Fish acute	Fish pro-longed	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic plants
D5/pond	0.026	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.034
D5/stream	0.577	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.749
D6/ditch	0.618	0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.803
R1/pond	0.025	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.032
R1/stream	0.581	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.755
R2/stream	1.454	0.001	0.001	< 0.001	< 0.001	0.001	**1.888**
R3/stream	0.752	0.001	0.001	< 0.001	< 0.001	0.001	0.977
R4/stream	0.639	0.001	0.001	< 0.001	< 0.001	< 0.001	0.830

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic plants**
Test species		***O. mykiss***	***P. promelas***	***D. magna***	***D. magna***	***P. subcapitata***	***L. gibba***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	EC_{50}
(µg/L)		120000	12500	622000	180000	13000	7.7
AF		100	10	100	10	10	10
RAC (µg/L)		1200	1250	6220	18000	1300	0.77
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)***						
Step 1							
	30.15	0.025	0.024	0.005	0.002	0.023	**39.156**
Step 2							
S-Europe	7.41	0.006	0.006	0.001	<0.001	0.006	**9.623**
N-Europe	3.95	0.003	0.003	0.001	<0.001	0.003	**5.130**
Step 3							
D3/ditch	0.472	<0.001	<0.001	<0.001	<0.001	<0.001	0.613
D4/pond	0.020	<0.001	<0.001	<0.001	<0.001	<0.001	0.026
D4/stream	0.460	<0.001	<0.001	<0.001	<0.001	<0.001	0.597
D5/pond	0.020	<0.001	<0.001	<0.001	<0.001	<0.001	0.026
D5/stream	0.426	<0.001	<0.001	<0.001	<0.001	<0.001	0.553
R2/stream	1.095	0.001	0.001	<0.001	<0.001	0.001	**1.422**

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Worst case obtained among all the pH options

Metabolites of Mesotrione

Table 9.5-18: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for MNBA for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of PRIMARY MX in maize

Group		Fish acute	Inverteb. acute	Algae	Aquatic plants
Test species		*O. mykiss*	*D. magna*	*P. subcapitata*	*L. gibba*
Endpoint (µg/L)		LC_{50} 120000	EC_{50} 130000	E_rC_{50} 42000	EC_{50} 97000
AF		100	100	10	10
RAC (µg/L)		1200	1300	4200	9700
FOCUS Scenario	PEC_{gl-max} (µg/L)*				
Step 1					
-	18.49	0.015	0.014	0.004	0.002
Step 2	-				
S-Europe	1.90	0.002	0.001	< 0.001	< 0.001
N-Europe	0.98	0.001	0.001	< 0.001	< 0.001

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Aquatic plants**
Test species		***O. mykiss***	***D. magna***	***P. subcapitata***	***L. gibba***
Endpoint (µg/L)		LC_{50} 120000	EC_{50} 130000	E_rC_{50} 42000	EC_{50} 97000
AF		100	100	10	10
RAC (µg/L)		1200	1300	4200	9700
FOCUS Scenario	**PEC_{gl-max} (µg/L)***				
Step 1					
-	14.10	0.012	0.011	0.003	0.001
Step 2					
S-Europe	2.15	0.002	0.002	0.001	<0.001
N-Europe	1.10	0.001	0.001	<0.001	<0.001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Worst case obtained among all the pH options

Table 9.5-19: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AMBA for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of PRIMARY MX in maize

Group		Fish acute	Inverteb. acute	Algae	Aquatic plants
Test species		*O. mykiss*	*D. magna*	*P. subcapitata*	*L. gibba*
Endpoint (µg/L)		LC_{50} 150000	EC_{50} 160000	E_bC_{50} 14000	EC_{50} 90000

Group		Fish acute	Inverteb. acute	Algae	Aquatic plants
AF		100	100	10	10
RAC (µg/L)		1500	1600	1400	9000
FOCUS Scenario	PEC gl-max (µg/L)*				
Step 1					
-	12.49	0.008	0.008	0.009	0.001
Step 2					
S-Europe	2.18	0.001	0.001	0.002	< 0.001
N-Europe	1.21	0.001	0.001	0.001	< 0.001

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Aquatic plants**
Test species		***O. mykiss***	***D. magna***	***P. subcapitata***	***L. gibba***
Endpoint		LC_{50}	EC_{50}	E_bC_{50}	EC_{50}
(µg/L)		150000	160000	14000	90000
AF		100	100	10	10
RAC (µg/L)		1500	1600	1400	9000
FOCUS Scenario	**PEC gl-max (µg/L)***				
Step 1					
-	6.47	0.004	0.004	0.005	0.001
Step 2					
S-Europe	1.64	0.001	0.001	0.001	<0.001
N-Europe	0.88	0.001	0.001	0.001	<0.001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Worst case obtained among all the pH options

Table 9.5-20: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for SYN546974 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of PRIMARY MX in maize

Group		Aquatic plants
Test species		*Lemna gibba*
Endpoint (µg/L)		EC_{50} 95000
AF		10
RAC (µg/L)		9500
FOCUS Scenario	PEC gl-max (µg/L)*	
Step 1		
-	1.26	< 0.001
Step 2		

Group		Aquatic plants
S-Europe	0.31	< 0.001
N-Europe	0.31	< 0.001

Group		**Aquatic plants**
Test species		***Lemna gibba***
Endpoint (µg/L)		EC_{50} 95000
AF		10
RAC (µg/L)		9500
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)***	
Step 1		
	0.96	< 0.001
Step 2		
S-Europe	0.23	< 0.001
N-Europe	0.23	< 0.001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Worst case obtained among all the pH options

For the intended uses on maize, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for higher plant as characterised by an EC_{50} for *Lemna gibba* of 7.7 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.

Table 9.5-21: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for Mesotrione (linear, pH 5.1) based on FOCUS Step 4 calculations and toxicity data for higher plant with mitigation of spray drift and run-off for the use of PRIMARY MX in maize

Intended use **Active substance** **Application rate (g/ha)**		**Maize** Mesotrione 1 x 118		
Nozzle reduction	**No-spray buffer (m)**	None	5	10
	Vegetated filter strip (m)	None	5	10
None	R2 stream (pH 5.1) Linear*	1.454	0.930	0.642
RAC (µg/L) 0.77		**PEC/RAC ratio**		
None	R2 stream (pH 5.1) Linear*	**1.888**	**1.208**	0.834

Intended use	**Maize**
Active substance	Mesotrione
Application rate (g/ha)	1 x 118

Nozzle reduction	**No-spray buffer (m)**	None	5
	Vegetated filter strip (m)	None	5
None	R2 stream (pH 5.1)	1.095	0.701
RAC (µg/L)			
0.77		**PEC/RAC ratio**	
None	R2 stream (pH 5.1)	**1.422**	0.910

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

zRMS comment:

Mesotrione

For the intended uses on maize, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for higher plant as characterised by an EC_{50} for *Lemna gibba* of 7.7 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. Based on the results of the risk assessment at step 4, the following conclusions regarding buffer zones and vegetative buffer strips may be drawn for maize use:

-R2 stream (pH 5.1) 5m no spray buffer zone and a 5m vegetative buffer strip are required.

For MNBA, AMBA and SYN546974 metabolites, all PEC/RAC values are below the trigger value of 1 at step 1-2. Therefore, no further assessment is necessary.

PRIMARY MX

Table 9.5-22: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for PRIMARY MX for each organism group for the use of PRIMARY MX in maize

Group			Fish acute	Inverteb. acute	Algae	Aquatic plants
Test species			*O. mykiss*	*D. magna*	*P. subcapitata*	*L. gibba*
Endpoint (µg/L)			LC_{50} 100000	IC_{50} 100000	E_rC_{50} 20211	E_rC_{50} 16.6
AF			100	100	10	10
RAC (µg/L)			1000	1000	2021.1	1.66
Distance	%Nozzles	PEC $_{gl-max}$ (µg/L)				
1m	None	3.047	0.003	0.003	0.002	**1.836**
	50%	1.524	0.002	0.002	0.001	0.918
	75%	0.762	0.001	0.001	< 0.001	0.459
	90%	0.305	< 0.001	< 0.001	< 0.001	0.184
5m	None	0.627	0.001	0.001	< 0.001	0.378
	50%	0.314	< 0.001	< 0.001	< 0.001	0.189
	75%	0.157	< 0.001	< 0.001	< 0.001	0.094
	90%	0.063	< 0.001	< 0.001	< 0.001	0.038

Group			**Fish acute**	**Inverteb. acute**	**Algae**	**Aquatic plants**	
Test species			***O. mykiss***	***D. magna***	***P. subcapitata***	***L. gibba***	
Endpoint (µg/L)			LC_{50} 100000	IC_{50} 100000	E_rC_{50} 20211	E_rC_{50} 16.6	14.9*
AF			100	100	10	10	10
RAC (µg/L)			1000	1000	2021.1	1.66	1.49
Distance	**%Nozzles**	**PEC $_{gl-max}$ (µg/L)**					
1m	None	2.308	0.002	0.002	0.001	**1.390**	**1.549**
	50%	1.154	0.001	0.001	0.001	0.695	0.774
	75%	0.577	0.001	0.001	<0.001	0.348	0.387
	90%	0.231	<0.001	<0.001	<0.001	0.139	0.155
5m	None	0.475	<0.001	<0.001	<0.001	0.286	0.319
	50%	0.238	<0.001	<0.001	<0.001	0.143	0.160
	75%	0.119	<0.001	<0.001	<0.001	0.072	0.080
	90%	0.048	<0.001	<0.001	<0.001	0.029	0.032

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

* Product endpoint recalculated as per the specified procedure for formulation tests in the "Outcome of pesticides peer review meeting on recurring issues in ecotoxicology" (EFSA Supporting publication 2019:EN-1673).

zRMS comment:

PRIMARY MX

For the endpoints from formulated product PRIMARY MX, any spray buffer zone with 50% of nozzles reduction OR a 5m no spray buffer zone are enough for acceptable risk.

In addition, for the combined was provided by the applicant.

Risk assessment for the combinations of a.s. in the formulation

Following the dilution and spraying of the formulated product, much of the formulation constituents are likely to be lost by volatilisation. Therefore, shortly after application of a formulated product, aquatic organisms are mainly exposed to the active substance present in the formulation. In addition, as demonstrated in the short-term studies here above there are no indications for interactions of the active substances (no synergisms or additional toxicity occurs due to the co-formulants) given that the formulation does not cause an (unexpected) increased toxicity compared to the active substances. An evaluation of the risk posed by the intact formulation is therefore relevant only for the acute/short-term assessment. The long-term risk was assessed considering data for the active substances in the formulation and no chronic combined risk assessment has been performed.

According to the new EFSA Scientific Opinion (EFSA, 2013) measured and calculated mixture toxicity should be compared to determine synergistic, additive or antagonistic effects of the formulation. In the following the concentration addition (CA) model is used as proposed by EFSA.

To determine the respective formulation effect, EFSA proposed to calculate the model deviation ratio (MDR), which divides the calculated mixture toxicity ($LC_{50}/_{EC50\ mix\text{-}CA}$) by the measured mix-ture toxicity ($LC_{50}/EC_{50\ PRIMARY\ MX}$). Ecotoxicity studies are biological test systems which underlie a certain natural biological variability when repeating a study. Hence, a threshold has to be defined when an increased/decreased mixture toxicity effect cannot be seen as only additive any longer. EFSA proposes a factor of 5, i.e. if the MDR is between 0.2 and 5 the observed and calculated mixture toxicities are considered in agreement.

~~The calculated MDR values are between 0.2 and 5 for each organism (see Table 9.5-23), indicating that the formulation does not cause an (unexpected) increased toxicity compared to the active substances for these organisms. No synergisms or additional toxicity occurs due to the co-formulants.~~

~~**Active susbtance / species**~~	~~**Test system**~~	~~**Endpoint (mg a.s./L)**~~
~~**Rimsulfuron**~~		
~~*Oncorhynchus mykiss*~~	~~LC_{50} 96h~~	~~390~~
~~*Daphnia magna*~~	~~EC_{50} 48h~~	~~360~~
~~*S. capricornutum*~~	~~EC_{50} 72h~~	~~1.2~~
~~*L. minor*~~	~~E_bC_{50} 14d~~	~~0.0046~~
~~**Nicosulfuron**~~		
~~*Oncorhynchus mykiss*~~	~~LC_{50} 96h~~	~~2.2~~
~~*Daphnia magna*~~	~~EC_{50} 48h~~	~~3.3~~
~~*A. flos-aquae*~~	~~E_bC_{50} 72h~~	~~4~~
~~*L. gibba*~~	~~EC_{50} 7d~~	~~0.0017~~
~~**Mesotrione**~~		
~~*Oncorhynchus mykiss*~~	~~LC_{50} 96h~~	~~120~~

Daphnia magna	EC_{50} 48h	622
P. subcapitata	E_rC_{50} 96h	13
L. gibba	E_bC_{50} 14d	0.0077

Table 9.5-23: Summary of results obtained in the studies with the formulated product PRIMARY MX and comparison of calculated and measured mixture toxicity

Test species	Endpoint & Test system	LC_{50} / EC_{50} [mg/L]			
		Measured toxicity of PRIMARY MX (LC_{50} PRIMARY MX or EC_{50} PRIMARY MX) (mg/L)	Measured toxicity of PRIMARY MX (converted to be a.i. based) (LC_{50} PRIMARY MX or EC_{50} PRIMARY MX) (mg a.s./L)	Calculated mixture toxicity[a] LC_{50} mix-CA or EC_{50} mix-CA	Model deviation ratio (MDR = EC_{50} mix-CA / EC_{50} PRIMARY MX)
O. mykiss	LC_{50}, acute, 96 h	100	51.000	8.851	0.174
D. magna	EC_{50}, acute, 48 h	100	51.000	13.774	0.270
P. subcapitata	E_rC_{50}, 72 h	20.211	10.308	6.167	0.598
L. gibba	E_rC_{50}, 7 d	0.0166	0.008	0.004	0.486

[a] The mixture toxicity of the formulation was re-calculated based on the nominal contents of Rimsulfuron (30 g/kg), Nicosulfuron (120 g/kg) and Mesotrione (360 g/kg) within the formulation.

Table 9.5-24: Comparison of mixture composition in the formulation study (giving the measured mixture toxicity) and mixture composition at the PEC_{mix}

Test species	Endpoint & Test system	LC_{50} / EC_{50} [mg/L]		
		Calculated mixture toxicity (a.s. in PRIMARY MX) LC_{50} mix-CA or EC_{50} mix-CA	Calculated mixture toxicity (a.s. in PEC_{mix})[b] LC_{50} mix-CA or EC_{50} mix-CA at lower exposure tier	Factors (EC_{50} mix-CA (a.s. in PRIMARY MX)/EC_{50} mix-CA (a.s. in PEC_{mix})) at lower exposure tier
O. mykiss	LC_{50}, acute, 96 h	8.851	8.050 9.259	1.099 1.072
D. magna	EC_{50}, acute, 48 h	13.774	12.446 12.807	1.107 1.075
P. subcapitata	E_rC_{50}, static, 72 h	6.167	5.258 5.989	1.173 1.030
L. gibba	E_rC_{50}, semi static 7d	0.004	0.004	1.060 1.035

[a] The mixture toxicity of the formulation was re-calculated based on the nominal contents of Rimsulfuron (30 g/kg), Nicosulfuron (120 g/kg) and Mesotrione (360 g/kg) within the formulation.

[b] The mixture toxicity of the formulation was re-calculated based on the mixture composition at the PEC_{mix} for Rimsulfuron (0.000870 0.00066 mg/L at Step 2 for SEU scenario), Nicosulfuron (0.002520 0.00274 mg/L at Step 2 for NEU SEU scenario) and Mesotrione (0.006270 0.00741 mg/L at Step 2 for SEU scenario, pH 5.1 6.5 (linear)).

Table 9.5-25: Comparison of calculated mixture toxicity and toxicity per fraction of a single a.s.

Test species	Endpoint & Test system	LC_{50} / EC_{50} [mg/L]		
		Calculated mixture toxicity (a.s. in PRIMARY MX) $LC_{50\ mix-CA}$ or $EC_{50\ mix-CA}$	Calculated toxicity per fraction of PRIMARY MX (based on each a.s.) $(1/TU_i)$[a]	Deviation from mixture toxicity $(1-EC_{x\ mix-CA} \times (1/EC_{x\ mix-CA} - TU_i))$ [%]
O. mykiss	LC_{50}, acute, 96 h	8.851	Rimsulfuron: 6630 Nicosulfuron: 9.35 Mesotrione: 170	Rimsulfuron: 0.1% Nicosulfuron: 94.66% Mesotrione: 5.2%
D. magna	EC_{50}, acute, 48 h	13.774	Rimsulfuron: 6120 Nicosulfuron: 14.025 Mesotrione: 881.167	Rimsulfuron: 0.2% Nicosulfuron: 98.21% Mesotrione: 1.6%
P. subcapitata	E_rC_{50}, static, 72 h	6.167	Rimsulfuron: 20.4 Nicosulfuron: 17 Mesotrione: 18.417	Rimsulfuron: 30.2% Nicosulfuron: 36.3% Mesotrione: 33.5%
L. gibba	E_rC_{50}, semi static 7d	0.004	Rimsulfuron: 0.0782 Nicosulfuron: 0.0072 Mesotrione: 0.0109	Rimsulfuron: 5.3% Nicosulfuron: 57.0% Mesotrione: 37.7%

[a] TUi is defined as the concentration of the i[th] a.s. at the $EC_{50\ PRIMARY\ MX}$ (re-calculated to the sum of a.s.) divided by the respective single substance toxicity ($EC_{50\ a.s.}$). This is calculated based on the nominal contents of Rimsulfuron (30 g/kg), Nicosulfuron (120 g/kg) and Mesotrione (360 g/kg) within the formulation.

Regarding PRIMARY MX, nicosulfuron clearly drives the acute risk for fish and aquatic invertebrates, but not for algae and aquatic plants. For these two last species, the studies performed with the formulated product PRIMARY MX do not reflect the toxicity of one particular active substance, as the formulation toxicity – endpoint recalculated to each active substance concentrations – does not come for 90 % (of more) from the toxicity per fraction of a single a.s. (TUi) (see Table 9.5-25).

Table 9.5-26: Conduct a mixture RA based on calculated mixture toxicity according to 10.3.8 from EFSA AGD in maize for fish

Exposure	Lower exposure tier			Higher exposure tier		
	Rimsulfuron	Nicosulfuron	Mesotrione	Rimsulfuron	Nicosulfuron	Mesotrione
Exposure tier (FOCUS step)	Step 2 (SEU)	Step 2 (NEU SEU)	Step 2 (SEU) pH 5.1 6.5 (linear)	Step 2 (NEU)	Step 2 (SEU NEU)	Step 2 (NEU) pH 5.1 6.5 (linear)
PECsw [mg a.s./L]	0.000870 0.00066	0.00252 0.00274	0.006270 0.00741	0.000470 0.00036	0.00143 0.00150	0.003440 0.00395
Relative proportions of the individual mixture components in the environment (pi PEC)	0.090 0.061	0.261 0.253	0.649 0.685	0.088 0.062	0.268 0.258	0.644 0.680
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]	0.009660 0.010810			0.005340 0.005810		
Calculated mixture toxicity (a.s. in PECmix) (ECx mix-CA = ∑ (pi PEC/ECx i)) [mg a.s./L] Toxicity of the product (a.s. based) (ECx PPP) [mg a.s./L]	8.050 51.000			7.854 51.000		
ETRmix = PECmix/ECx PPP	0.001 <0.001			0.001 <0.001		
Trigger	0.01					

No unacceptable risk to fish is expected from the exposure to the combined active substances following proposed uses of the product.

Applicability of such approach is justified following the EFSA AGD *Decision scheme for mixture toxicity risk assessment* for fish.

Step	EFSA AGD provisions	Option	Justification	Outcome
1	Are measured toxicity data (EC_x) available for the given endpoint (typically chronic data available only for a.s.)?	For both formulation ($EC_{x\text{-PRIMARY MX}}$) and a.s. ($EC_{xa.s.}$):	Please refer to tables 9.5-1 to 9.5-4	Go to 2
2	Check the plausibility of the measured formulation toxicity ($EC_{xPRIMARY\ MX}$) against the calculated mixture toxicity $EC_{xmix\text{-}CA}$ (assuming CA, Equation 13) for exactly the mixture composition of the a.s. in the formulation ($EC_{xPRIMARY\ MX}$) by means of the model deviation ratio (MDR = EC_{xmix}-CA/$EC_{xPRIMARY\ MX}$).	MDR = < 0.2	Please refer to table 9.5-23	Go to 9

9	Carefully recheck the apparent antagonism as observed in the measured mixture toxicity data ($EC_{xPRIMARY\ MX}$) regarding potential impacts of the default assumption of CA and/or heterogeneous input data used for the CA calculation. Does the apparent antagonism remain and no toxicologically plausible explanation is available (e.g. special feature of the formulation type)?	No (measured mixture toxicity plausible)		Go to 3
3	Check whether the mixture composition in the formulation study giving the measured mixture toxicity ($EC_{xPRIMARY\ MX}$) in terms of the relative proportions of the individual a.s. is similar to the mixture composition at the PEC_{mix}. As a direct comparison on the basis of the relative proportions of the a.s. at the $EC_{x\ PRIMARY\ MX}$ with the relative proportion at the PEC_{mix} is not informative as such, the comparison is done based on calculated mixture toxicity (assuming CA) for both mixture compositions. Therefore, calculate $EC_{x\ mix\ CA}$ (see Equation 13) for the mixture composition of the a.s. at the PEC_{mix} and compare with the estimate calculated for the formulation (as already done in step 2 above).	$EC_{x\ mix\ CA}$ (a.s. in product)/$EC_{x\ mix\ CA}$ (a.s. in PECmix) = 1.099 1.072 (0.8 - 1.2)	Please refer to table 9.5-24	Go to 4
4	Conduct a mixture RA based on measured mixture toxicity, with the exposure-toxicity ratio (ETR_{mix}) being defined as the PEC_{mix} divided by the measured $EC_{x\ PRIMARY\ MX}$ and compare the outcome with the acceptability criterion (trigger value) decisive for the specific end-point/exposure scenario combination.	If ETR_{mix} < 1 for aquatic plants: Low risk		**Low risk**

Table 9.5-27: Conduct a mixture RA based on calculated mixture toxicity according to 10.3.8 from EFSA AGD in maize for aquatic invertebrates

Exposure	Lower exposure tier			Higher exposure tier		
	Rimsulfuron	Nicosulfuron	Mesotrione	Rimsulfuron	Nicosulfuron	Mesotrione
Exposure tier (FOCUS step)	Step 2 (SEU)	Step 2 (NEU SEU)	Step 2 (SEU) pH 5.1 6.5 (linear)	Step 2 (NEU)	Step 2 (SEU NEU)	Step 2 (NEU) pH 5.1 6.5 (linear)
PECsw [mg a.s./L]	0.000870 0.00066	0.00252 0.00274	0.006270 0.00741	0.000470 0.00036	0.00143 0.00150	0.003440 0.00395
Relative proportions of the individual mixture components in the environment (pi PEC)	0.090 0.061	0.261 0.253	0.649 0.685	0.088 0.062	0.268 0.258	0.644 0.680
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]	0.009660 0.010810			0.005340 0.005810		
Calculated mixture toxicity (a.s. in PECmix) (ECx mix-CA = ∑ (pi PEC/ECx i)) [mg a.s./L]	8.050 51.000			7.854 51.000		
Toxicity of the product (a.s. based) (ECx PPP) [mg a.s./L]						
ETRmix = PECmix/ECx PPP	0.001 <0.001			0.001 <0.001		
Trigger	0.01					

No unacceptable risk to aquatic invertebrates is expected from the exposure to the combined active substances following proposed uses of the product.

Applicability of such approach is justified following the EFSA AGD *Decision scheme for mixture toxicity risk assessment* for aquatic invertebrates.

Step	EFSA AGD provisions	Option	Justification	Outcome
1	Are measured toxicity data (EC_x) available for the given endpoint (typically chronic data available only for a.s.)?	For both formulation ($EC_{x\text{-PRIMARY MX}}$) and a.s. ($EC_{xa.s.}$):	Please refer to tables 9.5-1 to 9.5-4	Go to 2
2	Check the plausibility of the measured formulation toxicity ($EC_{xPRIMARY\ MX}$) against the calculated mixture toxicity $EC_{xmix\text{-}CA}$ (assuming CA, Equation 13) for exactly the mixture composition of the a.s. in the formulation ($EC_{xPRIMARY\ MX}$) by means of the model deviation ratio ($MDR = EC_{xmix\text{-}CA}/EC_{xPRIMARY\ MX}$).	MDR = 0.2-5	Please refer to table 9.5-23	Go to 3

3	Check whether the mixture composition in the formulation study giving the measured mixture toxicity ($EC_{xPRIMARY\ MX}$) in terms of the relative proportions of the individual a.s. is similar to the mixture composition at the PEC_{mix}. As a direct comparison on the basis of the relative proportions of the a.s. at the $EC_{x\ PRIMARY\ MX}$ with the relative proportion at the PECmix is not informative as such, the comparison is done based on calculated mixture toxicity (assuming CA) for both mixture compositions. Therefore, calculate $EC_{xmix\ CA}$ (see Equation 13) for the mixture composition of the a.s. at the PECmix and compare with the estimate calculated for the formulation (as already done in step 2 above).	$EC_{x\ mix\ CA}$ (a.s. in product)/$EC_{x\ mix\ CA}$ (a.s. in PEC_{mix}) is 1.107 1.075 (0.8 – 1.2)	Please refer to table 9.5-24	Go to 4
4	Conduct a mixture RA based on measured mixture toxicity, with the exposure-toxicity ratio (ETR_{mix}) being defined as the PEC_{mix} divided by the measured $EC_{x\ PRIMARY\ MX}$ and compare the outcome with the acceptability criterion (trigger value) decisive for the specific end-point/exposure scenario combination.	If ETR_{mix} < 1 for aquatic plants: Low risk		Low risk

Table 9.5-28: Conduct a mixture RA based on calculated mixture toxicity according to 10.3.8 from EFSA AGD in maize for algae

Exposure	Lower exposure tier			Higher exposure tier		
	Rimsulfuron	Nicosulfuron	Mesotrione	Rimsulfuron	Nicosulfuron	Mesotrione
Exposure tier (FOCUS step)	Step 2 (SEU)	Step 2 (NEU SEU)	Step 2 (SEU) pH 5.1 6.5 (linear)	Step 2 (NEU)	Step 2 (SEU NEU)	Step 2 (NEU) pH 5.1 6.5 (linear)
PECsw [mg a.s./L]	0.000870 0.00066	0.00252 0.00274	0.006270 0.00741	0.000470 0.00036	0.00143 0.00150	0.003440 0.00395
Relative proportions of the individual mixture components in the environment (pi PEC)	0.090 0.061	0.261 0.253	0.649 0.685	0.088 0.062	0.268 0.258	0.644 0.680
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]	0.009660 0.010810			0.005340 0.005810		
Calculated mixture toxicity (a.s. in PECmix) (ECx mix-CA = ∑ (pi PEC/ECx i)) [mg a.s./L]	5.258 10.308			5.267 10.308		
Toxicity of the product (a.s. based) (ECx PPP) [mg a.s./L]						
ETRmix = PECmix/ECx PPP	0.002 0.001			0.001		
Trigger	0.1					

No unacceptable risk to algae is expected from the exposure to the combined active substances following proposed uses of the product.

Applicability of such approach is justified following the EFSA AGD *Decision scheme for mixture toxicity risk assessment* for algae.

Step	EFSA AGD provisions	Option	Justification	Outcome
1	Are measured toxicity data (EC_x) available for the given endpoint (typically chronic data available only for a.s.)?	For both formulation ($EC_{xPRIMARY\ MX}$) and a.s. ($EC_{xa.s.}$):	Please refer to tables 9.5-1 to 9.5-4	Go to 2
2	Check the plausibility of the measured formulation toxicity ($EC_{xPRIMARY\ MX}$) against the calculated mixture toxicity $EC_{xmix\text{-}CA}$ (assuming CA, Equation 13) for exactly the mixture composition of the a.s. in the formulation ($EC_{xPRIMARY\ MX}$) by means of the model deviation ratio ($MDR = EC_{xmix\text{-}CA}/EC_{xPRIMARY\ MX}$).	MDR = 0.2-5	Please refer to table 9.5-23	Go to 3
3	Check whether the mixture composition in the formulation study giving the measured mixture toxicity ($EC_{xPRIMARY\ MX}$) in terms of the relative proportions of the individual a.s. is similar to the mixture composition at the PEC_{mix}. As a direct comparison on the basis of the relative proportions of the a.s. at the $EC_{xPRIMARY\ MX}$ with the relative proportion at the PECmix is not informative as such, the comparison is done based on calculated mixture toxicity (assuming CA) for both mixture compositions. Therefore, calculate $EC_{xmix\text{-}CA}$ (see Equation 13) for the mixture composition of the a.s. at the PEC_{mix} and compare with the estimate calculated for the formulation (as already done in step 2 above).	$EC_{x\ mix\text{-}CA}$ (a.s. in product)/$EC_{x\ mix\text{-}CA}$ (a.s. in PEC_{mix}) is 1.173-1.030 (0.8-1.2)	Please refer to table 9.5-24	Go to 4
4	Conduct a mixture RA based on measured mixture toxicity, with the exposure-toxicity ratio (ETR_{mix}) being defined as the PEC_{mix} divided by the measured $EC_{x\ PRIMARY\ MX}$ and compare the outcome with the acceptability criterion (trigger value) decisive for the specific end-point/exposure scenario combination.	If ETR_{mix} < 1 for aquatic plants: Low risk		**Low risk**

Table 9.5-29: Conduct a mixture RA based on calculated mixture toxicity according to 10.3.8 from EFSA AGD in maize for aquatic plants

Exposure	Lower exposure tier			Higher exposure tier		
	Rimsulfuron	Nicosulfuron	Mesotrione	Rimsulfuron	Nicosulfuron	Mesotrione
Exposure tier (FOCUS step)	Step 2 (SEU)	Step 2 (NEU SEU)	Step 2 (SEU) pH 5.1 6.5 (linear)	Step 2 (NEU)	Step 2 (SEU NEU)	Step 2 (NEU) pH 5.1 6.5 (linear)
PECsw [mg a.s./L]	0.000870 0.00066	0.00252 0.00274	0.006270 0.00741	0.000470 0.00036	0.00143 0.00150	0.003440 0.00395
Relative proportions of the individual mixture components in the environment (pi PEC)	0.090 0.061	0.261 0.253	0.649 0.685	0.088 0.062	0.268 0.258	0.644 0.680
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]	0.009660 0.010810			0.005340 0.005810		
Calculated mixture toxicity (a.s. in PECmix) (ECx mix-CA = ∑ (pi PEC/ECx i)) [mg a.s./L] Toxicity of the product (a.s. based) (ECx PPP) [mg a.s./L]	0.004 0.008			0.004 0.008		
ETRmix = PECmix/ECx PPP	2.486 1.277			1.390 0.686		
Trigger	0.1					

An unacceptable risk to aquatic plants cannot be assumed after exposure to the combined active substances following proposed uses of the product.

A refinement was performed considering the results of recovery from a new study submitted with this application.

Based on the results of the recovery phase of the study on *Lemna* conducted with nicosulfuron (new report KCP 10.2.1-05 submitted with this application are listed in Appendix 1 and summarised in Appendix 2) the effects of nicosulfuron on it are expected to be reversible at concentrations lower than or equal to 0.74 µg nicosulfuron/L. Hence, the trigger considered after applying this new endpoint, would be 1, instead of 0.1.

Active susbtance / species	Test system	Endpoint (mg a.s./L)
Rimsulfuron		
Oncorhynchus mykiss	LC_{50} 96h	390
Daphnia magna	EC_{50} 48h	360
S. capricornutum	EC_{50} 72h	1.2
L. minor	E_rC_{50} 14d	0.0046
Nicosulfuron		
Oncorhynchus mykiss	LC_{50} 96h	2.2
Daphnia magna	EC_{50} 48h	3.3
A. flos-aquae	E_bC_{50} 72h	4

L. gibba	EC_{50} 7d	0.00074
Mesotrione		
Oncorhynchus mykiss	LC_{50} 96h	120
Daphnia magna	EC_{50} 48h	622
P. subcapitata	E_rC_{50} 96h	13
L. gibba	E_bC_{50} 14d	0.0077

Table 9.5-30: Summary of results obtained in the studies with the formulated product PRIMARY MX and comparison of calculated and measured mixture toxicity – *Lemna* endpoint refinement

Test species	Endpoint & Test system	LC_{50} / EC_{50} [mg/L]			
		Measured toxicity of PRIMARY MX (LC_{50} PRIMARY MX or EC_{50} PRIMARY MX) (mg/L)	Measured toxicity of PRIMARY MX (converted to be a.i. based) (LC_{50} PRIMARY MX or EC_{50} PRIMARY MX) (mg a.s./L)	Calculated mixture toxicity[a] LC_{50} mix-CA or EC_{50} mix-CA	Model deviation ratio (MDR = EC_{50} mix-CA / EC_{50} PRIMARY MX)
L. gibba	LOEC, 7d	0.0166	0.008	0.002	0.280

[a] The mixture toxicity of the formulation was re-calculated based on the nominal contents of Rimsulfuron (30 g/kg), Nicosulfuron (120 g/kg) and Mesotrione (360 g/kg) within the formulation.

Table 9.5-31: Comparison of mixture composition in the formulation study (giving the measured mixture toxicity) and mixture composition at the PEC_{mix} – *Lemna* endpoint refinement

Test species	Endpoint & Test system	LC_{50} / EC_{50} [mg/L]		
		Calculated mixture toxicity (a.s. in PRIMARY MX) LC_{50} mix-CA or EC_{50} mix-CA	Calculated mixture toxicity (a.s. in PEC_{mix})[b] LC_{50} mix-CA or EC_{50} mix-CA at lower exposure tier	Factors (EC_{50} mix-CA (a.s. in PRIMARY MX)/EC_{50} mix-CA (a.s. in PEC_{mix})) at lower exposure tier
L. gibba	LOEC, static 7d	0.002	0.002	1.080 1.052

[a] The mixture toxicity of the formulation was re-calculated based on the nominal contents of Rimsulfuron (30 g/kg), Nicosulfuron (120 g/kg) and Mesotrione (360 g/kg) within the formulation.

[b] The mixture toxicity of the formulation was re-calculated based on the mixture composition at the PEC_{mix} for Rimsulfuron (0.000870 0.00066 mg/L at Step 2 for SEU scenario), Nicosulfuron (0.002520 0.00274 mg/L at Step 2 for NEU SEU scenario) and Mesotrione (0.006270 0.00741 mg/L at Step 2 for SEU scenario, pH 5.16.5 (linear)).

Table 9.5-32: Comparison of calculated mixture toxicity and toxicity per fraction of a single a.s. – *Lemna* endpoint refinement

Test species	Endpoint & Test system	LC_{50} / EC_{50} [mg/L]		
		Calculated mixture toxicity (a.s. in PRIMARY MX) LC_{50} mix-CA or EC_{50} mix-CA	Calculated toxicity per fraction of PRIMARY MX (based on each a.s.) $(1/TU_i)$[a]	Deviation from mixture toxicity ($1-EC_{x\ mix-CA} \times (1/EC_{x\ mix-CA} - TU_i)$) [%]
L. gibba	LOEC, static 7d	0.002	Rimsulfuron: 0.078 Nicosulfuron: 0.003 Mesotrione: 0.011	Rimsulfuron: 3.0% Nicosulfuron: 75.3% Mesotrione: 21.7%

[a] TUi is defined as the concentration of the i^{th} a.s. at the $EC_{50\ PRIMARY\ MX}$ (re-calculated to the sum of a.s.) divided by the respective single-substance toxicity ($EC_{50\ a.s.}$). This is calculated based on the nominal contents of Rimsulfuron (30 g/kg), Nicosulfuron (120 g/kg) and Mesotrione (360 g/kg) within the formulation.

Table 9.5-33: Conduct a mixture RA based on calculated mixture toxicity according to 10.3.8 from EFSA AGD in maize for aquatic plants – *Lemna* endpoint refinement

Exposure	Lower exposure tier			Higher exposure tier (refinement)		
	Rimsulfuron	Nicosulfuron	Mesotrione	Rimsulfuron	Nicosulfuron	Mesotrione
Exposure tier (FOCUS step)	Step 2 (SEU)	Step 2 (NEU SEU)	Step 2 (SEU) pH 5.1-6.5 (linear)	Step 3 (R4 stream)	Step 4 (15-10 m VBZ, R4 stream)	Step 4 (10-5 m VBZ, R2 stream)
PECsw [mg a.s./L]	0.000870 0.00066	0.00252 0.00274	0.006270 0.00741	0.000413 0.000313	0.000598 0.000590	0.000642 0.000701
Relative proportions of the individual mixture components in the environment (pi PEC)	0.090 0.061	0.261 0.252	0.649 0.685	0.250 0.195	0.362 0.368	0.388 0.437
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]	0.009660 0.010810			0.001653 0.001604		
Calculated mixture toxicity (a.s. in PECmix) (ECx mix-CA = ∑ (pi PEC/ECx i)) [mg a.s./L] Toxicity of the product (a.s. based) (ECx PPP) [mg a.s./L]	0.004 0.008			0.002 0.008		
ETRmix = PECmix/ECx PPP	4.409 1.277			0.981 0.189		
Trigger	1					

The refinement is conducted by taking into account FOCUS PEC_{sw} values for Rimsulfuron (Step 3), Nicosulfuron (Step 4; 15-10 m vegetative buffer strip) and Mesotrione (Step 4; 10 m vegetative buffer strip) (see Table 9.5-33). No unacceptable risk to aquatic plants is expected from the exposure to the combined active substances following proposed uses of the product.

Applicability of such approach is justified following the EFSA AGD *Decision scheme for mixture toxicity risk assessment* for aquatic plants.

Step	EFSA AGD provisions	Option	Justification	Outcome
1	Are measured toxicity data (EC_x) available for the given endpoint (typically chronic data available only for a.s.)?	For both formulation ($EC_{xPRIMARY\ MX}$) and a.s. ($EC_{xa.s.}$):	Please refer to tables 9.5-1 to 9.5-4	Go to 2
2	Check the plausibility of the measured formulation toxicity ($EC_{xPRIMARY\ MX}$) against the calculated mixture toxicity $EC_{xmix\text{-}CA}$ (assuming CA, Equation 13) for exactly the mixture composition of the a.s. in the formulation ($EC_{xPRIMARY\ MX}$) by means of the model deviation ratio (MDR = $EC_{xmix\text{-}CA}/EC_{x\text{-}PRIMARY\ MX}$):	MDR = 0.2-5	Please refer to table 9.5-30	Go to 3

3	Check whether the mixture composition in the formulation study giving the measured mixture toxicity ($EC_{xPRIMARY\ MX}$) in terms of the relative proportions of the individual a.s. is similar to the mixture composition at the PEC_{mix}. As a direct comparison on the basis of the relative proportions of the a.s. at the $EC_{x\ PRIMARY\ MX}$ with the relative proportion at the PECmix is not informative as such, the comparison is done based on calculated mixture toxicity (assuming CA) for both mixture compositions. Therefore, calculate $EC_{x\ mix\ CA}$ (see Equation 13) for the mixture composition of the a.s. at the PEC_{mix} and compare with the estimate calculated for the formulation (as already done in step 2 above).	$EC_{x\ mix\ CA}$ (a.s. in product)/$EC_{x\ mix\ CA}$ (a.s. in PECmix) = 1.080 1.053 (0.8 – 1.2)	Please refer to table 9.5-31	Go to 4
4	Conduct a mixture RA based on measured mixture toxicity, with the exposure-toxicity ratio (ETR_{mix}) being defined as the PEC_{mix} divided by the measured $EC_{x\ PRIMARY\ MX}$ and compare the outcome with the acceptability criterion (trigger value) decisive for the specific end-point/exposure scenario combination.	If ETR_{mix} < 1 for aquatic plants: Low risk		**Low risk**

As an alternative to the nicosulfuron RAC of 0.74 µg/L, a different approach following new provided PECsw was followed using the agreed at EU level *Lemna* endpoint (E_rC_{50} = 2.7 µg/L, RAC = 0.27 µg/L).

Active susbtance / species	**Test system**	**Endpoint (mg a.s./L)**
Rimsulfuron		
Oncorhynchus mykiss	LC_{50} 96h	390
Daphnia magna	EC_{50} 48h	360
S. capricornutum	EC_{50} 72h	1.2
L. minor	E_rC_{50} 14d	0.0046
Nicosulfuron		
Oncorhynchus mykiss	LC_{50} 96h	2.2
Daphnia magna	EC_{50} 48h	3.3
A. flos-aquae	E_bC_{50} 72h	4
L. gibba	EC_{50} 7d	0.0027
Mesotrione		
Oncorhynchus mykiss	LC_{50} 96h	120
Daphnia magna	EC_{50} 48h	622
P. subcapitata	E_rC_{50} 96h	13
L. gibba	E_bC_{50} 14d	0.0077

Table 9.5-34: Summary of results obtained in the studies with the formulated product PRIMARY MX and comparison of calculated and measured mixture toxicity

Test species	Endpoint & Test system	LC_{50} / EC_{50} [mg/L]			
		Measured toxicity of PRIMARY MX ($LC_{50\ PRIMARY\ MX}$ or $EC_{50\ PRIMARY\ MX}$) (mg/L)	Measured toxicity of PRIMARY MX (converted to be a.i. based) ($LC_{50\ PRIMARY\ MX}$ or $EC_{50\ PRIMARY\ MX}$) (mg a.s./L)	Calculated mixture toxicity[a] $LC_{50\ mix\text{-}CA}$ or $EC_{50\ mix\text{-}CA}$	Model deviation ratio (MDR = $EC_{50\ mix\text{-}CA}$ / $EC_{50\ PRIMARY\ MX}$)
O. mykiss	LC_{50}, acute, 96 h	100	51.000	8.851	0.174
D. magna	EC_{50}, acute, 48 h	100	51.000	13.774	0.270
P. subcapitata	E_rC_{50}, 72 h	20.211	10.308	6.167	0.598
L. gibba	E_rC_{50}, 7 d	~~0.0166~~ 0.0149	0.008	0.005	~~0.616~~ 0.687

[a] The mixture toxicity of the formulation was re-calculated based on the nominal contents of Rimsulfuron (30 g/kg), Nicosulfuron (120 g/kg) and Mesotrione (360 g/kg) within the formulation.

Table 9.5-35: Comparison of mixture composition in the formulation study (giving the measured mixture toxicity) and mixture composition at the PEC_{mix}

Test species	Endpoint & Test system	LC_{50} / EC_{50} [mg/L]		
		Calculated mixture toxicity (a.s. in PRIMARY MX) $LC_{50\ mix\text{-}CA}$ or $EC_{50\ mix\text{-}CA}$	Calculated mixture toxicity (a.s. in PEC_{mix})[b] $LC_{50\ mix\text{-}CA}$ or $EC_{50\ mix\text{-}CA}$ at lower exposure tier	Factors ($EC_{50\ mix\text{-}CA}$ (a.s. in PRIMARY MX)/$EC_{50\ mix\text{-}CA}$ (a.s. in PEC_{mix})) at lower exposure tier
O. mykiss	LC_{50}, acute, 96 h	8.851	8.259	1.072
D. magna	EC_{50}, acute, 48 h	13.774	12.807	1.075
P. subcapitata	E_rC_{50}, static, 72 h	6.167	5.989	1.030
L. gibba	E_rC_{50}, semi static 7d	0.005	0.005	1.024

[a] The mixture toxicity of the formulation was re-calculated based on the nominal contents of Rimsulfuron (30 g/kg), Nicosulfuron (120 g/kg) and Mesotrione (360 g/kg) within the formulation.

[b] The mixture toxicity of the formulation was re-calculated based on the mixture composition at the PEC_{mix} for Rimsulfuron (0.000660 mg/L at Step 2 for SEU scenario), Nicosulfuron (0.002740 mg/L at Step 2 for SEU scenario) and Mesotrione (0.007410 mg/L at Step 2 for SEU scenario).

Table 9.5-36: Comparison of calculated mixture toxicity and toxicity per fraction of a single a.s.

Test species	Endpoint & Test system	LC_{50} / EC_{50} [mg/L]		
		Calculated mixture toxicity (a.s. in PRIMARY MX) $LC_{50\ mix\text{-}CA}$ or $EC_{50\ mix\text{-}CA}$	Calculated toxicity per fraction of PRIMARY MX (based on each a.s.) $(1/TU_i)$[a]	Deviation from mixture toxicity $(1\text{-}EC_{x\ mix\text{-}CA} \times (1/EC_{x\ mix\text{-}CA} - TU_i))$ [%]
O. mykiss	LC_{50}, acute, 96 h	8.851	Rimsulfuron: 6630 Nicosulfuron: 9.35 Mesotrione: 170	Rimsulfuron: 0.1% Nicosulfuron: 94.66% Mesotrione: 5.2%
D. magna	EC_{50}, acute, 48 h	13.774	Rimsulfuron: 6120 Nicosulfuron: 14.025 Mesotrione: 881.167	Rimsulfuron: 0.2% Nicosulfuron: 98.21% Mesotrione: 1.6%
P. subcapitata	E_rC_{50}, static, 72 h	6.167	Rimsulfuron: 20.4 Nicosulfuron: 17 Mesotrione: 18.417	Rimsulfuron: 30.2% Nicosulfuron: 36.3% Mesotrione: 33.5%
L. gibba	E_rC_{50}, semi static 7d	0.005	Rimsulfuron: 0.078 Nicosulfuron: 0.011 Mesotrione: 0.011	Rimsulfuron: 6.7% Nicosulfuron: 45.5% Mesotrione: 47.8%

[a] TUi is defined as the concentration of the i^{th} a.s. at the $EC_{50\ PRIMARY\ MX}$ (re-caculated to the sum of a.s.) divided by the respective single-substance toxicity ($EC_{50\ a.s.}$). This is calculated based on the nominal contents of Rimsulfuron (30 g/kg), Nicosulfuron (120 g/kg) and Mesotrione (360 g/kg) within the formulation.

Regarding PRIMARY MX, nicosulfuron clearly drives the acute risk for fish and aquatic invertebrates, but not for algae and aquatic plants. For these two last species, the studies performed with the formulated product PRIMARY MX do not reflect the toxicity of one particular active substance, as the formulation toxicity – endpoint recalculated to each active substance concentrations – does not come for 90 % (of more) from the toxicity per fraction of a single a.s. (TUi) (see Table 9.5-36).

Table 9.5-37: Conduct a mixture RA based on calculated mixture toxicity according to 10.3.8 from EFSA AGD in maize for fish

Exposure	Lower exposure tier			Higher exposure tier		
	Rimsulfuron	Nicosulfuron	Mesotrione	Rimsulfuron	Nicosulfuron	Mesotrione
Exposure tier (FOCUS step)	Step 2 (SEU)	Step 2 (SEU)	Step 2 (SEU)	Step 3 (R4 stream)	Step 3 (R4 stream)	Step 3 (R2 stream)
PECsw [mg a.s./L]	0.000660	0.002740	0.007410	0.000313	0.001296	0.001095
Relative proportions of the individual mixture components in the environment (pi PEC)	0.061	0.253	0.685	0.116	0.479	0.405
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]	0.010810			0.002704		
Toxicity of the product (a.s. based) (ECx PPP) [mg a.s./L]	51.000			51.000		
ETRmix = PECmix/ECx PPP	<0.001			<0.001		
Trigger	0.01					

No unacceptable risk to fish is expected from the exposure to the combined active substances following proposed uses of the product.

Applicability of such approach is justified following the EFSA AGD *Decision scheme for mixture toxicity risk assessment* for fish.

Step	EFSA AGD provisions	Option	Justification	Outcome
1	Are measured toxicity data (EC_x) available for the given endpoint (typically chronic data available only for a.s.)?	For both formulation ($EC_{x\ PRIMARY\ MX}$) and a.s. ($EC_{xa.s.}$):	Please refer to tables 9.5-1 to 9.5-4	Go to 2
2	Check the plausibility of the measured formulation toxicity ($EC_{xPRIMARY\ MX}$) against the calculated mixture toxicity $EC_{xmix\text{-}CA}$ (assuming CA, Equation 13) for exactly the mixture composition of the a.s. in the formulation ($EC_{xPRIMARY\ MX}$) by means of the model deviation ratio (MDR = EC_{xmix}-CA/$EC_{xPRIMARY\ MX}$).	MDR = < 0.2	Please refer to table 9.5-34	Go to 9
9	Carefully recheck the apparent antagonism as observed in the measured mixture toxicity data ($EC_{xPRIMARY\ MX}$) regarding potential impacts of the default assumption of CA and/or heterogeneous input data used for the CA calculation. Does the apparent antagonism remain and no toxicologically plausible explanation is available (e.g. special feature of the formulation type)?	No (measured mixture toxicity plausible)		Go to 3
3	Check whether the mixture composition in the formulation study giving the measured mixture toxicity ($EC_{xPRIMARY\ MX}$) in terms of the relative proportions of the individual a.s. is similar to the mixture composition at the PEC_{mix}. As a direct comparison on the basis of the relative proportions of the a.s. at the $EC_{x\ PRIMARY\ MX}$ with the relative proportion at the PEC_{mix} is not informative as such, the comparison is done based on calculated mixture toxicity (assuming CA) for both mixture compositions. Therefore, calculate $EC_{x\ mix\text{-}CA}$ (see Equation 13) for the mixture composition of the a.s. at the PEC_{mix} and compare with the estimate calculated for the formulation (as already done in step 2 above).	$EC_{x\ mix\text{-}CA}$ (a.s. in product)/$EC_{x\ mix\text{-}CA}$ (a.s. in PECmix) = 1.072 (0.8 - 1.2)	Please refer to table 9.5-35	Go to 4
4	Conduct a mixture RA based on measured mixture toxicity, with the exposure-toxicity ratio (ETR_{mix}) being defined as the PEC_{mix} divided by the measured $EC_{x\ PRIMARY\ MX}$ and compare the outcome with the acceptability criterion (trigger value) decisive for the specific endpoint/exposure scenario combination.	If ETR_{mix-} < 0.01 for ~~aquatic plants~~fish: Low risk		**Low risk**

Table 9.5-38: Conduct a mixture RA based on calculated mixture toxicity according to 10.3.8 from EFSA AGD in maize for aquatic invertebrates

Exposure	Lower exposure tier			Higher exposure tier		
	Rimsulfuron	Nicosulfuron	Mesotrione	Rimsulfuron	Nicosulfuron	Mesotrione
Exposure tier (FOCUS step)	Step 2 (SEU)	Step 2 (SEU)	Step 2 (SEU)	Step 3 (R4 stream)	Step 3 (R4 stream)	Step 3 (R2 stream)
PECsw [mg a.s./L]	0.000660	0.002740	0.007410	0.000313	0.001296	0.001095
Relative proportions of the individual mixture components in the environment (pi PEC)	0.061	0.253	0.685	0.116	0.479	0.405
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]	0.010810			0.002704		
Toxicity of the product (a.s. based) (ECx PPP) [mg a.s./L]	51.000			51.000		
ETRmix = PECmix/ECx PPP	<0.001			<0.001		
Trigger	0.01					

No unacceptable risk to aquatic invertebrates is expected from the exposure to the combined active substances following proposed uses of the product.

Applicability of such approach is justified following the EFSA AGD *Decision scheme for mixture toxicity risk assessment* for aquatic invertebrates.

Step	EFSA AGD provisions	Option	Justification	Outcome
1	Are measured toxicity data (EC_x) available for the given endpoint (typically chronic data available only for a.s.)?	For both formulation ($EC_{x\ PRIMARY\ MX}$) and a.s. ($EC_{xa.s.}$):	Please refer to tables 9.5-1 to 9.5-4	Go to 2
2	Check the plausibility of the measured formulation toxicity ($EC_{xPRIMARY\ MX}$) against the calculated mixture toxicity $EC_{xmix\text{-}CA}$ (assuming CA, Equation 13) for exactly the mixture composition of the a.s. in the formulation ($EC_{xPRIMARY\ MX}$) by means of the model deviation ratio (MDR = $EC_{xmix\text{-}CA}/EC_{xPRIMARY\ MX}$).	MDR = 0.2-5	Please refer to table 9.5-34	Go to 3

3	Check whether the mixture composition in the formulation study giving the measured mixture toxicity ($EC_{xPRIMARY\ MX}$) in terms of the relative proportions of the individual a.s. is similar to the mixture composition at the PEC_{mix}. As a direct comparison on the basis of the relative proportions of the a.s. at the $EC_{x\ PRIMARY\ MX}$ with the relative proportion at the PECmix is not informative as such, the comparison is done based on calculated mixture toxicity (assuming CA) for both mixture compositions. Therefore, calculate $EC_{xmix\text{-}CA}$ (see Equation 13) for the mixture composition of the a.s. at the PECmix and compare with the estimate calculated for the formulation (as already done in step 2 above).	$EC_{x\ mix\text{-}CA}$ (a.s. in product)/$EC_{x\ mix\text{-}CA}$ (a.s. in PEC_{mix}) is ~~1.061~~ 1.075 (0.8 - 1.2)	Please refer to table 9.5-35	Go to 4
4	Conduct a mixture RA based on measured mixture toxicity, with the exposure-toxicity ratio (ETR_{mix}) being defined as the PEC_{mix} divided by the measured $EC_{x\ PRIMARY\ MX}$ and compare the outcome with the acceptability criterion (trigger value) decisive for the specific endpoint/exposure scenario combination.	If $ETR_{mix\text{-}}$ < 0.01 for ~~aquatic plants~~aquatic invertebrates: Low risk		**Low risk**

Table 9.5-39: Conduct a mixture RA based on calculated mixture toxicity according to 10.3.8 from EFSA AGD in maize for algae

Exposure	Lower exposure tier			Higher exposure tier		
	Rimsulfuron	Nicosulfuron	Mesotrione	Rimsulfuron	Nicosulfuron	Mesotrione
Exposure tier (FOCUS step)	Step 2 (SEU)	Step 2 (SEU)	Step 2 (SEU)	Step 3 (R4 stream)	Step 3 (R4 stream)	Step 3 (R2 stream)
PECsw [mg a.s./L]	0.000660	0.002740	0.007410	0.000313	0.001296	0.001095
Relative proportions of the individual mixture components in the environment (pi PEC)	0.061	0.253	0.685	0.116	0.479	0.405
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]	0.010810			0.002704		
Toxicity of the product (a.s. based) (ECx PPP) [mg a.s./L]	10.308			10.308		
ETRmix = PECmix/ECx PPP	0.001			<0.001		
Trigger	0.1					

No unacceptable risk to algae is expected from the exposure to the combined active substances following proposed uses of the product.

Applicability of such approach is justified following the EFSA AGD *Decision scheme for mixture toxicity risk assessment* for algae.

Step	EFSA AGD provisions	Option	Justification	Outcome
1	Are measured toxicity data (EC_x) available for the given endpoint (typically chronic data available only for a.s.)?	For both formulation ($EC_{xPRIMARY\ MX}$) and a.s. ($EC_{xa.s.}$):	Please refer to tables 9.5-1 to 9.5-4	Go to 2
2	Check the plausibility of the measured formulation toxicity ($EC_{xPRIMARY\ MX}$) against the calculated mixture toxicity $EC_{xmix-CA}$ (assuming CA, Equation 13) for exactly the mixture composition of the a.s. in the formulation ($EC_{xPRIMARY\ MX}$) by means of the model deviation ratio ($MDR = EC_{xmix-CA}/EC_{xPRIMARY\ MX}$).	MDR = 0.2-5	Please refer to table 9.5-34	Go to 3
3	Check whether the mixture composition in the formulation study giving the measured mixture toxicity ($EC_{xPRIMARY\ MX}$) in terms of the relative proportions of the individual a.s. is similar to the mixture composition at the PEC_{mix}. As a direct comparison on the basis of the relative proportions of the a.s. at the $EC_{xPRIMARY\ MX}$ with the relative proportion at the PECmix is not informative as such, the comparison is done based on calculated mixture toxicity (assuming CA) for both mixture compositions. Therefore, calculate $EC_{xmix-CA}$ (see Equation 13) for the mixture composition of the a.s. at the PEC_{mix} and compare with the estimate calculated for the formulation (as already done in step 2 above).	$EC_{x\ mix-CA}$ (a.s. in product)/$EC_{x\ mix-CA}$ (a.s. in PEC_{mix}) is ~~1.144~~ 1.030 (0.8 - 1.2)	Please refer to table 9.5-35	Go to 4
4	Conduct a mixture RA based on measured mixture toxicity, with the exposure-toxicity ratio (ETR_{mix}) being defined as the PEC_{mix} divided by the measured $EC_{x\ PRIMARY\ MX}$ and compare the outcome with the acceptability criterion (trigger value) decisive for the specific endpoint/exposure scenario combination.	If ETR_{mix-} < 0.1 for ~~aquatic plants~~algae: Low risk		**Low risk**

Table 9.5-40: Conduct a mixture RA based on calculated mixture toxicity according to 10.3.8 from EFSA AGD in maize for aquatic plants

Exposure	Lower exposure tier			Higher exposure tier		
	Rimsulfuron	Nicosulfuron	Mesotrione	Rimsulfuron	Nicosulfuron	Mesotrione
Exposure tier (FOCUS step)	Step 2 (SEU)	Step 2 (SEU)	Step 2 (SEU)	Step 4 (5 m - R3 stream)	Step 4 (5 m - R3 stream)	Step 4 (5 m - R2 stream)
PECsw [mg a.s./L]	0.000660	0.002740	0.007410	0.000016	0.000064	0.000701
Relative proportions of the individual mixture components in the environment (pi PEC)	0.061	0.253	0.685	0.020	0.082	0.898
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]	0.010810			0.000781		
Toxicity of the product (a.s. based) (ECx PPP) [mg a.s./L]	0.008			0.008		
ETRmix = PECmix/ECx PPP	~~1.277~~ **1.351**			~~0.092~~ 0.098		
Trigger	0.1					

No unacceptable risk to aquatic plants is expected from the exposure to the combined active substances following proposed uses of the product and considering an unsprayed vegetated buffer zone of 5 m.

Applicability of such approach is justified following the EFSA AGD *Decision scheme for mixture toxicity risk assessment* for aquatic plants.

Step	EFSA AGD provisions	Option	Justification	Outcome
1	Are measured toxicity data (EC_x) available for the given endpoint (typically chronic data available only for a.s.)?	For both formulation ($EC_{xPRIMARY\ MX}$) and a.s. ($EC_{xa.s.}$):	Please refer to tables 9.5-1 to 9.5-4	Go to 2
2	Check the plausibility of the measured formulation toxicity ($EC_{xPRIMARY\ MX}$) against the calculated mixture toxicity $EC_{xmix\text{-}CA}$ (assuming CA, Equation 13) for exactly the mixture composition of the a.s. in the formulation ($EC_{xPRIMARY\ MX}$) by means of the model deviation ratio (MDR = $EC_{xmix\text{-}CA}/EC_{xPRIMARY\ MX}$).	MDR = 0.2-5	Please refer to table 9.5-34	Go to 3

3	Check whether the mixture composition in the formulation study giving the measured mixture toxicity ($EC_{xPRIMARY\ MX}$) in terms of the relative proportions of the individual a.s. is similar to the mixture composition at the PEC_{mix}. As a direct comparison on the basis of the relative proportions of the a.s. at the $EC_{xPRIMARY\ MX}$ with the relative proportion at the PECmix is not informative as such, the comparison is done based on calculated mixture toxicity (assuming CA) for both mixture compositions. Therefore, calculate $EC_{xmix\text{-}CA}$ (see Equation 13) for the mixture composition of the a.s. at the PEC_{mix} and compare with the estimate calculated for the formulation (as already done in step 2 above).	$EC_{x\ mix\text{-}CA}$ (a.s. in product)/$EC_{x\ mix\text{-}CA}$ (a.s. in PEC_{mix}) is ~~1.031~~ 1.024 (0.8 - 1.2)	Please refer to table 9.5-35	Go to 4
4	Conduct a mixture RA based on measured mixture toxicity, with the exposure-toxicity ratio (ETR_{mix}) being defined as the PEC_{mix} divided by the measured $EC_{x\ PRIMARY\ MX}$ and compare the outcome with the acceptability criterion (trigger value) decisive for the specific endpoint/exposure scenario combination.	If ETR_{mix-} < 0.1 for aquatic plants: Low risk		**Low risk**

zRMS comment:

We agree with mixture toxiocity assessment. Mixture toxicity assessment should be considered at MSs level.

9.5.3 Overall conclusions

Rimsulfuron
All PEC/RAC values for Rimsulfuron and its metabolite are below the trigger value of 1 at step 3, indicating that Rimsulfuron poses a low risk to aquatic organisms, as well as for IN-70941, IN-70942 and IN-E9260 metabolite.

Nicosulfuron
For the intended uses on maize, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for higher plant as characterised by an EC_{50} for *Lemna gibba* of 1.7 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. Based on the results of the risk assessment at step 4, the following conclusions regarding buffer zones and vegetative buffer strips may be drawn for maize use:

- D3 ditch~~, D4 stream, D5 stream and D6 ditch~~ scenarios: A 5 m no spray buffer zone is required.
- R1 stream scenario: A ~~15~~ 10 m no spray buffer zone and a ~~15~~ 10 m vegetative buffer strip are required.

- R2 stream, R3 stream and R4 stream: A 20 m no spray buffer zone and a 20 m vegetative buffer strip are reduction are not enough for acceptable risk. ~~After the refinement with the results of the recovery phase of the study on *Lemna* conducted with nicosulfuron (RAC equal to 0.74 µg nicosulfuron/L), the risk is considered acceptable with an unsprayed vegetated buffer zone of 15~~10 ~~m for R3 and R4 stream and an unsprayed vegetated buffer zone of 5 m for R2 stream~~
- After the refinement with the value agreed at EU level, based on 7 d E_rC_{50} of 2.7 µg/L (RAC equal to 0.27 µg nicosulfuron/L), the risk is considered acceptable with an unsprayed vegetated **buffer zone of 5 m for R1**, R2 R3 and R4 scenarios with PEC_{sw} VFSmod calculations.
  However, the refinement based on **these calculations should be decided at MSs level.**

For ASDM, AUSN, HMUD, ADMP and UCSN metabolites, all PEC/RAC values are below the trigger value of 1 at step 1-2. Therefore, no further assessment is necessary.

Mesotrione

For the intended uses on maize, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for higher plant as characterised by an EC_{50} for *Lemna gibba* of 7.7 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. Based on the results of the risk assessment at step 4, the following conclusions regarding buffer zones and vegetative buffer strips may be drawn for maize use:

- R2 stream (pH 5.1)-~~Linear~~: A ~~10~~5m no spray buffer zone and a ~~10~~5m vegetative buffer strip are required.

For MNBA, AMBA and SYN546974 metabolites, all PEC/RAC values are below the trigger value of 1 at step 1-2. Therefore, no further assessment is necessary.

Final risk mitigation meaures should be considered at MSs level.

PRIMARY MX

For the endpoints from formulated product PRIMARY MX, any spray buffer zone with 50% of nozzles reduction OR a 5 m no spray buffer zone are enough for acceptable risk. In addition, for the combined exposure the risk is considered acceptable with an unsprayed vegetated buffer zone of 5 m.

Conclusion

Maize – SPe 3: *To protect aquatic organisms respect an unsprayed vegetated buffer zone of 5 m to surface water bodies.*

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 Rimsulfuron, Nicosulfuron and Mesotrione. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on bees of PRIMARY MX were not evaluated as part of the EU assessment of Rimsulfuron, Nicosulfuron and Mesotrione. New data submitted with this application are listed and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
Apis mellifera	DPX-E9636 (rimsulfuron)	Oral (acute)	LD_{50} > 100 µg a.s./bee	EFSA Scientific Report (2005) 45, 1-61
Apis mellifera	DPX-E9636 (rimsulfuron)	Contact (acute)	Contact (24-96 h) – not available	
Apis mellifera	DPX-E9636 plus IN-KG691 (rimsulfuron)	Oral (acute)	**LD_{50} = 41.1 µg a.s./bee**	
Apis mellifera	DPX-E9636 plus IN-KG691 (rimsulfuron)	Contact (acute)	**LD_{50} = 27.9 µg a.s./bee**	
Apis mellifera	Rimsulfuron technical	Chronic, 10 d	LDD_{50} > 18.51 µg a.s./bee/day **NOEDD ≥ 18.51 µg a.s./bee/day**	KCP 10.3.1.2.1 Ansaloni, T., 2018, TRC16-193BA
Apis mellifera	Rimsulfuron technical	Larval, repeated exposure	**NOED ≥ 100.00 µg as/larva** EC_{10} Not determined	KCP 10.3.1.3.1 Aguilar-Alberola, J.A. & Marín Víllora, M. 2018, TRC16-162BA
Apis mellifera	Technical nicosulfuron	Oral (acute)	Study details did not allow calculation of oral LD_{50} in terms of µg a.s./bee [LC_{50} > 1000 mg a.s./litre in diet]	EFSA Scientific Report (2007) 120, 1-91
Apis mellifera	Technical nicosulfuron	Contact (acute)	**LD_{50} = 76 µg a.s./bee**	
Apis mellifera	Formulation: 'SL-950 4% SC'	Oral (acute)	LD_{50} >131 µg product/bee – **equivalent to 5.24 µg a.s./bee**	
Apis mellifera	Formulation: 'SL-950 4% SC'	Contact (acute)	Contact (24-96 h) – not available	
Apis mellifera	Nicosulfuron technical	Chronic, 10 d	LDD_{50} > 7.93 µg a.s./bee/day **NOEDD 7.93 µg a.s./bee/day**	KCP 10.3.1.2.2 Ansaloni, T., 2018, TRC16-049BA
Apis mellifera	Mesotrione	Oral (acute)	**LD_{50} > 11 µg/bee**	EFSA Journal 2016;14(3):4419
Apis mellifera	Mesotrione	Contact (acute)	**LD_{50} > 100 µg/bee**	
Apis mellifera	Callisto 100 SC (A12739A)	Oral (acute)	LD_{50} = 79.7 µg a.s./bee	
Apis mellifera	Callisto 100 SC (A12739A)	Contact (acute)	LD_{50} = 52.5 µg a.s./bee	
Apis mellifera	Callisto 100 SC (A12739A)	10 day (chronic)	LD_{50} = 19.2 µg a.s./bee	
Apis mellifera	Callisto 100 SC (A12739A)	Development (chronic)	$NOED_{larvae}$ = 57.8 µg a.s./larva	

Species	Substance	Exposure System	Results	Reference
Apis mellifera	Mesotrione technical	Chronic, 10 d	LDD_{50} > 57.21 µg a.s./bee/day **NOEDD ≥ 57.21 µg a.s./bee/day**	KCP 10.3.1.2.3 Gimeno, I., 2019, TRC17-006BA
Apis mellifera	Mesotrione technical	Larval, repeated exposure	**NOED = 1.8 µg as/larva** EC_{10} = 2.3 µg as/larva	KCP 10.3.1.3.2 Scheller, K., 2018, 17 48 BLC 0088
Apis mellifera	PRIMARY MX	Oral	**LD_{50} > 400 µg f.p./bee**	KCP 10.3.1.1.1 Stalmach, M. 2019, B/172/16
Apis mellifera	PRIMARY MX	Contact	**LD_{50} > 400 µg f.p./bee**	KCP 10.3.1.1.2 Stalmach, M. 2019, B/173/16
Apis mellifera	PRIMARY MX	Chronic, 10 d	LDD_{50} = 5.08 µg/bee/day NOEDD = 2.49 µg/bee/day	KCP 10.3.1.2.4 Radha, S., 2022, 7961/2020
Apis mellifera	PRIMARY MX	Larval, repeated exposure	NOED < 0.2 µg/larva	KCP 10.3.1.3.3 Radha, S., 2022, 7962/2020
Higher-tier studies (tunnel test, field studies)				
Rimsulfuron: Rimsulfuron had no impact on honeybee mortality, flight intesity, behaviour, colony condition or brood development following application to flowering *Phacelia tanacetifolia* in a cage test (80 g Rimsulfuron 25 WG or Rimsulfuron 25 WG + IN-KG 691 surfactant). Nicosulfuron: No bee field studies were conducted and non are required. Mesotrione: No bee field studies were conducted and non are required.				

9.6.1.1 Justification for new endpoints

The used endpoints are the EU agreed ones, except for formulation, corresponding to data proper to PRIMARY MX formulation.

9.6.2 Risk assessment

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

9.6.2.1 Hazard quotients for bees

Table 9.6-2: First-tier assessment of the risk for bees due to the use of PRIMARY MX in maize

Intended use	**Maize**
Active substance	Rimsulfuron

Application rate (g/ha)	1 x ~~9.9~~ 7.5		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	41.1	~~9.9~~ 7.5	~~0.24~~ 0.18
Contact toxicity	27.9		~~0.35~~ 0.27
Active substance	Nicosulfuron		
Application rate (g/ha)	1 x ~~39.6~~ 30		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	5.24	~~39.6~~ 30	~~7.56~~ 5.73
Contact toxicity	76		~~0.52~~ 0.39
Active substance	Mesotrione		
Application rate (g/ha)	1 x ~~118~~ 90		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	11	~~118~~ 90	~~10.73~~ 8.18
Contact toxicity	100		~~1.18~~ 0.90
Product	PRIMARY MX		
Application rate (g/ha)	1 x ~~330~~ 250 g f.p./ha		
Test design	**LD_{50} (lab.) (µg f.p./bee)**	**Single application rate (g f.p./ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	>400	~~330~~ 250	~~0.83~~ 0.63
Contact toxicity	>400		~~0.83~~ 0.63

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

Due to the results of laboratory tests rimsulfuron, nicosulfuron, mesotrione and the formulation PRIMARY MX are considered to be practically non-toxic to bees. All hazard quotients are clearly below the trigger of 50, indicating that the intended use poses a low risk to bees in the field.

The EPPO Standard PP 3/10(3) propose define a **bee brood-feeding test**. Effects on brood may be assessed qualitatively or quantitatively depending on the test that is performed. A larvae bees study has been performed by the Applicant and effects were assessed quantitatively. According to EPPO Standard PP 3/10(3), a calculation of the ratio (TER) between the no observed effect level (NOEL) and exposure should be performed. Exposure is assessed by estimating the amount of residues that may be ingested by a bee in 1 day. Since residues in plant material are not available, a generic worst-case value of 1 mg a.s./kg plant matrix was proposed. This value is deduced from a compilation of the data generated in various plant species treated with systemic insecticides.

The oral NOED is measured in µg active substance per bee and residues in plant parts are expressed in mg/kg. Therefore, a conversion of residue data is necessary to express exposure as an amount of residue ingested. This conversion may be done by multiplying the residue concentration (mg a.s./kg plant part) by the daily food ingestion that reflects the dietary need in sugar for a larvae bee. The maximum food ingestion may be estimated from Rortais et al., 2005 at 59.4 mg sugar/larvae for five days for workers. The data set provided by Rortais et al. (2005) is considered to satisfyingly represent food consumption estimates of the different categories of bees. Considering the maximum amount of sugar a worker larvae bee consumes per day (11.88 mg/larva/day) and the amount of sugar in nectar of 15% (worst-case sugar content based on the available scientific literature (Maccagnani et al., 2003; Monzon et al., 2004; Nicolson,

2009)), adults consume an amount of nectar of 79.2 mg/larva/day (thus will be exposed to 0.0792 µg a.s./larva/day). The relevant calculations are presented below.

- 1 kg (=1000000 mg) of plant matrix contains 1 mg of a.s. (=1000 µg a.s.) → 1 mg plant matrix (=nectar) contain 0.001 µg a.s.
- Consumption of 11.88 mg sugar/larva/day and 15% sugar content in nectar → 79.2 mg nectar/larva/day → 79.2*0.001 µg a.s. = 0.0792 µg a.s.

In addition, according to Rortais et al. (2005) a worker larvae might consume up to 5.4 mg of pollen in 5 days which corresponds to 1.08 mg pollen/larva/day.

Tier-1 calculations based on consumption of both nectar and pollen are presented below:

Table 9.6-3: Risk assessment of the risk for larvae bees due to the use of rimsulfuron and mesotrione

Test design	**NOED (lab.) (µg a.s./larva)**	**Consumption (µg a.s./larva)**		**TER criterion: TER≥ 1**
		Nectar	**Pollen**	
Larvae	100 (Rimsulfuron)	0.0792	0.00108	1245.64
	1.8 (Mesotrione)			22.42

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

Applicability of such approach is justified following the risk assessment scheme for Identification of potential risks to larvae according to *EPPO Standard PP 3/10(3).*

Question	**EPPO Standard PP 3/10 provisions**	**Option**	**Justification**	**Outcome**
4	Can effects on growth or development of bees be excluded (risk assessment for bee brood triggered)?	No		Go to 5
5	Conduct a bee brood-feeding test (see Note 8). Effects on brood may be assessed qualitatively or quantitatively depending on the test that is performed. In the case where effects are assessed quantitatively, calculate the ratio (TER) between the no observed effect level (NOEL) and exposure. Exposure is assessed by estimating the amount of residues that may be ingested by a bee in 1 day.	Ratio≥1		Go to 11
11	Categorize as low risk to bees	No		Low risk demonstrated

The EPPO 2010 scheme does not recommend a **chronic assessment for adults** for foliar spray applications. However, as an approach is proposed as an assessment refinement for seed coatings/soil treatments (point 7, on the scheme), this approach can be adapted to provide a worst-case assessment for foliar sprays.

A worst-case of potential exposure via residues in pollen and nectar can be estimated, as before, based on the default worst-case value of 1 mg a.s./kg proposed in the EPPO 2010 scheme (see Note 6), based on a database of measured values from aerial plant parts as a surrogate for nectar and pollen.

The default residues can then be combined with a measure of consumption in order to estimate the exposure. Worst case data from Rortais et al., 2005, as proposed in the EPPO 2010 scheme, have been used to estimate the consumption by bee foragers: 898.8 mg sugar/bee for seven days (worst case for nectar foragers). Considering the maximum amount of sugar a nectar foragers bee consumes per day (128.4 mg/bee/day) and the amount of sugar in nectar of 15% (worst-case sugar content based on the available scientific literature (Maccagnani et al., 2003; Monzon et al., 2004; Nicolson, 2009)), adults consume an amount of nectar of 856 mg/bee/day (thus will be exposed to 0.856 µg a.s./bee/day). The relevant calculations are presented below.

- 1 kg (=1000000 mg) of plant matrix contains 1 mg of a.s. (=1000 µg a.s.) → 1 mg plant matrix (=nectar) contain 0.001 µg a.s.
- Consumption of 128.4 mg sugar/bee/day and 15% sugar content in nectar → 856 mg nectar/bee/day → 856*0.001 µg a.s. = 0.856 µg a.s.

In addition, according to Rortais et al. (2005) honeybees might consume several milligrams of pollen per day. Then as a worst case scenario, the nurses pollen consumption was considered, which might be up to 65 mg of pollen in 10 days, which corresponds to 6.5 mg pollen/bee/day.
Tier-1 calculations based on consumption of both nectar and pollen are presented below:

Table 9.6-4: Risk assessment of the risk for adult bees due to the use of rimsulfuron, nicosulfuron and mesotrione

Test design	NOED (lab.) (µg a.s./bee)	Consumption (µg a.s./bee)		TER criterion: TER≥ 1
		Nectar	Pollen	
Foraging bees (nectar foragers)	≥18.51 (Rimsulfuron)	0.856	0.0065	21.46
	7.93 (Nicosulfuron)			9.19
	≥57.21 (Mesotrione)			66.33

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

According to the trigger proposed by the EPPO 2010 scheme it is clear that with above TER values there is a wide safety margin, indicating that the proposed uses of rimsulfuron, nicosulfuron and mesotrione pose an acceptable chronic risk to adult bees.

zRMS comments:

The risk assessment for adult bees based on the laboratory tests with rimsulfuron, nicosulfuron, mesotrione and the formulation PRIMARY MX are considered acceptable.

All hazard quotients are clearly below the trigger of 50, indicating that the intended use poses a low risk to bees.

According to the trigger proposed by the EPPO 2010 scheme the TER values have a wide safety margin, indicating that the proposed uses of rimsulfuron, nicosulfuron and mesotrione pose an acceptable chronic risk to adult bees.

However, according to Reg 284/2009 the chronic tests for adult and larvae bees should be submitted for product PRIMARY MX. During commenting period process the Applicant provided these studies. Fur-

ther consideration should be decided at MSs level.

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

Not relevant.

9.6.3 Effects on bumble bees

Not required.

9.6.4 Effects on solitary bees

Not required.

9.6.5 Overall conclusions

First-tier assessments indicate that no unacceptable risk for bees exposed to PRIMARY MX is expected according to the proposed intended uses.

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 Rimsulfuron, Nicosulfuron, Mesotrione. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target arthropods of PRIMARY MX were not evaluated as part of the EU assessment of Rimsulfuron, Nicosulfuron and Mesotrione. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
Typhlodromus pyri (Protonymph)	DPX-E9636 25 WG + IN KG691* (Rimsulfuron)	Laboratory test, glass plates, 14 d	Mortality/ Fecundity (14d, protonymph to adult, glass plate): 9/0% at 1,1 g as/ha, 8/0% at 27,5 g as/ha, 3/0% at 1,1 g as/ha + 0,4*, 11/0% at 27,5 g as/ha + 0,4*, 2/0% at 0.016*, 11/10% at 0.4* LR_{50}>27,5 g as/ha	EFSA Scientific Report (2005), 45, 1-61

Species	Substance	Exposure System	Results	Reference
Aphidius Rhopalosiphi (adult)	DPX-E9636 25 WG (Rimsulfuron)	Laboratory test, glass plates, 48 h	Mortality/ parasitisation capacity (48h, adult, glass plate): 14/1% at 37,5 g as/ha LR_{50}>37,5 g as/ha	
Chrysoperla carnea	DPX-E9636 25 WG (Rimsulfuron)	Laboratory test, glass plates	Mortality/fertility (exposure till hatching, adult, glass plate): 4/22% at 37,5 g as/ha	
Aleochara bilineata (adult)	DPX-E9636 25 WG (Rimsulfuron)	Extended laboratory, 28 d	Mortality/parasitation capacity (28d, adult, sand): 0/5% at 1,1 g as/ha, 0/22% at 27,5 g as/ha	
Aphidius rhopalosiphi	Nicosulfuron formulation 'SL-950 4% SC'	Laboratory test, glass plates, 48 h	<u>% mortality</u> Water ctrl: 0% 60 g a.s./ha: 15% (n.s.) <u>Parasitism (no. aphid mummies /female)</u> Water ctrl: 33.3% 60 g a.s./ha: 16.6 – red. of 50% (sig. at P=0.05)	EFSA Scientific report (2007) 120, 1-91
Aphidius rhopalosiphi	Nicosulfuron formulation 'SL-950 4% SC'	48 h exposure to deposit on freshly sprayed barley seedlings)	<u>% mortality</u> Water ctrl: 0% 60 g a.s./ha: 5% (n.s.) <u>Parasitism (nº aphid mummies /female)</u> Water ctrl: 21.1 60 g a.s./ha: 17.6% (n.s.)	
Typhlodromus pyri	Nicosulfuron formulation 'SL-950 4% SC'	Proto-nymph through to adult stage (14 day exposure to glass plate residue)	<u>% mortality (after 7 days exposure)</u> Water ctrl: 17% 1.5 L prod./ha: 41% - ctrl corr. 29% (n.s.) <u>Fecundity (nº of eggs per female during days 7-14)</u> Control: 9.0 1.5 L prod./ha: 9.1 (n.s.)	
Poecilus cupreus	Nicosulfuron formulation 'SL-950 4% SC'	Adult (28 day exposure to initial spray & residues in moist sand substrate)	<u>% mortality (after 28 day exposure)</u> Water ctrl: 33% 1.5 L prod./ha: 40% -control corr. 10% (n.s.) <u>Mean prey consumption per beetle over study period:</u> Water ctrl: 8.6 1.5 L prod./ha: 8.4 (n.s.)	

Species	Substance	Exposure System	Results	Reference
Coccinella septempunctata	Nicosulfuron formulation 'SL-950 4% SC'	3 day old larvae through to pupae stage (15-20 day exposure to glass plate residue)	% mortality during exposure phase (based on nº of emerging adults): Water ctrl: 18% 1.5 L prod./ha: 16% -ctrl corr. - 6% (n.s.) 3.0 L prod./ha: 40% -ctrl corr. 19% (n.s.) Fecundity (nº of eggs per female during 8-9 week postexposure phase) & % hatch Control: 137.7 & 60.4% hatch 1.5 L prod./ha: 91.5 & 84.6% hatch (n.s.) 3.0 L prod./ha: 123.4 & 91.2% hatch (n.s.)	
Aleochara bilineata	Nicosulfuron formulation 'SL-950 4% SC'	Adult plus developing F1 beetles present in treated substrate (28 day exposure to residues in moist sand substrate)	% mortality (after 28 day exposure) Water ctrl: 0% 1.5 L prod./ha: 0% Parasitism rate (mean nº per treatment group of F1 beetles emerging from Delia pupae) Water ctrl.: 356 1.5 L prod./ha: 284 – equivalent to 20% red. (n.s.)	
Typhlodromus pyri	Callisto 100 SC (Mesotrione)	Laboratory test glass plates (2D)	LR_{50} = 93.11 g as/ha ER_{50} > 81 g as/ha	EFSA Journal 2016;14(3):4419
Aphidius rhopalosiphi	Callisto 100 SC (Mesotrione)	Laboratory test glass plates (2D)	LR_{50} = 43.56 g as/ha ER_{50} > 25.6 g as/ha	
Typhlodromus pyri	Callisto 100 SC (Mesotrione)	Leaf discs	Mortality: 12% at 75 g as/ha 19% at 150 g as/ha 42% at 300 g as/ha Reproduction: 41.6% at 75 g as/ha 47.4% at 150 g as/ha 56.9% at 300 g as/ha	
Aphidius rhopalosiphi	Callisto 100 SC (Mesotrione)	Barley seedlings	Mortality: 0% at 100 g as/ha 3.3% at 150 g as/ha 3.3% at 225 g as/ha Reproduction: -9.6% at 100 g as/ha -3.5% at 150 g as/ha -8.0% at 225 g as/ha	
Aleochara bilineata	Callisto 100 SC (Mesotrione)	Soil	Reproduction: 11.7% at 50 g as/ha 7.4% at 100 g as/ha 10.1% at 200 g as/ha	

<table>
<tr><th>Species</th><th>Substance</th><th>Exposure System</th><th>Results</th><th>Reference</th></tr>
<tr><td>Pardosa sp.</td><td>Callisto 100 SC (Mesotrione)</td><td>Soil</td><td><u>Mortality</u>:
9% at 37.5 g as/ha
26% at 75 g as/ha
41% at 150 g as/ha
<u>Feeding</u>:
-2.2% at 37.5 g as/ha
6.7% at 75 g as/ha
13.3% at 150 g as/ha</td><td></td></tr>
<tr><td>Aphidius rhopalosiphi (adults)</td><td>PRIMARY MX</td><td>Extended laboratory test (3D)</td><td>LR_{50} > 660 g/ha
ER_{50} = 564.9 g/ha</td><td>KCP 10.3.2.1-01 Kulec-Ploszczyca, E. 2018, B/174/16</td></tr>
<tr><td>Typhlodromus pyri</td><td>PRIMARY MX</td><td>Extended laboratory test (2D)</td><td>LR_{50} = 147.0 g/ha
ER_{50} > 20.6 g/ha</td><td>KCP 10.3.2.1-02 Stalmach, M. 2019, B/175/16</td></tr>
<tr><td>Chrysoperla carnea</td><td>PRIMARY MX</td><td>Laboratory test glass plates (2D)</td><td>LR_{50} = 168.2 g/ha
ER_{50} = 200 g/ha</td><td>KCP 10.3.2.1-03 Mohanraj, M. 2020, 7554/2020</td></tr>
<tr><td>Aleochara bilineata</td><td>PRIMARY MX</td><td>Extended laboratory test (2D)</td><td>LR_{50} = 373.75 g/ha
ER_{50} = 378.77 g/ha</td><td>KCP 10.3.2.1-04 Sonali, G. 2020, 7555/2020</td></tr>
<tr><td>Typhlodromus pyri</td><td>PRIMARY MX</td><td>Aged residues test</td><td><u>0.33 kg f.p./ha</u><table><tr><td>Res. aged:</td><td>% mort.</td><td>% repr. red.</td></tr><tr><td>0 DAA1</td><td>11.96</td><td>35.26</td></tr><tr><td>7 DAA1</td><td>4.55</td><td>18.53</td></tr><tr><td>14 DAA1</td><td>6.32</td><td>11.43</td></tr></table>0 DAA1: when compared to the control group, a fecundity reduction ≤ 50 % was observed at the application rate of 0.33 kg/ha.</td><td>KCP 10.3.2.1-05 Varela, S. 2021, S20-07857</td></tr>
<tr><td colspan="5">Field or semi-field tests</td></tr>
<tr><td colspan="5"><u>Rimsulfuron:</u>
Not required.
<u>Nicosulfuron:</u>
No non-target arthropods studies were conducted and none are required.
<u>Mesotrione:</u>
Not required.</td></tr>
</table>

(n.s.): not significant

9.7.1.1 Justification for new endpoints

The used endpoints are the EU agreed ones, except for formulation, corresponding to data proper to PRIMARY MX formulation.

9.7.2 Risk assessment

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

9.7.2.1 Risk assessment for in-field exposure

Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods regarding rimsulfuron due to the use of PRIMARY-MX in maize

Intended use	Maize
Active substance	Rimsulfuron
Application rate (g/ha)	1 x 9.9
MAF	1

Test species Tier-1	LR_{50} (lab.) (g/ha)	$PER_{in\ field}$ (g/ha)	$HQ_{in\ field}$ criterion: HQ ≤ 2
T. pyri	37.5	9.9	0.26
A. rhopalosiphi	37.5		0.26
P. cupreus	37.5		0.26
Test species Higher-tier	Rate with ≤ 50 % effect* (g/ha)	$PER_{in\ field}$ (g/ha)	$PER_{in\ field}$ below rate with ≤ 50 % effect?
A. bilineata	37.5	9.9	yes

Table 9.7-3: First- and higher-tier assessment of the in-field risk for non-target arthropods regarding nicosulfuron due to the use of PRIMARY-MX in maize

Intended use	Maize
Active substance	Nicosulfuron
Application rate (g/ha)	1 x 39.6
MAF	1

Test species Tier-1	LR_{50} (lab.) (g/ha)	$PER_{in\ field}$ (g/ha)	$HQ_{in\ field}$ criterion: HQ ≤ 2
T. pyri	80	39.6	0.66
A. rhopalosiphi	80		0.66
P. cupreus	80		0.66
C. septempunctata	120		0.33
A. bilineata	80		0.66
Test species Higher-tier	Rate with ≤ 50 % effect* (g/ha)	$PER_{in\ field}$ (g/ha)	$PER_{in\ field}$ below rate with ≤ 50 % effect?
A. rhopalosiphi	80	39.6	yes

Table 9.7-4: First- and higher-tier assessment of the in-field risk for non-target arthropods regarding mesotrione due to the use of PRIMARY-MX in maize

Intended use	Maize
Active substance	Mesotrione
Application rate (g/ha)	1 x 118
MAF	1

Test species Tier-1	LR_{50} (lab.) (g/ha)	$PER_{in\ field}$ (g/ha)	$HQ_{in\ field}$ criterion: HQ ≤ 2

~~*T. pyri*~~	~~93.11~~	~~118~~	~~1.27~~
~~*A. rhopalosiphi*~~	~~43.56~~		~~2.71~~
~~*A. bilineata*~~	~~200~~		~~0.59~~
~~*Pardosa sp.*~~	~~150~~		~~0.79~~
~~**Test species Higher tier**~~	~~**ER_{50} (g/ha)**~~	~~**$PER_{in-field}$ (g/ha)**~~	~~**$PER_{in-field}$ below rate with ≤ 50 % effect?**~~
~~*T. pyri*~~	~~150~~	~~118~~	~~yes~~
~~*A. rhopalosiphi*~~	~~225~~		~~yes~~

Table 9.7-5: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of PRIMARY MX in maize

Intended use	**Maize**		
Product	PRIMARY MX		
Application rate (g/ha)	1 x ~~330~~ 250		
MAF	1		
Test species Tier I	**LR_{50} (lab.) (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
C. carnea	168.2	~~330~~ 250	~~1.96~~ 1.49
A. bilineata	373.75		~~0.88~~ 0.67
Test species Higher-tier	**ER_{50} (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$PER_{in\text{-}field}$ below rate with ≤ 50 % effect?**
T. pyri	20.6	~~330~~ 250	no
A. rhopalosiphi	564.9		yes
Test species Higher-tier	**Rate with ≤ 50 % effect (g/ha) at 0 DAA1**	**$PER_{in\text{-}field}$ (g/ha)**	**$PER_{in\text{-}field}$ below rate with ≤ 50 % effect?**
T. pyri	330	~~330~~ 250	yes

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.

~~The resulting in-field HQ values for *T. pyri* is well above the trigger of 2, showing unacceptable in-field risk to non-target arthropods after application of PRIMARY MX.~~

~~Refinement of in-field risk~~

~~There are two extended laboratory tests, one on *Typhlodromus pyri* and the other on *Aphidius rhopalosiphi* submitted to support this application. In the study with *Aphidius rhopalosiphi*, the mortality and effects on reproduction were below 50% at 330 g/ha. At our maximum $PER_{in\text{-}field}$, there were effects on reproduction higher than 50%.~~

~~Several lab studies with representative formulations (DPX-E9636 25-WG for rimsulfuron, SL-950 4% SC for nicosulfuron and Callisto 100 SC for mesotrione) on *T.Pyri* and *A. rhopalosiphi* were submitted in Monograph and showed harmless effects at concentrations higher than the application rates corresponding to each of the active substances found in the formula (i.e. 9.9, 39.6 and 118 g/ha for rimsulfuron, nicosulfuron and mesotrione, respectively).~~

~~In addition, extended studies performed for each active substances in the above mentioned formulations, both on *T. pyri* and *A. rhopalosiphi* and additional species (*A. bilineata*, *C. carnea*, *C. septempunctata*, *P. cupreus* and *Pardosa sp.*), neither did show adverse effects on reproduction at the highest tested concentrations.~~

A new study assessing the effects of aged residues on *T. pyri* was presented (KCP 10.3.2.1-05, code S20-07857). According to the obtained results, the application of PRIMARY MX at a rate of 0.33 kg/ha, will not cause mortality greater than 50 % and reduction on reproduction will be less than 50 % from 0 days after the test item application (exposure 0 DAA1).

Therefore, based on the results presented above, the application of PRIMARY MX shows acceptable risk in-field to non-target arthropods.

9.7.2.2 Risk assessment for off-field exposure

Table 9.7-6: First- and higher-tier assessment of the off-field risk for non-target arthropods regarding rimsulfuron due to the use of PRIMARY MX in maize

Intended use	Maize
Active substance	rimsulfuron
Application rate (g/ha)	1 × 9.6
MAF	1
wdl	10 (2D) / 1 (3D)

Test species Tier-1	LR_{50} (lab.) (g/ha)	Drift rate	$PER_{arthropod}$ (g/ha)	CF	$HQ_{arthropod}$ criterion: HQ ≤ 2
T. pyri	37.5	0.0277	0.027	10	0.04
A. rhopalosiphi	37.5				0.007
C. carnea	37.5				0.007
Test species Higher-tier	**Rate with ≤ 50 % effect* (g/ha)**	**Drift rate**	**$PER_{arthropod}$ (g/ha)**	**CF**	**corr. $PER_{arthropod}$ below rate with ≤ 50 % effect?**
A. bilineata	37.5	0.0277	0.274	3	yes

Table 9.7-7: First- and higher-tier assessment of the off-field risk for non-target arthropods regarding nicosulfuron due to the use of PRIMARY MX in maize

Intended use	Maize
Active substance	Nicosulfuron
Application rate (g/ha)	1 × 39.6
MAF	1
wdl	10 (2D) / 1 (3D)

Test species Tier-1	LR_{50} (lab.) (g/ha)	Drift rate	$PER_{arthropod}$ (g/ha)	CF	$HQ_{arthropod}$ criterion: HQ ≤ 2
T. pyri	50	0.0277	0.110	10	0.018
A. rhopalosiphi	50				0.018
P. cupreus	50				0.018
C. septempunctata	120				0.008
A. bilineata	50				0.018
Test species Higher-tier	**Rate with ≤ 50 % effect* (g/ha)**	**Drift rate**	**$PER_{arthropod}$ (g/ha)**	**CF**	**corr. $PER_{arthropod}$ below rate with ≤ 50 % effect?**
A. rhopalosiphi	50	0.0277	1.00692	3	yes

Table 9.7-8: First- and higher-tier assessment of the off-field risk for non-target arthropods regarding mesotrione due to the use of PRIMARY MX in maize

Intended use	Maize

Active substance	Mesotrione
Application rate (g/ha)	1 x 112
MAF	1
vdf	10 (2D) / 1 (3D)

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
T. pyri	98.14	0.0277	2.7686	10	0.028
A. rhopalosiphi	42.56				0.065
A. bilineata	200				0.014
Pardosa sp.	150				0.019
Test species Higher-tier	**Rate with ≤ 50 % effect* (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**CF**	**corr. $PER_{off\text{-}field}$ below rate with ≤ 50 % effect?**
T. pyri	150	0.0277	2.7686	5	yes
A. rhopalosiphi	225				yes

Table 9.7-9: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of PRIMARY MX in maize

Intended use	**Maize**
Product	PRIMARY MX
Application rate (g/ha)	1 x 330 250
MAF	1
vdf	10 (2D) / 1 (3D)

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**
C. carnea	168.2	0.0277	9.314 0.693	5	0.055 0.021
A. bilineata	373.75		9.314 0.693		0.012 0.009
Test species Tier I	**Rate with ≤ 50 % effect* (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**CF**	**corr. $PER_{off\text{-}field}$ below rate with ≤ 50 % effect?**
T. pyri	20.6	0.0277	0.9141 0.693	5	yes
A. rhopalosiphi	564.9		9.141 6.925		yes

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

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

zRMS comments:

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. Two extended laboratory tests for Primary MX for *Typhlodromus pyri and Aphidius rhopalosiphi species were* submitted to support this application.

For refined in-field risk assessment the age residue study for T.pyri was submitted.According to the ob-

tained results, the application of PRIMARY MX at a rate of 0.25 kg/ha, will not cause mortality greater than 50 % and reduction on reproduction will be less than 50 % from 0 days after the test item application (exposure 0 DAA1).

In addition, according to recommendation given in ESCORT 2 in case when extended laboratory test are provided for two indicator species two additional species should be tested for Primary MX.

The risk for two additional species Aleochara bilineata and C. carnea indicated acceptable risk.

No risk mitigation measures are required for product PRIMARY MX.

9.7.2.3 Additional higher-tier risk assessment

Not relevant.

9.7.2.4 Risk mitigation measures

Not relevant.

9.7.3 Overall conclusions

The results of the risk assessment for non-target arthropods showed an acceptable in-field and off-field risk after the application of PRIMARY MX. ~~The absence of risk in the in-field area has been demonstrated according to the data from the monograph.~~

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 Rimsulfuron, Nicosulfuron, Mesotrione and their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of PRIMARY MX were not evaluated as part of the EU assessment of Rimsulfuron, Nicosulfuron and Mesotrione. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
Eisenia fetida	Rimsulfuron	Acute	LC_{50} > 1000 mg as/kg	EFSA Scientific

Species	Substance	Exposure System	Results	Reference
Eisenia fetida	Rimsulfuron 25% WG	Acute	LC_{50} > 1000 mg Prod./kg LC_{50} > 250 mg as./kg	Report (2005), 45, 1-61
Eisenia fetida	Rimsulfuron 25% WG + Exell	Acute	LC_{50} > 1000 mg Prod./kg LC_{50} > 22.5 mg as./kg	
Eisenia fetida	IN-70941	Chronic	NOEC 0.18 mg/kg	
Eisenia fetida	IN-70942	Chronic	NOEC 0.18 mg/kg	
Eisenia fetida	IN-E9260	Chronic	NOEC 0.18 mg/kg	
Folsomia candida	IN-70941	Chronic	NOEC ≥ 0.183 mg/kg	
Folsomia candida	IN-70942	Chronic	NOEC ≥ 0.183 mg/kg	
Folsomia candida	IN-E9260	Chronic	NOEC ≥ 0.183 mg/kg	
Eisenia fetida	Technical nicosulfuron	Acute, 14 d	LC_{50} > 1000 mg a.s. /kg d.w. soil (highest test dose, no affects reported)	EFSA Scientific Report (2007) 120, 1-91
Eisenia fetida	ASDM	Acute, 14 d	LC_{50} > 1000 mg ASDM /kg d.w. soil (highest test dose, no affects reported)	
Eisenia fetida	ADMP, AUSN, HMUD, MU-466 & UCSN	Acute, 14 d	LC_{50} > 1250 mg metabolite /kg d.w. soil (highest test dose, no affects reported)	
Eisenia fetida	'SL-950 4% SC'	Acute, 14 d	LC_{50} > 1000 mg formulation /kg d.w. soil (highest test dose, no affects reported)	
Eisenia fetida	AUSN	Chronic (8 weeks) (reproductive toxicity study)	NOEC 0.100 mg AUSN /kg d.w. soil (highest test dose)	
Eisenia fetida	UCSN	Chronic (8 weeks) (reproductive toxicity study)	NOEC 0.050 mg UCSN /kg d.w. soil (highest test dose)	
Eisenia fetida	ASDM	Chronic (8 weeks) (reproductive toxicity study)	NOEC 0.350 mg ASDM /kg d.w. soil (highest test dose)	
Folsomia candida	AUSN	Chronic (28 days) (reproductive toxicity study)	NOEC 0.100 mg AUSN /kg d.w. soil (highest test dose)	

Species	Substance	Exposure System	Results	Reference
Folsomia candida	UCSN	Chronic (28 days) (reproductive toxicity study)	NOEC 0.050 mg UCSN /kg d.w. soil (highest test dose)	
Folsomia candida	ASDM	Chronic (28 days) (reproductive toxicity study)	NOEC 0.100 mg ASDM /kg d.w. soil (highest test dose)	
Eisenia fetida	Mesotrione	Mixed with soil as a solution Acute 10% peat content	LC_{50} > 2000 mg as/kg dw soil	EFSA Journal 2016;14(3):4419
Eisenia fetida	MNBA	-	LC_{50} > 1000 mg as/kg dw soil	
Eisenia fetida	Callisto 100 SC (Mesotrione)	Mixed with soil as a solution Chronic 10% peat content	NOEC = 10.85 mg as/kg dw soil EC_{10}=5.91 mg a.s/kg dws	
Eisenia fetida	AMBA	Mixed with soil using quartz sand 5% peat content	NOEC = 1050 mg/kg dw soil	
Eisenia fetida	MNBA	Mixed with soil using quartz sand 5% peat content	NOEC = 1050 mg/kg dw soil	
Folsomia candida	Callisto 100 SC (Mesotrione)	Mixed with soil as a solution 14 days 5% peat content	NOEC = 50.54 mg as/kg dw soil EC_{10}=413 mg prod/kg dws (correspond to EC10= 37.54 mg as/kg dw soil)	
Hypoaspis aculeifer	Callisto 100 SC (Mesotrione)	Mixed with soil as a solution 28 days 5% peat content	NOEC = 90.9 mg as/kg dw soil	
Eisenia fetida	PRIMARY MX	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 10 mg/kg dw **EC_{10} = 7.7 mg/kg dw**	KCP 10.4.1.1 Dec, W., 2019 G/265/17
Folsomia candida	PRIMARY MX	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 56 mg/kg dw **EC_{10} = 44.2 mg/kg dw**	KCP 10.4.2.1-01 Dec, W., 2019 G/266/17
Field studies				
Not required.				
Litter bag test				
Not required.				

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

9.8.1.1 Justification for new endpoints

The used endpoints are the EU agreed ones, except for formulation, corresponding to data proper to PRIMARY MX formulation.

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, tables from 8.7-2 to 8.7-19. According to the assessment of environmental-fate data, multi-annual accumulation in soil is considered for Rimsulfuron, Nicosulfuron, Mesotrione.

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 PRIMARY MX in maize

Intended use	**Maize**		
~~**Acute effects on earthworms**~~			
~~**Product/active substance**~~	~~**LC_{50} (mg/kg dw)**~~	~~**PEC_{soil} (mg/kg dw)**~~	~~**TER_a (criterion TER ≥ 10)**~~
~~Rimsulfuron~~	~~> 1000~~	~~0.010~~ 0.008	~~100000~~ 125000
~~Nicosulfuron~~	~~> 1000~~	~~0.040~~ 0.030	~~25000~~ 33333
~~ASDM~~	~~> 1000~~	~~0.016~~ 0.013	~~62500~~ 76923
~~ADMP~~	~~> 1250~~	~~0.001~~	~~1250000~~
~~AUSN~~	~~> 1250~~	~~0.009~~ 0.007	~~138888.9~~ 178571
~~HMUD~~	~~> 1250~~	~~0.006~~ 0.004	~~208333.3~~ 312500
~~UCSN~~	~~> 1250~~	~~0.004~~ 0.003	~~312500~~ 416666
~~Mesotrione~~	~~> 2000~~	~~0.118~~ 0.090	~~16949.2~~ 22222
~~MNBA~~	~~> 1000~~	~~0.049~~	~~20408.2~~
Chronic effects on earthworms			
Product/active substance	**NOEC/EC10 (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
IN-70941	0.18	~~0.006~~ 0.005	~~30~~ 36
IN-70942	0.18	~~0.002~~ 0.001	~~90~~ 180
IN-E9260	0.18	0.002	90
AUSN	0.100	~~0.009~~ 0.007	~~11.1~~ 14.3
UCSN	0.050	~~0.004~~ 0.003	~~12.5~~ 16.7
ASDM	0.350	~~0.016~~ 0.013	~~21.9~~ 26.9

Callisto 100 SC (Mesotrione)	10.85	~~0.118~~ 0.090	~~91.9~~ 120.6
	5.91	0.090	65.66
AMBA	1050	0.007	150000
MNBA	1050	0.049	21428.6
PRIMARY MX	~~10~~ 7.7	~~0.330~~ 0.250	~~30.3~~ 40
Chronic effects on other soil macro- and mesofauna			
Product/active substance	**NOEC/EC10 (mg/kg dw)**	**PEC_{soil}** (mg/kg dw)	**TER_{lt} (criterion TER ≥ 5)**
IN-70941 (*Folsomia candida*)	> 0.183	~~0.006~~ 0.005	~~30.5~~ 36.6
IN-70942 (*Folsomia candida*)	> 0.183	~~0.002~~ 0.001	~~91.5~~ 183
IN-E9260 (*Folsomia candida*)	> 0.183	0.002	91.5
AUSN (*Folsomia candida*)	0.100	~~0.009~~ 0.007	~~11.1~~ 14.3
UCSN (*Folsomia candida*)	0.050	~~0.004~~ 0.003	~~12.5~~ 16.7
ASDM (*Folsomia candida*)	0.100	~~0.016~~ 0.013	~~6.25~~ 7.7
Callisto 100 SC (Mesotrione) (*Folsomia candida*)	50.54	~~0.118~~ 0.090	~~428.3~~ 561.6
	37.54	0.090	417.11
Callisto 100 SC (Mesotrione) (*Hypoaspis aculeifer*)	90.9	~~0.118~~ 0.090	~~770.3~~ 1010
PRIMARY MX (*Folsomia candida*)	~~56~~ 44	~~0.330~~ 0.250	~~169.7~~ 224

TER values shown in bold fall below the relevant trigger.

Chronic studies with PRIMARY MX on earthworms and collembolan were submitted by the Applicant and no unacceptable risk was obtained after the risk assessment. Moreover, the risk assessment for NTA was acceptable with endpoints for tested indicator species including the ground dwelling arthropod *Aleochara bilineata* after exposure to the three active substances in the mixture. Therefore, according to SANCO/10329/2002 rev 2 final, the Applicant considers that an acceptable risk to *Hypoaspis aculeifer* for formulation PRIMARY MX can be concluded based on low risks to earthworms and other soil macro-organisms and ground dwelling arthropod *Aleochara bilineata*.

Therefore, it is expected that chronic toxicity on *Hypoaspis* will result from prolonged exposure and the formulation is not expected to remain intact in the environment.

zRMS comment:

The risk assessment for soil macro- and meso-fauna is agreed by the zRMS with following corrections:

1. Acute risk assessment for earthworms is no longer required so it was struck through in Table 9.8-2 above.
2. The chronic risk assessment for earthworms for PRIMARY MX based on NOEC value derived has been struck through as EC_{10} value was lower and thus more relevant for the risk assessment.
3. The risk assessment for *F. candida* for PRIMARYMX based on NOEC values has been struck through as EC_{10} values were lower and thus more relevant for the risk assessment.
4. The lowest EC10 value for a.s.-mesotrione for Folsomia and E. fetida was added after comentig period process.

All these corrections have no impact on the outcome of the calculations and acceptable risk from intended uses of Primary MX may be concluded for all soil macro-organisms.

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

The TER values for earthworms and other soil macro- and mesofauna for PRIMARY MX were above the relevant Annex VI trigger of 10 and 5, respectively. Therefore, it is concluded that active substance Rimsulfuron, Nicosulfuron and Mesotrione do not pose acute and chronic risk to earthworms and other soil macro- and mesofauna.

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 Rimsulfuron, Nicosulfuron, Mesotrione and their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on soil microorganisms of PRIMARY MX were not evaluated as part of the EU assessment of Rimsulfuron, Nicosulfuron and Mesotrione. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Rimsulfuron 25 WG + EXELL	28 d, aerobic	< 25% effect up to 0.25 kg prep/ha + 2.5 L Exell	EFSA Scientific Report (2005) 45, 1-61
N-mineralisation	Rimsulfuron 25 WG	28 d, aerobic	< 25% effect up to 0.6 kg prep/ha (0.2 mg/kg dw soil)*	
N-mineralisation	IN-70941	28 d, aerobic	< 25% effect at day 28 at 0.150 kg/ha (0.2 mg/kg dw soil)	
N-mineralisation	IN-E9260	28 d, aerobic	< 25% effect at day 28 at 0.150 kg/ha (0.2 mg/kg dw soil)	
~~C-mineralisation~~	~~Rimsulfuron 25 WG + EXELL~~	~~28 d, aerobic~~	~~< 25% effect up to 0.25 kg prep/ha + 2.5 L Exell~~	
~~C-mineralisation~~	~~Rimsulfuron 25 WG~~	~~28 d, aerobic~~	~~< 25% effect up to 0.6 kg prep/ha (0.150 kg a.s./ha)~~	
~~C-mineralisation~~	~~IN-70941~~	~~28 d, aerobic~~	~~< 25% effect at day 28 at 0.150 kg/ha (0.2 mg/kg dw soil)~~	
~~C-mineralisation~~	~~IN-E9260~~	~~28 d, aerobic~~	~~< 25% effect at day 28 at 0.150 kg/ha (0.2 mg/kg dw soil)~~	
N-mineralisation	Nicosulfuron	29 d, aerobic	At 0.08 & 0.8 mg as/kg soil dw < 25% deviation from control by study end (day 28)	EFSA Scientific Report (2007) 120, 1-91
N-mineralisation	SL-950 4% SC	28 day study	At doses equivalent to 0.08 & 0.8 mg a.s. /kg soil d.wt. < 25% deviation from control by study end (day 29)	
N-mineralisation	AUSN	29 d, aerobic	0.082 mg AUSN + 0.034 mg UCSN + 0.191 mg ASDM/kg dry soil: < 25% deviation from control by study end (day 28)	
N-mineralisation	UCSN	28 day study	0.082 mg AUSN + 0.034 mg UCSN + 0.191 mg ASDM/kg dry soil: < 25% deviation from control by study end (day 28)	
N-mineralisation	ASMD	28 day study	0.082 mg AUSN + 0.034 mg UCSN + 0.191 mg ASDM/kg dry soil: < 25% deviation from control by study end (day 28)	
~~C-mineralisation~~	~~Nicosulfuron~~	~~29 d, aerobic~~	~~At 0.08 & 0.8 mg as/kg soil dw < 25% deviation from control by study end (day 28)~~	
~~C-mineralisation~~	~~SL-950 4% SC~~	~~28 day study~~	~~At doses equivalent to 0.08 & 0.8 mg a.s. /kg soil d.wt. < 25% deviation from control by study end (day 29)~~	

Endpoint	Substance	Exposure System	Results	Reference
~~C mineralisation~~	~~AUSN~~	~~29 d, aerobic~~	~~0.082 mg AUSN + 0.034 mg UCSN + 0.191 mg ASDM/kg dry soil: < 25% deviation from control by study end (day 28)~~	
~~C mineralisation~~	~~UCSN~~	~~28 day study~~	~~0.082 mg AUSN + 0.034 mg UCSN + 0.191 mg ASDM/kg dry soil: < 25% deviation from control by study end (day 28)~~	
~~C mineralisation~~	~~ASMD~~	~~28 day study~~	~~0.082 mg AUSN + 0.034 mg UCSN + 0.191 mg ASDM/kg dry soil: < 25% deviation from control by study end (day 28)~~	
N-mineralisation	Callisto 100 SC (Mesotrione)	28 d, aerobic	7.8% effect at day 28 at 0.53 mg as/kg dw soil	EFSA Journal 2016;14(3):4419
N-mineralisation	AMBA	28 d, aerobic	-7.6% effect at day 28 at 1.13 mg/kg dw soil	
N-mineralisation	MNBA	28 d, aerobic	-4.8 effect at day 28 at 1.13 mg/kg dw soil	
N-mineralisation	PRIMARY MX	42 d, aerobic soil type	No effects >25% on nitrogen transformation at 0.9 and 4.5 mg test item/kg dry soil weight.	KCP 10.5-01 Dec, W., 2019 G/264/17
~~C mineralisation~~	~~PRIMARY MX~~	~~28 d, aerobic soil type~~	~~No effects >25% on carbon transformation at 0.9 and 4.5 mg test item/kg dry soil weight.~~	~~KCP 10.5-02 Dec, W., 2019 G/263/17~~

* Conversion of endpoint (g/ha) in endpoint (mg a.s./kg soil)
Endpoint 2 = 150 / (100 x Soil depth (cm) x Soil dry bulk density (g/cm^3))
= 150 / 750
= 0.2 mg a.s./kg soil

9.9.1.1 Justification for new endpoints

The used endpoints are the EU agreed ones, except for formulation, corresponding to data proper to PRIMARY MX formulation

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, tables from 8.7-2 to 8.7-19 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.8).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of PRIMARY MX in maize

Intended use	**Maize**		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
Rimsulfuron 25 WG (Rimsulfuron)	0.2 (at 28 d)	~~0.010~~ 0.008	yes
IN-70941	0.2 (at 28 d)	~~0.006~~ 0.005	yes
IN-E9260	0.2 (at 28 d)	0.002	yes
Nicosulfuron	0.8 (at 28 d)	~~0.040~~ 0.030	yes
AUSN	0.082 (at 29 d)	~~0.009~~ 0.007	yes
UCSN	0.034 (at 28 d)	~~0.004~~ 0.003	yes
ASDM	0.191 (at 28 d)	~~0.016~~ 0.013	yes
Callisto 10 SC (Mesotrione)	0.53 (at 28 d)	~~0.118~~ 0.090	yes
AMBA	1.13 (at 28 d)	0.007	yes
MNBA	1.13 (at 28 d)	0.049	yes
PRIMARY MX	4.5 (at 42 d)	~~0.330~~ 0.250	yes
C-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
Rimsulfuron 25 WG (Rimsulfuron)	0.2 (at 28 d)	~~0.010~~ 0.008	yes
IN-70941	0.2 (at 28 d)	~~0.006~~ 0.005	yes
IN-E9260	0.2 (at 28 d)	0.002	yes
Nicosulfuron	0.8 (at 28 d)	~~0.040~~ 0.030	yes
AUSN	0.082 (at 29 d)	~~0.009~~ 0.007	yes
UCSN	0.034 (at 28 d)	~~0.004~~ 0.003	yes
ASDM	0.191 (at 28 d)	~~0.016~~ 0.013	yes
PRIMARY MX	4.5 (at 28 d)	~~0.330~~ 0.250	yes

zRMS comments:

The risk assessment presented in Table 9.9-2 is agreed by the zRMS.

Maximum PEC_{soil} values are considerably lower than concentrations at which effects were < 25%.

On this basis acceptable risk to soil micro-organisms may be concluded from intended uses of PRIMARY MX. Risk assessment for effects on carbon transformation has been struck through as being no longer a data requirement.

9.9.3 Overall conclusions

Risk assessments conducted with relevant PEC_{soil} for the active substance Rimsulfuron, Nicosulfuron and Mesotrione indicate a low risk to soil microorganisms when applied according to the proposed use rates. The use of PRIMARY MX at the proposed rates poses no unacceptable risk to non-target soil micro-organisms.

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

9.10.1 Toxicity data

Studies on the toxicity to non-target terrestrial plants have been carried out with Rimsulfuron, Nicosulfuron, Mesotrione and their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target terrestrial plants of PRIMARY MX were not evaluated as part of the EU assessment of Rimsulfuron, Nicosulfuron and Mesotrione.

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

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

Species	Substance	Exposure System	Results	Reference
Sorghum bicolor	Rimsulfuron	Green House Test	ED_{50} technical rimsulfuron 0.17 g as/ha ED_{50} 25 WG formulation 4.89 g product/ha (equal to 1.22 g as/ha)	EFSA Scientific Report (2005), 45, 1-61
Rice	SL-950 4% SC (Nicosulfuron)	Post-emergence (vegetative vigour)	ER_{50} = 0.47 g as/ha (based on % of plants showing visible adverse effects in glasshouse test)	EFSA Scientific Report (2007) 120, 1-91
Most sensitive species not ascertained (equivalent endpoint for six tested dicot / monocot crop species	SL-950 4% SC (Nicosulfuron)	Pre-emergence (emergence)	$ER_{50\ emergence}$ > 20 g a.s./ha (no adverse effects at 20 g a.s./ha)	
Lactuca sativa (worst case species)	Callisto 100 SC (Mesotrione)	Vegetative vigour and emergence (lab.)	ER_{50} = 0.883 g as/ha	EFSA Journal 2016;14(3):4419
Lactuca sativa (worst case species)	Callisto 100 SC (Mesotrione)	Seedling emergence (lab.)	ER_{50} = 13.8 g as/ha	
SSD		Vegetative vigour	HC5 = 0.173 g a.s./ha	

Species	Substance	Exposure System	Results	Reference
Brassica oleracea var. capitata	PRIMARY MX	21 d Vegetative vigour	ER_{50} = 6.6 g/ha (plant dry weight) ER_{50} = 21.4 g/ha (plant damage in *Alium cepa*)*	KCP 10.6.2-01 Wróbel, A., 2020 G/269/17
Allium cepa	PRIMARY MX	14 d Seedling emergence	ER_{50} = 41.2 g/ha (plant dry weight) ER_{50} = 16.7 g/ha (plant damage in *Brassica oleracea var. capitata*)*	KCP 10.6.2-02 Wróbel, A., 2020 G/268/17

* New endpoints calculated based on the observed plant damage for each study. As this endpoint in the seedling emergence study is lower than the endpoint derived from the dry weight, it is also used in the risk assessment below. For further detail, please refer to the study summaries for KCP 10.6.2-01 and KCP 10.6.2-02.

9.10.1.1 Justification for new endpoints

The used endpoints are the EU agreed ones.

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Not relevant.

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

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

Table 9.10-2: Assessment of the risk for non-target plants due to the use of PRIMARY MX in maize

Intended use	**Maize**			
Active substance	Rimsulfuron			
Application rate (g/ha)	1 x ~~9.9~~ 7.5			
MAF	1			
Test species	**ER_{50} (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**TER criterion: TER ≥ 5**
Sorghum bicolor	0.17	0.0277	~~0.2742~~ 0.208	~~0.62~~ **0.82**
Active substance	Nicosulfuron			
Application rate (g/ha)	1 x ~~39.6~~ 30			
MAF	1			

Test species	**ER_{50} (g/ha)**	**Drift rate**	**$PER_{off-field}$ (g/ha)**	**TER criterion: TER ≥ 5**
Rice	0.47 (vegetative vigour)	0.0277	~~1.0969~~ 0.831	~~0.43~~ **0.57**
	20 (emergence)			~~18.23~~ 24.07
Active substance	Mesotrione			
Application rate (g/ha)	1 x ~~118~~ 90			
MAF	1			
Test species	**ER_{50} (g/ha)**	**Drift rate**	**$PER_{off-field}$ (g/ha)**	**TER criterion: TER ≥ 5**
Lactuca sativa	0.883 (vegetative vigour)	0.0277	~~3.2686~~ 2.493	~~0.27~~ **0.35**
Lactuca sativa	13.8 (emergence)	0.0277	~~3.2686~~ 2.493	**4**~~.22~~ 5.54
Active substance	PRIMARY MX			
Application rate (g/ha)	1 x ~~330~~ 250			
MAF	1			
Test species	**ER_{50} (g/ha)**	**Drift rate**	**$PER_{off-field}$ (g/ha)**	**TER criterion: TER ≥ 5**
Brassica oleracea var. capitata	6.6 g/ha (plant dry weight)	0.0277	~~9.141~~ 6.925	~~0.72~~ **0.95**
Allium cepa	41.2 g/ha (plant dry weight)	0.0277	~~9.141~~ 6.925	**4**~~.51~~ 5.95
Brassica oleracea var. capitata	16.7 g/ha (**phytotoxic effects**)	0.0277	6.925	**2.41**

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

zRMS comments:

Deterministic risk assessment for PRIMARY MX presented in Tables 9.10-2 above is agreed by the zRMS. An acceptable risk could be not concluded with E_rC_{50} of 6.6 g/ha value from vegetative vigour test from seedling emergence test for the max. application rate of 250 g product/ha ($PER_{in-field}$).

Therefore, further refinement was needed to concluded the acceptable risk to non target plants.

For this reason, the applicant provided the risk migitation measures in the Table 9.10-3 below.

9.10.2.3 Higher-tier risk assessment

According to EFSA Journal 2016;14(3):4419, a Species Sensitivity Distribution (SSD) risk assessment was performed using the lowest endpoints for each species (biomass) from the vegetative vigour test with mesotrione. The model on the www.webfram.com website was used to determine the HC_5 (the concentration hazardous to 5% of species), obtaining an **HC_5 of 0.173 g a.s./ha**. Where the TER is > 1 in mesotrione assessment, an acceptable risk is concluded.

9.10.2.4 Risk mitigation measures

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

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

Intended use **Active substance** **Application rate (g/ha)** **MAF**		**Maize** Rimsulfuron 1 x ~~9.9~~ 7.5 1			
Buffer strip (m)	**Drift rate (%)**	**$PER_{off-field}$ (g/ha)**	**$PER_{off-field}$ 50 % drift red. (g/ha)**	**$PER_{off-field}$ 75 % drift red. (g/ha)**	**$PER_{off-field}$ 90 % drift red. (g/ha)**
1	0.0277	~~0.274~~ 0.208	~~0.137~~ 0.104	~~0.069~~ 0.052	~~0.027~~ 0.021
5	0.0057	~~0.056~~ 0.043	~~0.028~~ 0.021	~~0.014~~ 0.011	-
10	0.0029	~~0.029~~ 0.022	-	-	-
Toxicity value ER_{50} = 0.17 g a.s./ha		**TER** **criterion: TER ≥ 5**			
1		~~0.62~~ 0.82	~~1.24~~ 1.64	~~2.48~~ 3.27	~~6.20~~ 8.18
5		~~3.01~~ 3.98	~~6.03~~ 7.95	~~12.05~~ 15.91	-
10		~~5.92~~ 7.82	-	-	-
Active substance **Application rate (g/ha)** **MAF**		Nicosulfuron 1 x ~~39.6~~ 30 1			
Buffer strip (m)	**Drift rate (%)**	**$PER_{off-field}$ (g/ha)**	**$PER_{off-field}$ 50 % drift red. (g/ha)**	**$PER_{off-field}$ 75 % drift red. (g/ha)**	**$PER_{off-field}$ 90 % drift red. (g/ha)**
1	0.0277	~~1.097~~ 0.831	~~0.548~~ 0.416	~~0.274~~ 0.208	~~0.110~~ 0.083
5	0.0057	~~0.226~~ 0.171	~~0.113~~ 0.086	~~0.056~~ 0.043	~~0.023~~ 0.017
10	0.0029	~~0.115~~ 0.087	~~0.057~~ 0.044	-	-
Toxicity value ER_{50} = 0.47 g a.s./ha		**TER** **criterion: TER ≥ 5**			
1		~~0.43~~ 0.57	~~0.86~~ 1.13	~~1.71~~ 2.26	~~4.28~~ 5.66
5		~~2.08~~ 2.75	~~4.16~~ 5.50	8.33	~~20.82~~
10		~~4.09~~ 5.40	~~8.19~~	-	-
Active substance **Application rate (g/ha)** **MAF**		Mesotrione 1 x ~~118~~ 90 1			

Buffer strip (m)	Drift rate (%)	$PER_{off-field}$ (g/ha)	$PER_{off-field}$ 50 % drift red. (g/ha)	$PER_{off-field}$ 75 % drift red. (g/ha)	$PER_{off-field}$ 90 % drift red. (g/ha)
1	0.0277	~~3.269~~ 2.493	~~1.634~~ 1.247	~~0.817~~ 0.623	~~0.327~~ 0.249
5	0.0057	~~0.673~~ 0.513	~~0.336~~ 0.257	~~0.168~~ 0.128	~~0.067~~ 0.051
10	0.0029	~~0.342~~ 0.261	~~0.171~~ 0.131	-	-
Toxicity value HC_5 = 0.173 g a.s./ha		TER criterion: TER ≥ 1			
1		~~0.05~~ **0.07**	~~0.11~~ **0.14**	~~0.21~~ **0.28**	~~0.53~~ **0.69**
5		~~0.26~~ **0.34**	~~0.51~~ **0.67**	~~1.03~~ 1.35	~~2.57~~ 3.37
10		~~0.51~~ **0.66**	~~1.01~~ 1.33	-	-
Active substance **Application rate (g/ha)** **MAF**		PRIMARY MX 1 x ~~333~~ 250 1			
Buffer strip (m)	**Drift rate (%)**	**$PER_{off-field}$ (g/ha)**	**$PER_{off-field}$ 50 % drift red. (g/ha)**	**$PER_{off-field}$ 75 % drift red. (g/ha)**	**$PER_{off-field}$ 90 % drift red. (g/ha)**
1	0.0277	~~9.141~~ 6.925	~~4.5705~~ 3.463	~~2.28525~~ 1.731	~~0.9141~~ 0.693
3	0.0095	~~3.135~~ 2.375	~~1.5675~~ 1.188	~~0.78375~~ 0.594	~~0.3135~~ 0.238
5	0.0057	~~1.881~~ 1.425	~~0.9405~~ 0.713	~~0.47025~~ 0.356	~~0.1881~~ 0.143
10	0.0029	~~0~~.957 0.725	~~0.4785~~ 0.363	~~0.23925~~ 0.181	~~0.0957~~ 0.073
Toxicity value ~~HC_5~~ ER50 = 6.6 g/ha		**TER** **criterion: TER ≥ 5**			
1		~~0.72~~ **0.95**	~~1.44~~ **1.91**	~~2.89~~ **3.81**	~~7.22~~ 9.53
3		~~2.11~~ **2.78**	~~4.24~~ 5.56	~~8.42~~ 11.12	-
5		~~3.51~~ **4.63**	~~7.02~~ 9.26	-	-
10		~~8.00~~ 9.10	-	-	-

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

zRMS comments:

Based on the lowest toxicity endpoint- E_rC_{50} of 6.6. g product/ha value form vegetative vigour test and determistic risk assessment, the rsi for NTP is acceptable when following risk migitation measures are applied to non - agricultural land.

-90% drift reducing nozzles or

- an unsprayed buffer zone of 3m with 50 % drift reducing nozzles or

- an unsprayed buffer zone of 10m to non-agricultural land.

The risk mitigation measure should be decided on MS level

~~**To protect non-target plants respect 90% drift reducing nozzles OR an unsprayed buffer zone of 3m with 50% drift reducing nozzles OR an unsprayed buffer zone of 10m to non-agricultural land.**~~

9.10.3 Overall conclusions

~~Risk assessment conducted with relevant toxicity data on non-target terrestrial plants for Rimsulfuron, Nicosulfuron and Mesotrione shows that the Annex VI trigger value of 5 is exceeded. Therefore, mitigation measures are needed. When there is 5m buffer zone with 75% nozzle reduction OR 10 m buffer zone with 50% nozzle reduction, PRIMARY MX poses a low risk to non-target plants when applied according to the proposed use rates.~~

~~***Maize – SPe 3:** To protect non-target plants respect an unsprayed buffer zone of 5m with 75% drift reducing nozzles OR 10m with 50% drift reducing nozzles to non-agricultural land.~~

Risk assessment conducted with relevant toxicity data on non-target terrestrial plants for Rimsulfuron, Nicosulfuron and Mesotrione shows that the Annex VI trigger value of 5 is exceeded. Therefore, mitigation measures are needed. When there is 90% nozzle reduction OR 3m buffer zone with ~~a~~ 50% nozzle reduction OR 5m buffer zone with 50% nozzle reduction OR 10 m buffer zone, PRIMARY MX poses a low risk to non-target plants when applied according to the proposed use rates.

~~***Maize – SPe 3:** To protect non-target plants respect 90% drift reducing nozzles OR an unsprayed buffer zone of 3m with 7550% drift reducing nozzles OR an unsprayed buffer zone of 5m with 50% drift reducing nozzles OR an unsprayed buffer zone of 10m to non-agricultural land.~~

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

Rimsulfuron:
Data from a test with activated sludge are available and indicate that the risk to biological methods of sewage treatment plants is low.

Nicosulfuron:
Effects on biological methods for sewage treatment

Test type/organism	**End point**
Activated sludge	--
Pseudomonas putida	Nicosulfuron EC_{50} > 250 mg as/L (no reported effects) ASDM, AUSN, UCSN, MU-466, HMUD > 100 mg metabolite/L (no significant inhibition)

Mesotrione:
Effects on biological methods for sewage treatment

Test type/organism	**End point**
Activated sludge	$EC_{50} \geq 160$ mg as/L
Pseudomonas sp.	NOEC = 100 mg as/L

9.12 Monitoring data (KCP 10.8)

Not relevant.

9.13 Classification and Labelling

	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG
Common Name	PRIMARY MX
Classification ad proposed labelling	
With regard to ecotoxicological endpoints (according to the criteria Reg. 1272/2008, as amended)	Hazards classe(s), categories: Aquatic Acute Category 1, M factor 10 Aquatic Chronic Category 1, M factor 10 Code(s) for hazard pictogram(s): GHS09 Signal word: Warning Hazard statement(s): H400, H410 Precautionary statement: ~~P273~~, P391, P501

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

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

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.1-01	xxx	2018	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG Rainbow trout Acute toxicity test xxx report No. W/204/17 GLP, unpublished	Y	Sharda Cropchem Limited
KCP 10.2.1-02	Bak, P.	2018	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG *Raphidocelis subcapitata* (formely *Pseudokirchneriella subapitata*) SAG 61.81 Growth inhibition test Institute of Industrial Organic Chemistry Branch Pszczyna report No. W/205/17 GLP, unpublished	N	Sharda Cropchem Limited
KCP 10.2.1-03	Bak, P.	2018	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG *Daphnia magna*, acute immobilisation test Institute of Industrial Organic Chemistry Branch Pszczyna report No. W/206/17 GLP, unpublished	N	Sharda Cropchem Limited
KCP 10.2.1-04	Bak, P.	2018	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG *Lemna gibba* CPCC 310, growth inhibition test Institute of Industrial Organic Chemistry Branch Pszczyna report No. W/207/17 GLP, unpublished	N	Sharda Cropchem Limited
KCP 10.2.1-05	Bätscher, R.	2008	"Toxicity of Nicosulfuron technical to the Aquatic Higher Plant *Lemna gibba* in a 7-Day Growth Inhibition Test, Supplemented With Testing for Recovery of Growth" B75341. GLP, unpublished	N	Sharda Cropchem Limited

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.1.1.1	Stalmach, M.	2019	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG Honeybees (*Apis mellifera* L.), Acute Oral Toxicity Test Institute of Industrial Organic Chemistry Branch Pszczyna report No. B/172/16 GLP, unpublished	N	Sharda Cropchem Limited
KCP 10.3.1.1.2	Stalmach, M.	2019	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG Honeybees (*Apis mellifera* L.), Acute Contact Toxicity Test Institute of Industrial Organic Chemistry Branch Pszczyna report No. B/173/16 GLP, unpublished	N	Sharda Cropchem Limited
KCP 10.3.1.2.1	Ansaloni, T.	2018	Rimsulfuron Technical - Chronic Toxicity to the Honey Bee, *Apis mellifera* Trialcamp S.L.U. TRC16-193BA GLP, unpublished	N	Sharda Cropchem Limited
KCP 10.3.1.2.2	Ansaloni, T.	2018	Nicosulfuron Technical - Chronic Toxicity to the Honey Bee, *Apis mellifera* L. Trialcamp S.L.U. TRC16-049BA GLP, unpublished	N	Sharda Cropchem Limited
KCP 10.3.1.2.3	Gimeno, I.	2019	Mesotrione Technical - Chronic Toxicity to the Honey Bee, *Apis mellifera* L. Trialcamp S.L.U. TRC17-006BA GLP, unpublished	N	Sharda Cropchem Limited
KCP 10.3.1.2.4	Radha, S.	2022	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG on adult honey bee (*Apis mellifera* L.), Chronic Oral Toxicity Test. Bioscience Research foundation. 7961/2020 GLP, unpublished	N	Sharda Cropchem Limited
KCP 10.3.1.3.1	Aguilar-Alberola, J.A. & Marín Víllora, M.	2018	Toxicity of Rimsulfuron Technical on honeybee larvae (*Apis mellifera* L.) after repeated exposure under laboratory conditions Trialcamp S.L.U. TRC16-162BA GLP, unpublished	N	Sharda Cropchem Limited
KCP 10.3.1.3.2	Scheller, K.	2018	Mesotrione Technical - Repeated exposure of honey bee (*Apis mellifera* L.) larvae under laboratory conditions (*in vitro*) BioChem agrar 17 48 BLC 0088 GLP, unpublished	N	Sharda Cropchem Limited

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.1.3.3	Radha, S.	2022	Effect of Rimsulfuron 3%+ Nicosulfuron12% + Mesotrione 36% WG on larvae of honey bee, *Apis mellifera* (L.) following repeated exposure. Bioscience Research foundation. 7962/2020 GLP, unpublished	N	Sharda Cropchem Limited
KCP 10.3.2.1-01	Kulec-Płoszczyca, E.	2018	An extended laboratory test for evaluating the effects of Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani - Perez) Institute of Industrial Organic Chemistry Branch Pszczyna report No. B/174/16 GLP, unpublished	N	Sharda Cropchem Limited
KCP 10.3.2.1-02	Stalmach, M.	2019	An extended laboratory test for evaluating the effects of Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG on the predatory mite, *Typhlodromus pyri* (Sch.) Institute of Industrial Organic Chemistry Branch Pszczyna report No. B/175/16 GLP, unpublished	N	Sharda Cropchem Limited
KCP 10.3.2.1-03	Mohanraj, M.	2020	A laboratory test for evaluating the effects of Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG on larvae of the green lacewing *Chrysoperla carnea* (L.) (Neuroptera: Chrysopidae). Bioscience Research Foundation. 7554/2020 GLP, unpublished	N	Sharda Cropchem Limited
KCP 10.3.2.1-04	Sonali, G.	2020	An extended laboratory test for evaluating the effects of Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG on the rove beetle, *Aleochara bilineata* (Gyllenhal). Bioscience Research Foundation. 7555/2020 GLP, unpublished	N	Sharda Cropchem Limited
KCP 10.3.2.1-05	Varela, S.	2021	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG: Toxicity to the Predatory Mite, *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) after Exposure to Freshly Applied and Aged Spray Deposits under Extended Laboratory Conditions Trialcamp S.L.U. S20-07857 GLP, unpublished	N	Sharda Cropchem Limited
KCP 10.4.1.1	Dec, W.	2019	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WDG Earthworm Reproduction Test (*Eisenia andrei*) Institute of Industrial Organic Chemistry Branch Pszczyna report No. G/265/17 GLP, unpublished	N	Sharda Cropchem Limited

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 10.4.2.1-01	Dec, W.	2019	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WDG Collembolan (*Folsomia candida*) Reproduction Test Institute of Industrial Organic Chemistry Branch Pszczyna report No. G/266/17 GLP, unpublished	N	Sharda Cropchem Limited
KCP 10.5-01	Dec, W.	2019	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WDG Soil Microorganisms: Carbon Transformation Test Institute of Industrial Organic Chemistry Branch Pszczyna report No. G/263/17 GLP, unpublished	N	Sharda Cropchem Limited
KCP 10.5-02	Dec, W.	2019	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WDG Soil Microorganisms: Nitrogen Transformation Test Institute of Industrial Organic Chemistry Branch Pszczyna report No. G/264/17 GLP, unpublished	N	Sharda Cropchem Limited
KCP 10.6.2-01	Wróbel, A.	2020	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WDG. Terrestrial Plant Test: Vegetative Vigour Test Institute of Industrial Organic Chemistry Branch Pszczyna report No. G/269/17 GLP, unpublished	N	Sharda Cropchem Limited
KCP 10.6.2-02	Wróbel, A.	2020	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WDG. Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test Institute of Industrial Organic Chemistry Branch Pszczyna report No. G/268/17 GLP, unpublished	N	Sharda Cropchem Limited

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

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

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of the new studies

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

Comments of zRMS:	The LoA was provided by the applicant to this study. The study was previously evaluated by zRMS-PL for the other product with mesotrione (Calisto avalible on Circa platform, company Syngenta). The short zRMS-PL's previously evaluation of this study was summerised below: The aim of the study by xxx (2019) was to determine the proportion of time the brown hares spent potentially foraging in early germinated maize fields (BBCH <20). The study was well performed and is considered acceptable by the zRMS-PL previously. Initial site selection in the study was based on the presence of the European hare, high proportions of maize fields within the landscape and the suitability for performing radio-tracking. To increase representativeness and variability of landscape parameters among Central European maize growing areas, two different study areas in two different countries and five different study sites were chosen in areas of high proportions of maize within Central Europe. The region used for the study represented typical maize growing region in Germany and Hungary and may be thus considered representative for conditions of the Central Zone. However, concerned Member States may wish to re-consider representativeness of the conditions of the study for agronomic conditions in their countries. At test sites the maize fields represented on average 36% of the landscape surface within the investigated hares home ranges and at some test sites their proportion in the total landscape exceeded 45 or even 50%. The study was performed at early stages of maize and included BBCH from 00 to 19. However, as the aim of the study was to determine the time that brown hares potentially feed on maize shoots, results for fields with BBCH <09 were excluded from calculation of PT values. For purposes of the radio-tracking, 23 individual adult brown hares were trapped and equipped with the radio tags. Trapping locations were chosen in areas with high number of maize/future maize fields and hares were captured either in such fields or nearby (e.g. in adjacent off-crop structures or neighbouring fields). The total weight of the hare collar was about 40 g, representing approximately 1% of the bodyweight of the tagged animals. Due to the low weight of the tags (far below the recommended maximum of 5% of the total bodyweight) it was not expected that they would have influence the animals' behaviour. Visual observations confirmed normal behaviour of the animals. In order to give animal time to acclimatize, the radio-tracking started no earlier

	than 2 days after tagging with single check telemetry of the individuals. During the telemetry sessions each individual was tracked continuously for 24 hours, which is in line with recommendations of EFSA (2009). During this time all movements between different habitats and changes of the behaviour (e.g. foraging, resting) were recorded. In addition to that, animals were observed with binoculars, scopes and night observation devices. During radio-tracking without visual contact, all instances of an active signal were interpreted as potential foraging behaviour and thus included in the calculation of PT values. However, based on the behaviour confirmed by visual contacts during the 24h telemetry, animals foraged for just 32.0% of their visually observed time and showed active behaviour other than foraging in 18.5% of the time. Therefore, the time spent potentially foraging in maize is rather overestimated for this habitat. This confirms that the PT values are conservative and rather overestimate the actual PT values for early maize (BBCH growth stages up to 20) than being a minimum value. In general, results of the study indicate that brown hares do utilise early maize fields as the feeding habitat. During the 24-hours radio-tracking session most of 23 radio-tagged hares were observed in maize fields with individual PT values ranging from 0.02 to 0.94. One individual (or signal) could not be tracked after tagging and most probably the animal left the study site. One individual was found at the end of May far outside the study site. To increase the number of radio-tracking sessions and to cover wider range of BBCH stages, two individuals were radio-tracked twice, giving 23 radio-tracking sessions in total. One session was excluded from further calculations as being not "consumer session" (animal was never located in a maize field being active during the session, had no maize in the 24h home range and was not caught on a maize field). Taking into account that 21 individuals (i.e. >20 recommended by EFSA, 2009) were observed potentially foraging during radio-tracking sessions (with one animal observed twice), in opinion of the zRMS-PL the 90th percentile PT value is sufficiently reliable and may be used for purposes of the refinement of the risk for the brown hare. It should be noted that PT values were derived for maize stages ranging from BBCH 09 to 19, while PRIMARY MX is intended to applied at BBCH 12-18. Nevertheless, obtained results show that different BBCH growth stages up to <20 did not have an impact on the use of maize as foraging habitat by the brown hare. Taking this into account, the overall 90th percentile PT of 0.62 is considered acceptable for purposes of refinement of the risk to the brown hare exposed after application of PRIMARY MX according to the intended use pattern. **Agreed endpoints:** PT= 0.62 (hare)

Reference:	KCP 10.1.2.2/07
Report	xxx (2019) Generic monitoring of European hares to determine proportion of time spent foraging in early maize in Central Europe Syngenta Limited; unpubl. RIFCON GmbH report No. R1740045, March 2019. Syngenta File No. NA_14950
Guideline(s):	No official test guideline(s) available at present Conducted under consideration of the EFSA Guidance Document on Risk Assessment for Birds & Mammals (EFSA 2009).
Deviations:	Not relevant
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Comments of zRMS:	The applicant has access to this study. The kinetic evaluation of the study was provided by the applicant and DT_{50} was estimated to be 0.813 d. The Chi^2 error in trials S15-02057-01, S15-02057-03 and S15-02057-04 was >15%, however Chi^2 above 15% is not the reason for rejection of obtained results when the statistical analyses and visual fits are acceptable. As this is the case for trials mentioned and in general, SFO kinetics is preferred to derive DT_{50} values for residue decline trials in plants, consideration of only SFO is accepted by the zRMS. As results from 5 trials performed in only 2 countries are available (of which one is Northern France not belonging to the Central Zone, although conditions in Northern France are similar to the Central Europe), it was proposed by the zRMS-PL to use the worst case DT_{50} of 0.813 days for purposes of the risk refinement. **Agreed endpoints:** **DT50=0.813 d**

Reference:	KCP 10.1.2.2/06
Report	North L (2016). Mesotrione – Foliage Decline Study with A12739A on Maize in Northern France and the United Kingdom in 2015. Report Number S15-02057. Eurofins Agroscience Services Ltd., Slade Lane, Wilson, Melbourne, Derbyshire, DE73 8AG, UK. Syngenta File No. A12739A_11065
Guideline(s):	Commission of the European Communities, General Recommendations for the Design, Preparation and Realization of Residue Trials; 7029/VI/95 (rev. 5, working document). OECD Guidelines for the Testing of Chemicals – Crop Field Trial, No. 509, OECD, Paris 2009. OECD Guidance Document on Crop Field Trials, Series on Pesticides No. 66 and Series on Testing and Assessment No. 164, ENV/JM/MONO(2011)50. OECD Guidance Document on Overview of Residue Chemistry Studies (as revised 2009), Series on Testing and Assessment (No. 64) and Series on Pesticides (No. 32), ENV/JM/MONO(2009)31. Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009. OECD Guidance Document on Pesticide Residue Analytical Methods, ENV/JM/MONO(2007)17 (Unclassified, 13 Aug 2007).
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Summary:

Five residue decline field trials on maize were successfully conducted in Northern France and the United Kingdom during 2015. Each trial consisted of a control and a treated plot, with the exception of trial S15-02057-05, where the control samples were taken from the treated plot immediately prior to application of the formulation.

One application was made at 150 g a.s./ha for mesotrione at BBCH 14-16.

Following the application, treated maize whole plant samples were collected at < 1 hour after application (HAA), 4 HAA, 10 HAA, 24 HAA, 34 HAA, 48-51 HAA, 72-78 HAA and 96-99 HAA, with untreated maize whole plant samples being collected < 1 hour before application (HBA).

(Nominal sampling intervals for treated maize whole plant samples: < 1 HAA, 4 HAA, 10 HAA, 24 HAA, 34 HAA, 48 HAA, 72 HAA, and 96 HAA).

Residue analysis

Results analysis field trial samples are given below.

Number and rate of Application rate (g a.s./ha)	Sampling intervals (hours)	Crop part	Mesotrione residue (mg/kg)				
			Trial S15-02057-01 (UK)	Trial S15-02057-03 (UK)	Trial S15-02057-04 (UK)	Trial S15-02057-05 (France)	Trial S15-02057-06 (UK)
1 x 150	< 1 HAA	Whole plant	7.09	13.96	4.24	14.99	3.09
1 x 150	4 HAA	Whole plant	8.48	7.75	2.98	12.63	2.74
1 x 150	10 HAA	Whole plant	4.11	6.25	3.33	8.61	2.05
1 x 150	24 HAA	Whole plant	3.86	3.57	1.69	4.30	0.91
1 x 150	34 HAA	Whole plant	2.79	2.95	0.50	2.19	0.80
1 x 150	48-51 HAA	Whole plant	0.92	1.37	0.41	1.07	0.36
1 x 150	72-78 HAA	Whole plant	0.16	0.63	0.14	0.31	< 0.01
1 x 150	96-99 HAA	Whole plant	0.12	0.11	0.06	0.13	0.10
1 x 150	< 1 HAA	Whole plant	7.09	13.96	4.24	14.99	3.09
Control	< 1 HBA	Whole plant	<0.01	< 0.01	< 0.01	< 0.01	< 0.01

The half life calculations have been done using Cake v 3.4. For calculations, results at less than one hour have been considered as at time 0, for intervals the average of intervals has been used (e.g. 48-51h the average 49.5h) for calculations. Below the calculated DT_{50} and DT_{90} for the trials.

Trial	DT_{50} (h)	DT_{50} (d)	DT_{90} (h)	χ^2 (%)	Kinetic model
Trial S15-02057-01(UK)	19.5	0.813	64.8	20.3	SFO
Trial S15-02057-03 (UK)	12.3	0.513	40.9	19.1	
Trial S15-02057-04 (UK)	16	0.679	53.3	16.3	
Trial S15-02057-05 (France)	12.8	0.533	42.5	2.93	
Trial S15-02057-06 (UK)	15.4	0.642	51.1	6.01	
Geomean (n=5)	**15.0**	**0.627**	-	-	

In the next tables and figures are given the data and the summary of the graphics used for half life modelling. The modelling has been done without any improvement, using the data as such (Detailed Cake v3.4 reports will be sent separately).

Table 1:Data used for modelling

Sampling intervals (hours)	Mesotrione residue (mg/kg)				
	Trial S15-02057-01 (UK)	Trial S15-02057-03 (UK)	Trial S15-02057-04 (UK)	Trial S15-02057-05 (France)	Trial S15-02057-06 (UK)
0	7.09	13.96	4.24	14.99	3.09
4	8.48	7.75	2.98	12.63	2.74
10	4.11	6.25	3.33	8.61	2.05
24	3.86	3.57	1.69	4.30	0.91
34	2.79	2.95	0.50	2.19	0.80
49.5	0.92	1.37	0.41	1.07	0.36

75	0.16	0.63	0.14	0.31	< 0.01
97.5	0.12	0.11	0.06	0.13	0.10

The half life calculations have been done using Cake v 3.4. For calculations, results at less than one hour have been considered as at time 0, for intervals the average of intervals has been used (e.g. 48-51h the average 49.5h) for calculations. Below the calculated DT_{50} and DT_{90} for the trials.

Trial		**DT_{50} (h)**	**DT_{50} (d)**	**DT_{90} (h)**	**χ^2 (%)**	**Kinetic model**
Trial S15-02057-01(UK)		19.5	0.813	64.8	20.3	SFO
Trial S15-02057-03 (UK)		12.3	0.513	40.9	19.1	
Trial S15-02057-04 (UK)		16	0.679	53.3	16.3	
Trial S15-02057-05 (France)		12.8	0.533	42.5	2.93	
Trial S15-02057-06 (UK)		15.4	0.642	51.1	6.01	
Geomean (n=5)		**15.0**	**0.627**	-	-	

Table 1: Data used for modelling

Sampling intervals (hours)	**Mesotrione residue (mg/kg)**				
	Trial S15-02057-01 (UK)	**Trial S15-02057-03 (UK)**	**Trial S15-02057-04 (UK)**	**Trial S15-02057-05 (France)**	**Trial S15-02057-06 (UK)**
0	7.09	13.96	4.24	14.99	3.09
4	8.48	7.75	2.98	12.63	2.74
10	4.11	6.25	3.33	8.61	2.05
24	3.86	3.57	1.69	4.30	0.91
34	2.79	2.95	0.50	2.19	0.80
49.5	0.92	1.37	0.41	1.07	0.36
75	0.16	0.63	0.14	0.31	< 0.01
97.5	0.12	0.11	0.06	0.13	0.10

Trial S15-02057-01 (UK)

Estimated Values:

Parameter	**Value**	**s**	**Prob. > t**	**Lower (90%) CI**	**Upper (90%) CI**	**Lower (95%) CI**	**Upper (95%) CI**
Parent_0	7.834	0.7603	N/A	6.357	9.312	5.974	9.695
k_Parent	0.03553	0.00797	0.002145	0.02005	0.05102	0.01603	0.055

Sum of Squared Residuals: 6.119

χ^2

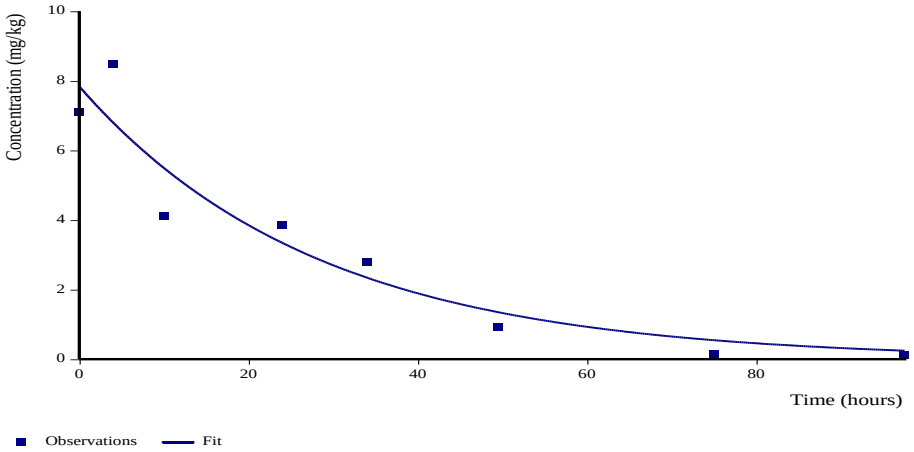
Parameter	**Error %**	**Degrees of Freedom**
All data	20.3	6
Parent	20.3	6

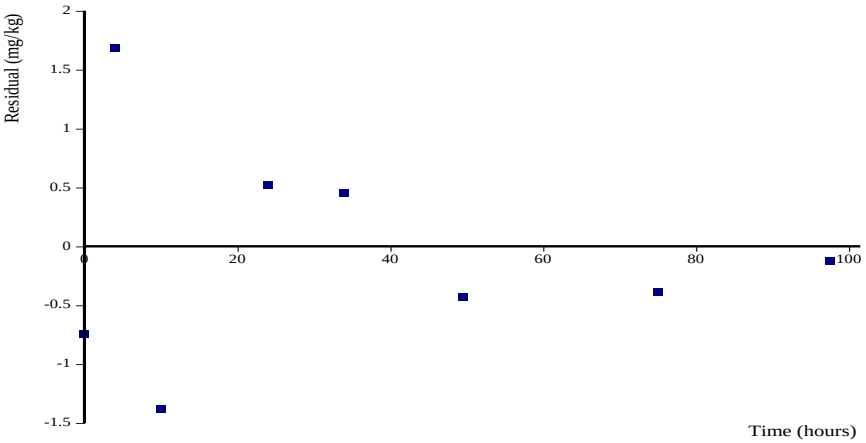
Decay Times:

Compartment	**DT50 (hours)**	**DT90 (hours)**
Parent	19.5	64.8

Graphical Summary:

Observations and Fitted Model:





Trial S15-02057-03 (UK)

Estimated Values:

Parameter	Value	s	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	12.25	1.048	N/A	10.21	14.28	9.68	14.81
k_Parent	0.05628	0.01134	0.001271	0.03425	0.07831	0.02854	0.084

Sum of Squared Residuals: 9.623

χ^2

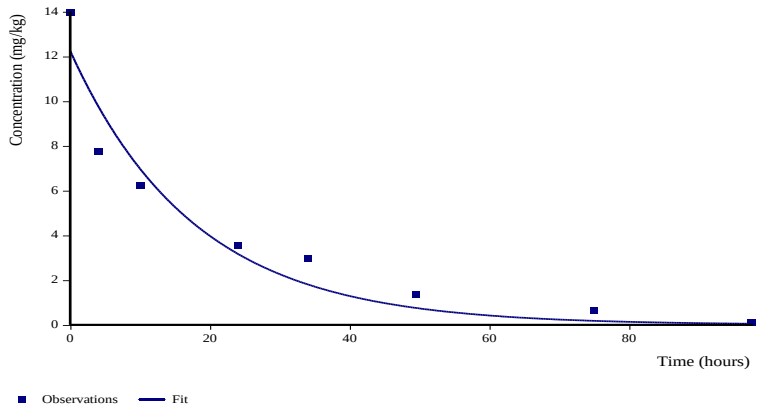
Parameter	Error %	Degrees of Freedom
All data	19.1	6
Parent	19.1	6

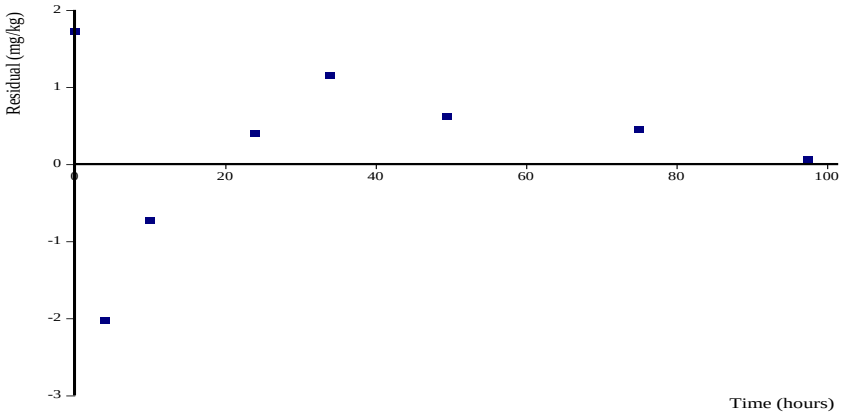
Decay Times:

Compartment	DT50 (hours)	DT90 (hours)
Parent	12.3	40.9

Graphical Summary:

Observations and Fitted Model:





Trial S15-02057-04 (UK)

Estimated Values:

Parameter	Value	s	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	4.166	0.3093	N/A	3.565	4.767	3.409	4.922
k_Parent	0.04322	0.007437	5.70E-004	0.02877	0.05768	0.02503	0.061

Sum of Squared Residuals: 0.9352

χ^2

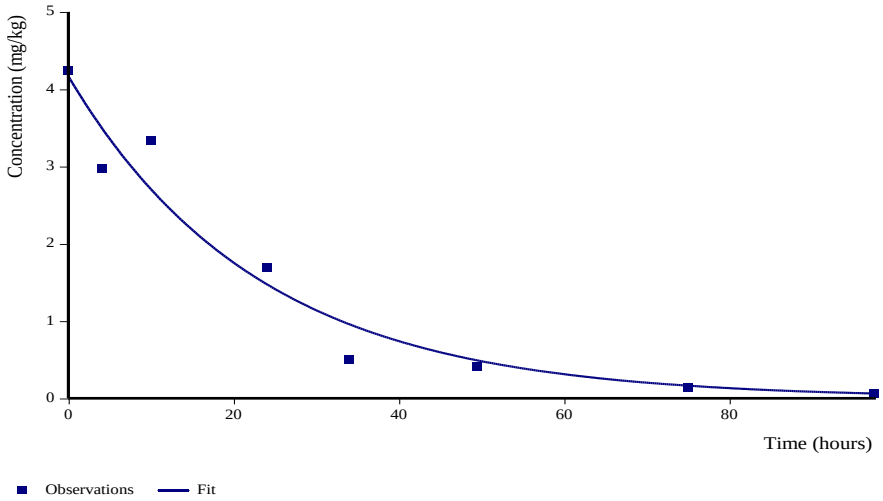
Parameter	Error %	Degrees of Freedom
All data	16.3	6
Parent	16.3	6

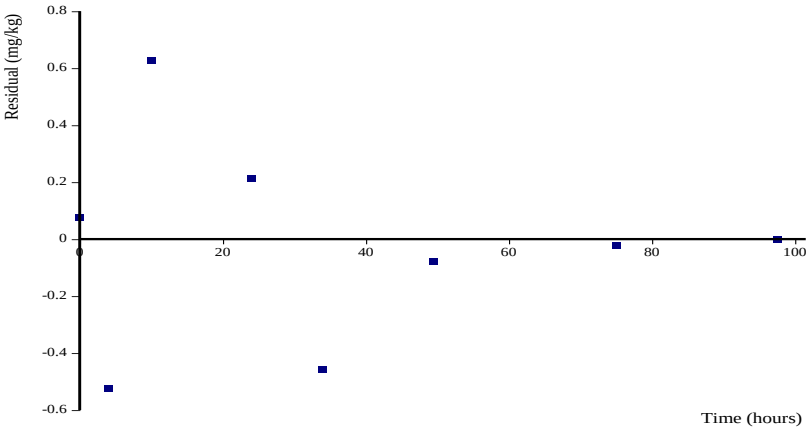
Decay Times:

Compartment	DT50 (hours)	DT90 (hours)
Parent	16	53.3

Graphical Summary:

Observations and Fitted Model:





Trial S15-02057-05 (France)

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	15.19	0.1926	N/A	14.82	15.56	14.72	15.66
k_Parent	0.05415	0.001611	2.31E-008	0.05102	0.05728	0.05021	0.058

Sum of Squared Residuals: 0.3303

χ^2

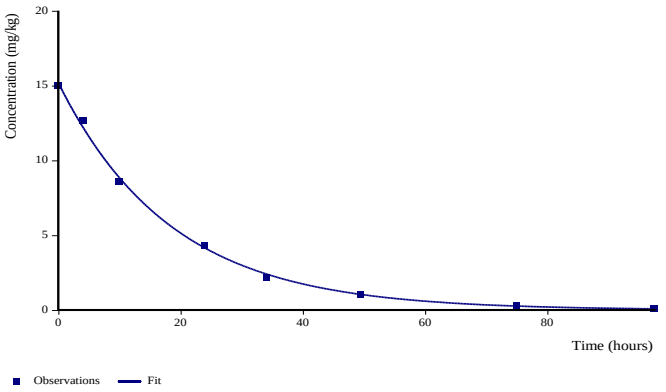
Parameter	Error %	Degrees of Freedom
All data	2.93	6
Parent	2.93	6

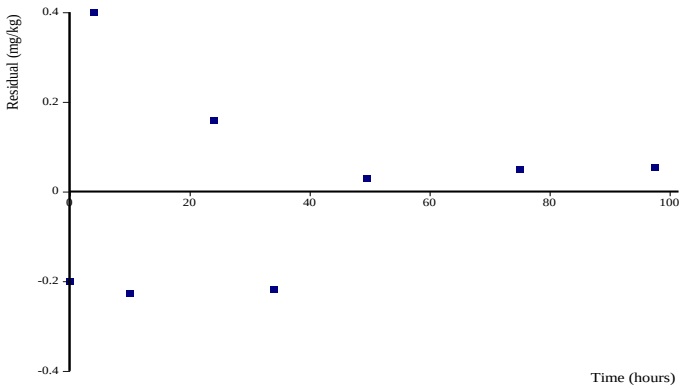
Decay Times:

Compartment	DT50 (hours)	DT90 (hours)
Parent	12.8	42.5

Graphical Summary:

Observations and Fitted Model:





Trial S15-02057-06 (UK)

Estimated Values:

Parameter	Value	s	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	3.164	0.08643	N/A	2.996	3.332	2.952	3.375
k_Parent	0.04509	0.002859	2.06E-006	0.03954	0.05065	0.0381	0.052

Sum of Squared Residuals: 0.07175

χ^2

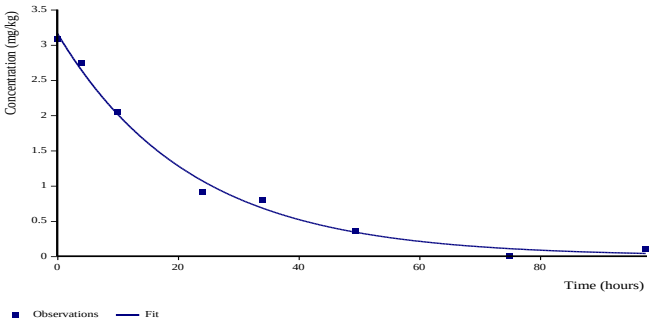
Parameter	Error %	Degrees of Freedom
All data	6.01	6
Parent	6.01	6

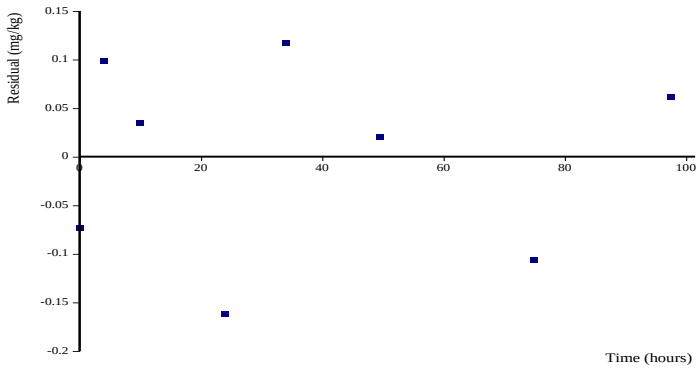
Decay Times:

Compartment	DT50 (hours)	DT90 (hours)
Parent	15.4	51.1

Graphical Summary:

Observations and Fitted Model:





zRMS comments:

The LoA to this study was provided by the Applicant. The study was previously evaluated by zRMS-PL for the other products with mesotrione by Syngenta (ppp Calisto, zRMS-PL, please see on Circa platform for details of study summary). The evaluation was provided below:

The residue trials were performed in various Central Zone countries (UK, Hungary, Germany, Poland and Belgium) as well as in one Southern Zone country (Northern France). However, in opinion of the zRMS-PL, environmental conditions in the Northern France are comparable with conditions of the Central Zone and for this reason results for this trial may be included in the overall analysis.
The aim of the study was determination of the decline of the residues of mesotrione on clover, which may be considered as representative species for dicotyledonous weeds consumed by birds and mammals.
The study was performed with formulation containing 480 g mesotrione/L. In most of trials the application was made to early growth stages of clover (BBCH 15-18). In two trials the application was performed at BBCH 12-61 or 12-81 (it is not specified in the report at which stage exactly the product was applied). Nevertheless, the study does need to simulate the growth stages of the target crop (maize), as at the time of application weeds may be at various growth stages. Furthermore, residues in trials performed at later BBBCH stages were at level comparable with trials where the product was applied earlier, which gives additional reassurance that the residue decline on clover does not depend on the growth stage.
Due to expected rapid decline of mesotrione, intensive sampling was performed during the first days after application, with two samplings performed on the day of application. The sampling schedule gave together 8 data points for each trial, which is sufficient ot perfom the reliable kinetic analysis.
In some trials residues during first 24 hours were quite variable with slightly higher residue levels observed on later samplings. No explanation regarding this issue was provided in the study report. In some trials the variability of residues resulted with poor or unacceptable kinetic fits.
The applicant Sharda company provided the kinetic evaluation of this study which is presented below.
In general, the kinetic evaluation is considered acceptable by zRMS-PL.
Kinetic fit for one trial performed in Germany (SRDE18-002-037HR) is, in opinion, of the zRMS, unacceptable, which is confirmed by very high Chi^2 (>40%). Results for this trial are excluded from further considerations, as only unacceptable SFO fit is available.
The Chi^2 error in several trials was >15%, however Chi^2 above 15% is not the reason for rejection of obtained results when the statistical analyses and visual fits are acceptable. As this is the case for trials mentioned and in general, SFO kinetics is preferred to derive DT_{50} values for residue decline trials in plants, consideration of only SFO is accepted by the zRMS. As after exclusion of unacceptable fit results from 10 trials are available, it is proposed by the zRMS that mean DT_{50} value of **2.19 days** may be used for purposes of the risk refinement.

Agreed endpoint:

DT50=2.19 days

Reference:	KCP 10.1.2.2/11
Report	Allen L. (2019). Mesotrione – Foliage Decline Study on Clover in Hungary, Germany, United Kingdom, Northern France and Belgium in 2018. Report Number CEMR-8397. CEMAS), Imperial House, Oaklands Park, Wokingham, Berkshire, RG41 2FD, UK. Syngenta File No. A12738A_10535
Guideline(s):	Commission of the European Communities, General Recommendations for the Design, Preparation and Realization of Residue Trials; 7029/VI/95 (rev. 5, working document). OECD Guidance Document on Crop Field Trials, Series on Pesticides No. 66 and Series on Testing and Assessment No. 164, ENV/JM/MONO(2011)50. OECD Guidance Document on Overview of Residue Chemistry Studies (as revised 2009), Series on Testing and Assessment (No. 64) and Series on Pesticides (No. 32), ENV/JM/MONO(2009)31. Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009. OECD Guidelines for the Testing of Chemicals – Crop Field Trial, No. 509, OECD, Paris 2009.

	European Commission Guidance for Generating and Reporting Methods of Analysis in Support of Pre-registration Requirements for Annex II (Part A, Section 4) of Directive 91/414, SANCO/3029/99 revision 4 (11 Jul 2000). OECD Guidance Document on Pesticide Residue Analytical Methods, ENV/JM/MONO(2007)17 (Unclassified, 13 Aug 2007). The Application of the OECD Principles of GLP to the Organisation and Management of Multi-Site Studies, ENV/JM/MONO (2002) 9. OECD Series on Principles of GLP and Compliance Monitoring No. 1 (as revised in 1997) "OECD Principles on Good Laboratory Practice", Paris 1998. ENV/MC/CHEM(98)17 and respective national regulations. The national GLP requirements are based on the OECD Principles of Good Laboratory Practice, which are accepted by regulatory authorities throughout the European Community, the United States of America (FDA and EPA) and Japan (MHW, MAFF and METI) on the basis of intergovernmental agreements
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Summary

Twelve (10 + 2 contingency) foliar decline residue field trials on clover were planned, eleven (10 + 1 contingency) were successfully conducted in Northern France, Germany, Poland, Hungary, United Kingdom and Belgium during 2018. One trial was cancelled due to poor crop growth.

Mesotrione was applied to clover in one application at BBCH 16-18 with a nominal rate of 150 g ai/ha for mesotrione, with the exception of trial SRDE18-001-037HR which was applied at BBCH 12-61 and trial SRDE18-002-037HR which was applied at BBCH 16-81. Untreated immature clover samples were taken from the plot at 0 DBA (days before application). Treated samples of immature clover were taken at 0, 8, 24, 32 and 48 HAA (hours after application) and at 3, 4 and 7 DAA (days after application).

Residue analysis

Residues of mesotrione in treated clover taken at < 1 hour after application (HAA) were in the range 3.63 to 11.97 mg/kg. At 8 HAA they were in the range 2.71 to 11.41 mg/kg, at 24 HAA in the range 2.28 to 11.02 mg/kg, at 32 HAA in the range 2.06 to 9.02 mg/kg, at 48 HAA they were in the range 1.78 to 7.14 mg/kg, at 3 days after application (DAA) they were in the range 0.22 – 6.06, 5 DAA in the range 0.08 – 4.37 and 7 DAA 0.05 – 1.70 mg/kg.

Results of analysis are given below

Time	Mesotrione residue (mg/kg)										
	001-037HR	SRUK18-002-037HR	SRHU18-053-037HR	SRHU18-054-037HR	SRFR18-010-037HR	SRFR18-011-037HR	SRDE18-001-037HR	SRDE18-002-037HR	SRPL18-014-037HR	SRPL18-015-037HR	G006-18H
0	6.10	3.63	11.97	11.69	11.51	8.75	4.46	9.11	6.15	6.50	8.58
8 HAA	6.03	4.20	11.41	8.99	8.78	9.98	5.66	2.71	4.34	4.58	8.48
24 HAA	4.58	3.39	11.02	8.76	9.86	8.73	4.59	2.59	2.28	4.72	8.17
32 HAA	2.69	4.09	9.02	8.80	6.72	4.77	3.98	3.29	2.06	6.37	5.65
48 HAA	2.73	2.61	7.14	6.89	5.47	4.66	4.21	2.54	1.78	5.78	5.54
72 HAA	1.95	2.17	6.06	5.51	2.00	4.77	0.22	2.61	3.82	1.8	3.26
96 HAA	0.58	2.76	0.14	0.12	0.58	4.37	0.08	0.43	1.67	1.66	3.43
168 HAA	0.27	0.19	0.11	0.07	0.09	0.70	0.09	0.05	1.23	0.27	1.70
0 DBA	<0.01										

DBA: days before application, HAA: hours after application
No correction of results for either control residues or recovery values has been performed

The half life calculations have been done using Cake v 3.4. Below the calculated DT_{50} and DT_{90} for the trials.

Trial	DT_{50} (h)	DT_{90} (h)	χ^2 (%)	MODEL
SRUK18-001-037HR	35.9	119	11.4	SFO
SRUK18-002-037HR	85.8	285	16	
SRHU18-053-037HR	47.7	159	17	
SRHU18-054-037HR	48.3	160	17.8	
SRFR18-010-037HR	37.1	123	16.7	
SRFR18-011-037HR	61.7	205	15.8	
SRDE18-001-037HR	42.5	141	29.2	
SRDE18-002-037HR*	25.3	83.9	40.1	Unreliable
SRPL18-014-037HR	57.6	192	29.7	SFO
SRPL18-015-037HR	63.2	210	23.8	
G006-18H	63.6	211	8.94	
Geometric mean	**52.53 (2.19 d)**	-	-	-

***Not used for Geomean calculations**

In the next tables and figures are given the data and the summary of the graphics used for half life modelling. The modelling has been done without any improvement, using the data as such (Detailed Cake v3.4 reports will be sent separately).

Table 1:Data used for modelling

Time	Mesotrione residue (mg/kg)										
	SRUK18-001-037HR	SRUK18-002-037HR	SRHU18-053-037HR	SRHU18-054-037HR	SRFR18-010-037HR	SRFR18-011-037HR	SRDE18-001-037HR	SRDE18-002-037HR	SRPL18-014-037HR	SRPL18-015-037HR	G006-18H
0	6.10	3.63	11.97	11.69	11.51	8.75	4.46	9.11	6.15	6.50	8.58
8 HAA	6.03	4.20	11.41	8.99	8.78	9.98	5.66	2.71	4.34	4.58	8.48
24 HAA	4.58	3.39	11.02	8.76	9.86	8.73	4.59	2.59	2.28	4.72	8.17
32 HAA	2.69	4.09	9.02	8.80	6.72	4.77	3.98	3.29	2.06	6.37	5.65
48 HAA	2.73	2.61	7.14	6.89	5.47	4.66	4.21	2.54	1.78	5.78	5.54
72 HAA	1.95	2.17	6.06	5.51	2.00	4.77	0.22	2.61	3.82	1.8	3.26
96 HAA	0.58	2.76	0.14	0.12	0.58	4.37	0.08	0.43	1.67	1.66	3.43
168 HAA	0.27	0.19	0.11	0.07	0.09	0.70	0.09	0.05	1.23	0.27	1.70

Trial SRUK18-001-037HR

Estimated Values:

Parameter	Value	s	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	6.48	0.4018	N/A	5.7	7.261	5.497	7.464
k_Parent	0.01933	0.002596	1.51E-004	0.01429	0.02438	0.01298	0.026

Sum of Squared Residuals: 1.601

χ^2

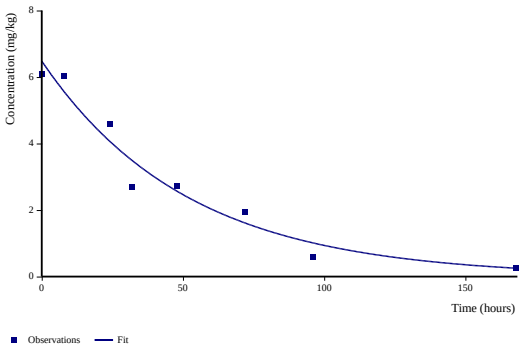
Parameter	Error %	Degrees of Freedom
All data	11.4	6
Parent	11.4	6

Decay Times:

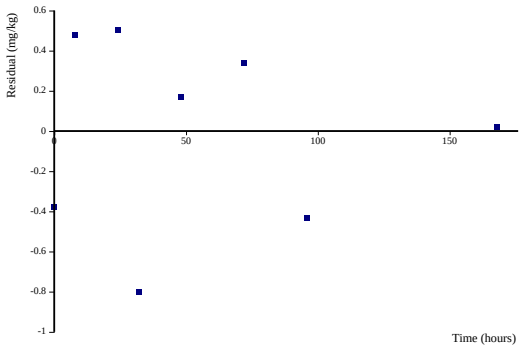
Compartment	DT50 (hours)	DT90 (hours)
Parent	35.9	119

Graphical Summary:

Observations and Fitted Model:



Residuals:



Trial SRUK18-002-037HR

Estimated Values:

Parameter	Value	s	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	4.239	0.4357	N/A	3.392	5.085	3.172	5.305
k_Parent	0.008079	0.002424	0.007878	0.003368	0.01279	0.002147	0.014

Sum of Squared Residuals: 2.675

χ^2

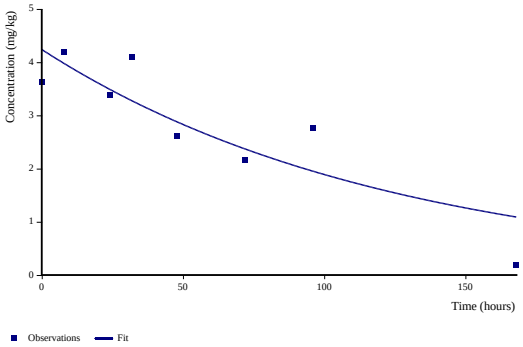
Parameter	Error %	Degrees of Freedom
All data	16	6
Parent	16	6

Decay Times:

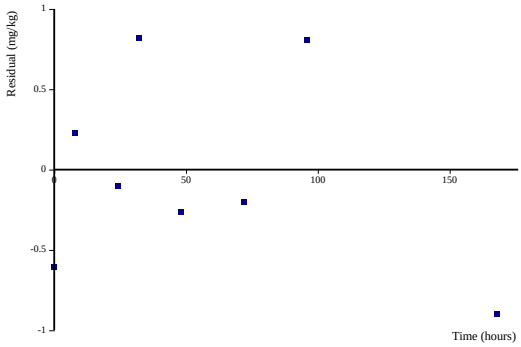
Compartment	DT50 (hours)	DT90 (hours)
Parent	85.8	285

Graphical Summary:

Observations and Fitted Model:



Residuals:



Trial SRHU18-053-037HR

Estimated Values:

Parameter	Value	s	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	13.21	1.282	N/A	10.72	15.7	10.08	16.35
k_Parent	0.01453	0.00328	0.002215	0.008151	0.0209	0.006499	0.023

Sum of Squared Residuals: 18.31

χ^2

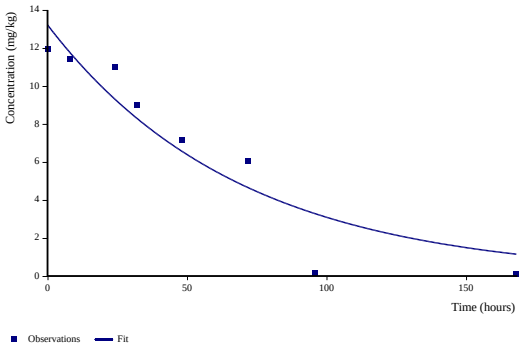
Parameter	Error %	Degrees of Freedom
All data	17	6
Parent	17	6

Decay Times:

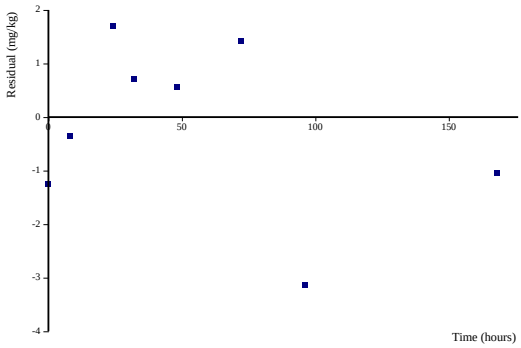
Compartment	DT50 (hours)	DT90 (hours)
Parent	47.7	159

Graphical Summary:

Observations and Fitted Model:



Residuals:



Trial SRHU18-054-037HR

Estimated Values:

Parameter	Value	s	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	11.74	1.199	N/A	9.41	14.07	8.806	14.67
k_Parent	0.01437	0.003426	0.002865	0.007707	0.02102	0.005982	0.023

Sum of Squared Residuals: 16.09

χ^2

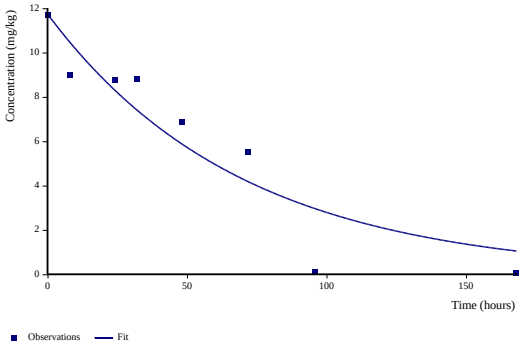
Parameter	Error %	Degrees of Freedom
All data	17.8	6
Parent	17.8	6

Decay Times:

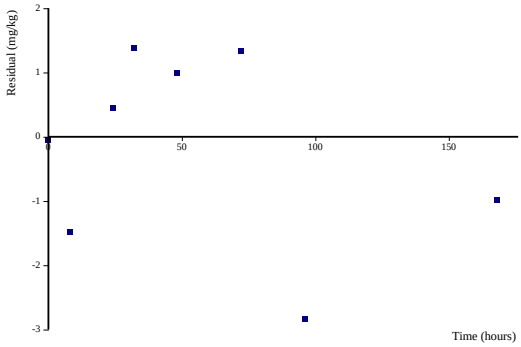
Compartment	DT50 (hours)	DT90 (hours)
Parent	48.3	160

Graphical Summary:

Observations and Fitted Model:



Residuals:



Trial SRFR18-010-037HR

Estimated Values:

Parameter	Value	s	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	11.76	1.052	N/A	9.713	13.8	9.183	14.33
k_Parent	0.01871	0.003649	0.001083	0.01161	0.0258	0.009776	0.028

Sum of Squared Residuals: 11.12

χ^2

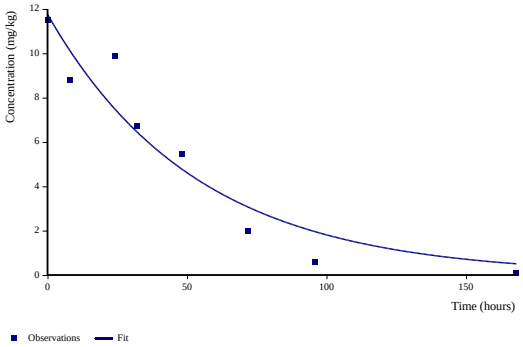
Parameter	Error %	Degrees of Freedom
All data	16.7	6
Parent	16.7	6

Decay Times:

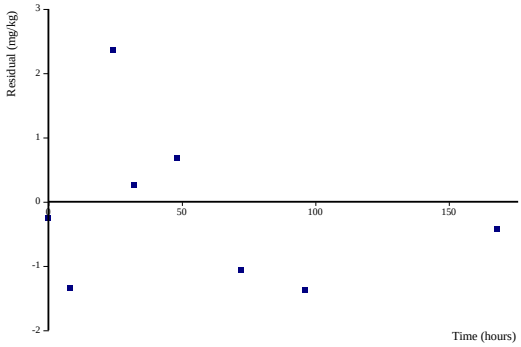
Compartment	DT50 (hours)	DT90 (hours)
Parent	37.1	123

Graphical Summary:

Observations and Fitted Model:



Residuals:



Trial SRFR18-011-037HR

Estimated Values:

Parameter	Value	s	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	9.575	0.9324	N/A	7.763	11.39	7.293	11.86
k_Parent	0.01124	0.002775	0.003366	0.005845	0.01663	0.004447	0.018

Sum of Squared Residuals: 10.77

χ^2

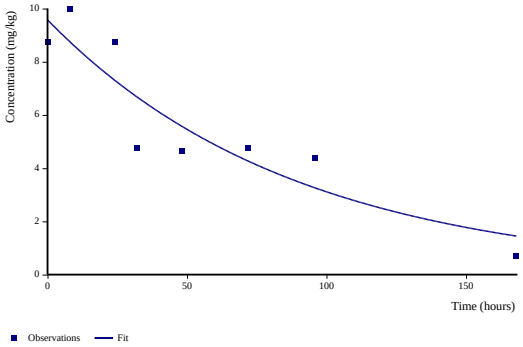
Parameter	Error %	Degrees of Freedom
All data	15.8	6
Parent	15.8	6

Decay Times:

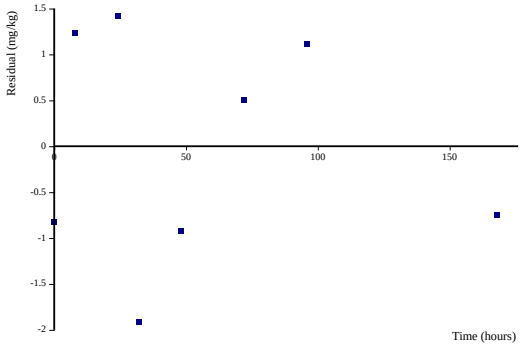
Compartment	DT50 (hours)	DT90 (hours)
Parent	61.7	205

Graphical Summary:

Observations and Fitted Model:



Residuals:



Trial SRDE18-001-037HR

Estimated Values:

Parameter	Value	s	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	5.765	0.9263	N/A	3.965	7.565	3.499	8.032
k_Parent	0.0163	0.005902	0.01641	0.004826	0.02776	0.001853	0.031

Sum of Squared Residuals: 9.118

χ^2

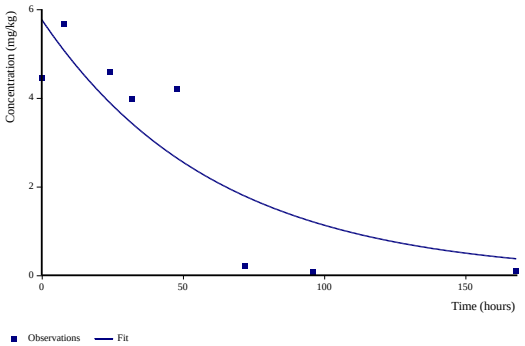
Parameter	Error %	Degrees of Freedom
All data	29.2	6
Parent	29.2	6

Decay Times:

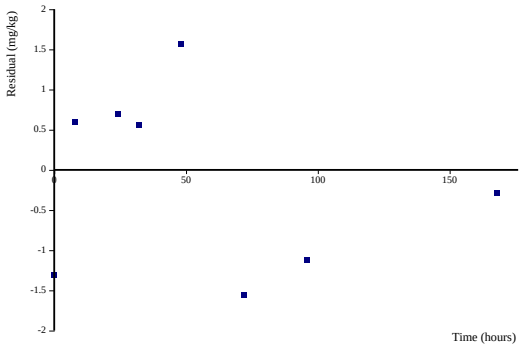
Compartment	DT50 (hours)	DT90 (hours)
Parent	42.5	141

Graphical Summary:

Observations and Fitted Model:



Residuals:



Trial SRDE18-002-037HR

Estimated Values:

Parameter	Value	s	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	6.907	1.409	N/A	4.169	9.644	3.459	10.35
k_Parent	0.02744	0.01149	0.02707	0.005114	0.04976	-0.0006724	0.056

Sum of Squared Residuals: 17.25

χ^2

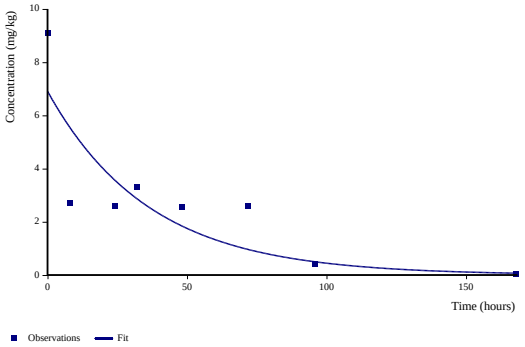
Parameter	Error %	Degrees of Freedom
All data	40.1	6
Parent	40.1	6

Decay Times:

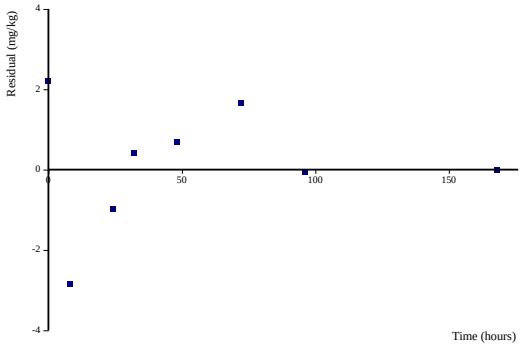
Compartment	DT50 (hours)	DT90 (hours)
Parent	25.3	83.9

Graphical Summary:

Observations and Fitted Model:



Residuals:



Trial SRPL18-014-037HR

Estimated Values:

Parameter	Value	s	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	4.77	0.8862	N/A	3.048	6.492	2.601	6.938
k_Parent	0.01202	0.005528	0.03627	0.001282	0.02277	-0.001502	0.026

Sum of Squared Residuals: 9.466

χ^2

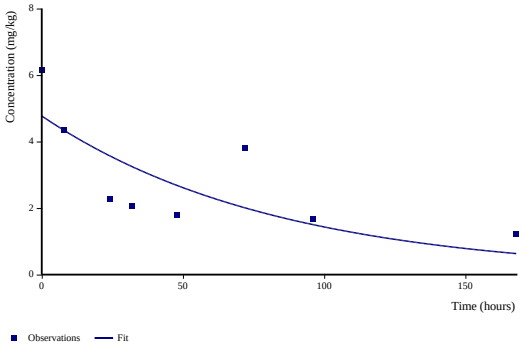
Parameter	Error %	Degrees of Freedom
All data	29.7	6
Parent	29.7	6

Decay Times:

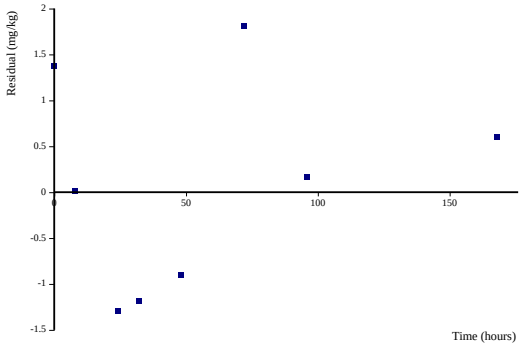
Compartment	DT50 (hours)	DT90 (hours)
Parent	57.6	192

Graphical Summary:

Observations and Fitted Model:



Residuals:



Trial SRPL18-015-037HR

Estimated Values:

Parameter	Value	s	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	6.522	0.9466	N/A	4.683	8.361	4.206	8.838
k_Parent	0.01096	0.004072	0.01798	0.003048	0.01887	9.97E-004	0.021

Sum of Squared Residuals: 11.22

χ^2

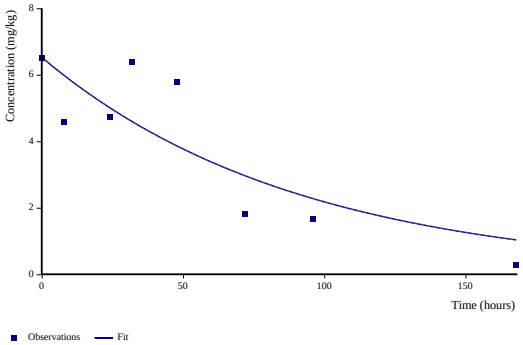
Parameter	Error %	Degrees of Freedom
All data	23.8	6
Parent	23.8	6

Decay Times:

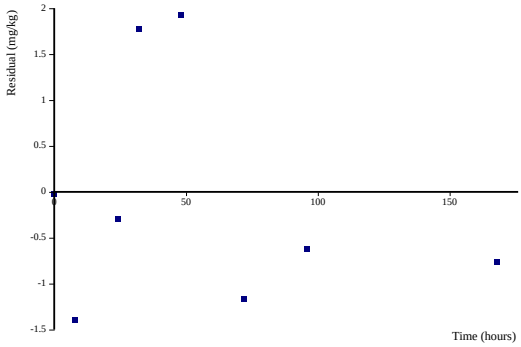
Compartment	DT50 (hours)	DT90 (hours)
Parent	63.2	210

Graphical Summary:

Observations and Fitted Model:



Residuals:



Trial G006-18H

Estimated Values:

Parameter	Value	s	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	9.035	0.5014	N/A	8.061	10.01	7.808	10.26
k_Parent	0.0109	0.001552	2.08E-004	0.007885	0.01392	0.007103	0.015

Sum of Squared Residuals: 3.154

χ^2

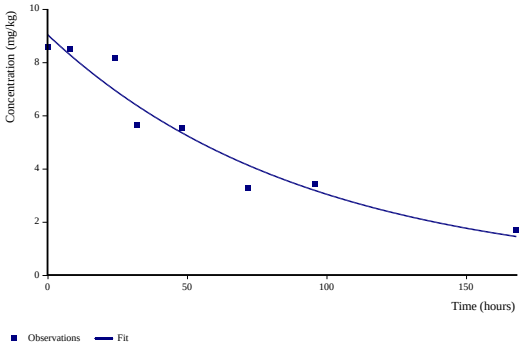
Parameter	Error %	Degrees of Freedom
All data	8.94	6
Parent	8.94	6

Decay Times:

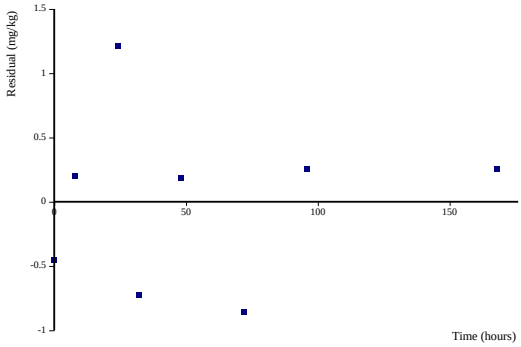
Compartment	DT50 (hours)	DT90 (hours)
Parent	63.6	211

Graphical Summary:

Observations and Fitted Model:



Residuals:



zRMS evaluation and conclusion:

Trial	DT_{50} [days]	DT_{90} [days]	Remarks
SRUK18-001-037HR	1.49	4.96	Acceptable, good visual fit
SRUK18-002-037HR	3.57	11.9	Acceptable, acceptable visual fit,
SRHU18-053-037HR	1.99	6.61	Acceptable, acceptable visual fit
SRHU18-054-037HR	2.01	6.68	Acceptable, acceptable visual fit
SRFR18-010-037HR	1.55	5.13	Acceptable, good visual fit,
SRFR18-011-037HR	2.57	8.54	Acceptable but poor visual fit,
SRDE18-001-037HR	1.77	5.89	Acceptable, but poor visual fit,
SRDE18-002-037HR	1.06	3.51	Unacceptable, unacceptable visual fit, Chi^2 >40%,
SRPL18-014-037HR	2.41	7.99	Acceptable but poor visual fit,
SRPL18-015-037-HR	2.64	8.75	Acceptable but poor visual fit,
G006-18H	2.65	8.8	Acceptable, good visual fit
Geometric mean	**2.19**	-	**Results of trial SRDE18-002-037HR excluded from the calculation**

Comments of zRMS:	The study has been already evaluated at the EU level and re-evaluation at the zonal level was deemed not necessary. For the study summary and respective evaluation, please refer to mesotrione RAR of 2015. **Agreed endpoints:** **PT=0.139 (wood mouse)**

Reference:	KCP 10.1.2.2/09
Report	xxx (2013) Generic field study on small mammals - focal species and wood mouse (*Apodemus sylvaticus*) PT in maize fields in Germany. Rifcon GmbH. Oxon unpublished Report No.: R12225. Syngenta File Number NA_13410 (Data owner: Oxon Italia, S.p.A., Syngenta access)
Guideline(s):	No guidelines available, but following recommendations in the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009) and its appendices
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Duplication (if vertebrate study)	No

Comments of zRMS:	The study has been already evaluated at the EU level and re-evaluation at the zonal level was deemed not necessary. For the study summary and respective evaluation, please refer to mesotrione RAR of 2015.

Reference:	KCP 10.1.2.2/08
Report	Wolf C (2005) Generic field monitoring of birds and mammals on maize and beet fields in Austria. Bayer CropScience AG. Unpublished Report No: WFC/FS 017, 20 January 2005. Study dates: 19 March 2004 – 14 December 2004. BCS reference: MO-05-001258 (Owner: Bayer Crop Science, Syngenta have access Syngenta file No:. N/1155)
Guideline(s):	No guidelines available, but following recommendations in the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009) and its appendices
Deviations:	No
GLP:	Yes
Acceptability:	Already evaluated at the EU level
Duplication (if vertebrate study)	No

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

A 2.2 KCP 10.2 Effects on aquatic organisms

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

A 2.2.1.1.1 Study 1

Comments of zRMS:	The study is considered valid. All validity criteria were met. • the mortality in the control was 0% at exposure termination (should not exceed 10% or 1 fish if less than 10 fish are used) • the dissolved oxygen concentrations were within the range of 93 – 100% of air saturation value (obligatory above 60% of air saturation value) **Agreed endpoints:** LC_{50}/96h >100 mg product/L correspond to: LC_{50}/96h for rimsulfuron >3.0 mg/L, LC_{50}/96h for nicosulfuron >12.0 mg/L LC_{50}/96h for mesotrione >36.0 mg/L.

Reference:	KCP 10.2.1-01
Report	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG Rainbow trout

	Acute toxicity test, xxx, 2018, report No. W/204/17
Guideline(s):	Yes, OECD guideline No. 203
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

The aim of the study is to demonstrate that the test item concentration causing 50% mortality of rainbow trout (*Oncorhynchus mykiss*), i.e. LC_{50} value after 96 h of exposure, is higher than 100 mg/L (limit test).

Test item:

The test item, Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG, I a light brown powder. Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WDG; batch number: SCL-58176; rimsulfuron content: 30 g/Kg; nicosulfuron content: 120 g/Kg; mesotrione content: 360 g/Kg; density: 512 kg/m3; manufacturing ,date: 15 March 2018, expiry date: 14 March 2020.

Test organism:
Test organisms is rainbow trout (*Oncorhynchus mykiss*) originating from 'The culture of the Salmonidae fish in Zawoja', Poland. Rainbow trout (Oncorhynchus mykiss Walb.), age: approximately 4.5 months, average weight: 0.89 g ± 0.08 g, average body length:4.43 cm ± 0.21 cm.

Preliminary non-GLP test:
The test was performed as a static test. The time of exposure was 96 h. Three test item concentrations of 100, 10 and 1.0 mg/L and a control were used. The test medium was water. The test was conducted at a daily cycle of 16 h day and 8 h night. The temperature was in the range of 14.7-15.1°C, pH of the control: 7.71 – 7.89; dissolved oxygen concentration in the test item concentration and the control: 93 – 100% ASV.

Definitive test:
The definitive test will be conducted in a single test item concentration: 100 mg/L plus control in a static design. The amount of the test item will be weighted and mixed with the conditioned water in a glass aquarium of a total volume 10 L to result in the test item concentration for exposure.

Chemical determinations:
The concentrations of rimsulfuron, nicosulfuron and mesotrione were chemically determined using a validated high performance liquid chromatographic method with DAD detection [SOP/C/499]. The validated analytical method was performed according to SANCO/3029/99 rev.4 [6].
The concentrations of rimsulfuron, nicosulfuron and mesotrione were chemically determined in samples of the test item concentration and the control collected at exposure initiation and at exposure termination.
Sample preparation for the chemical determinations.
Each sample in a volume between 10 and 100 mL (i.e. control sample, test sample, sample fortified with standards) was acidified by hydrochloric acid and applied to column (ENVI-18 3 mL, 500 mg), which was previously conditioned. The conditioning of the column was by sequential washing twice with 5 mL of methanol, twice with 5 mL of deionized water, pH ≤ 2 (acidified HCl). Following the sample introduction, the column was dried for 5 minutes by vacuum. The part of sample with affinity to the column was eluted with 15 mL of methanol. Eluate was evaporated to dryness using vacuum rotary evaporator. The dry residue was dissolved in mixture of acetonitrile : 0.05% ortho-phosphoric

acid (30:70, v/v) and 15 µL was applied to chromatographic column.

Linearity:
The working solutions of rimsulfuron, nicosulfuron and mezotrione at the concentrations of 0.05, 0.1, 0.5, 1.0, 5.0, 10.0 and 20.0 µg/mL were injected successively to the chromatographic column and the chromatograms were recorded. The standard curve (peak area versus quantity of the standard) are linear with coefficient (r2) of 0.9998269 for rimsulfuron, 0.9995570 for nicosulfuron and 0.9995297 for mezotrione . The range of linearity of the analytical graphs is from 0.05 µg/mL to 20.0 µg/mL.
Specificity:
The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control water samples and fortified samples. Considering the results of the analysis, no signal of detected substance was overlapping with the matrix signal of the control samples under the experimental conditions. Therefore, the specificity of the method was demonstrated.

Extraction recovery level:
In order to study the recovery level, the solution of the detected substance was added to non-treated water samples and then analyzed using the method described above.
Precision:
Precision of a particular method is defined as its repeatability (RSD – relative standard deviation [%]). The repeatability for rimsulfuron, nicosulfuron and mesotrione analyzed in the test samples are provided.
Limit of Quantification and Detection:
The Limit of Quantification was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery is obtained (normally 70 – 110% with a relative standard deviation of preferably ≤ 20%).
The Limit of Detection was estimated as the lowest concentration of a detected substance that the analytical procedure can reliably differentiate from the background noise.
The Limit of Quantification (LoQ) for rimsulfuron, nicosulfuron and mesotrione analyzed in water is 0.001 mg/L and the Limit of Detection (LoD) is 0.0003mg/L.

Results and discussions

Definitive test:

Exposure time	**Number of dead fish**	**Number of alive fish**	**Total mortality of fish [%]**	**Symptom category**				
				Loss of balance	**Nontypical swimming**	**Respiratory problems**	**Pigmentation change**	
3 h	0	10	0	0	0	0	0	**Number of fish with symptoms**
				10	10	10	10	**Number of fish with-out symptoms**
6 h	0	10	0	0	0	0	0	**Number of fish with symptoms**
				10	10	10	10	**Number of fish with-out symptoms**
24 h	0	10	0	0	0	0	0	**Number of fish with symptoms**
				10	10	10	10	**Number of fish with-out symptoms**
48 h	0	10	0	0	0	0	0	**Number of fish with symptoms**

				10	10	10	10	**Number of fish without symptoms**
72 h	0	10	0	0	0	0	0	**Number of fish with symptoms**
				10	10	10	10	**Number of fish without symptoms**
96 h	0	10	0	0	0	0	0	**Number of fish with symptoms**
				10	10	10	10	**Number of fish without symptoms**

Analytical results:

At exposure initiation, the determined concentration of rimsulfuron was in the range 97.6% of the nominal concentration, the determined concentration of nicosulfuron was in the range 98.4% of the nominal concentration and the determined concentration of mesotrione was in the range 91.2% of the nominal concentration.
At exposure termination, the determined concentration of rimsulfuron was in the range 87.4% of the nominal concentration, the determined concentration of nicosulfuron was in the range 98.0% of the nominal concentration and the determined concentration of mesotrione was in the range 91.3% of the nominal concentration.
Therefore, the concentrations of rimsulfuron, nicosulfuron and mesotrione were stable under test conditions.

Conclusion

The endpoint values determined on the basis of the nominal test item concentration and mortality of fish: The LC_{50}/96h value is higher than 100 mg/L.
The endpoint values determined on the basis of the nominal concentration of Rimsulfuron and mortality fish: The LC_{50}/96h value is higher than 3.0 mg/L.
The endpoint values determined on the basis of the nominal concentration of Nicosulfuron and mortality fish: The LC_{50}/96h value is higher than 12.0 mg/L.
The endpoint values determined on the basis of the nominal concentration of Mesotrione and mortality fish: The LC_{50}/96h value is higher than 36.0 mg/L.

A 2.2.1.1.2 Study 2

Comments of zRMS:	The study is considered valid. All validity criteria were met. • The biomass in the control increased by a factor of 131.7 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 3.5% (criterion: it must not exceed 7%), • The mean coefficient of variation for the section-by-section growth rate in the control culture was 24.0% (criterion: it must not exceed 35%). **Agreed endpoints:** E_rC_{50} = 20.211 mg/L nom E_yC_{50} = 2.657 mg/L nom

Reference:	KCP 10.2.1-02
Report	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG *Raphidocelis subcapitata* (formely *Pseudokirchneriella subapitata*) SAG 61.81 Growth inhibition test, Pawel Bak, 2018, Report No. W/205/17
Guideline(s):	Yes, OECD guideline No. 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:
Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG, is light brown powder.

Test organism:
The freshwater unicellular green algae, *Raphidocelis subcapitata* (formely *Pseudokirchneriella subcapitata*) (Reinsch) Korshikov (SAG 61.81) formely known as *Selenastrum capricornutum* Prinz are obtained from a standard laboratory culture cultivated at the Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicology.

Preliminary test:
In the preliminary exposure test, the *Raphidocelis subcapitata* (formely *Pseudokirchneriella subcapitata*) was exposed to the following test item concentrations of 100, 10, 1.0, 0.1 and 0.01 mg/L plus the control for 72 h.

Definitive test:
The definitive test will be performed with test item concentrations of 100, 31.25, 9.77, 3.05, 0.95, 0.30, 0.093 mg/L plus the control (with a separation factor of 3.2).

Test medium:
Composition of AAP medium

Micronutrient stock solution		
Solution	**Ingredient**	**Concentration [mg/500mL]**
1	H_3BO_3	92.760
	$MnCl_2 . 4 H_2O$	207.69
	$FeCl_3 . 6 H_2O$	79.880
	$Na_2EDTA . 2 H_2O$	150.00
	$ZnCl_2$	1.635
	$CoCl_2 . 6 H_2O$	0.714
	$Na_2MoO_4 . 2 H_2O$	3.630
	$CuCl_2 . 2 H_2O$	0.006
Macronutrient stocks solutions		
Solution	**Ingredient**	**Concentration [g/100 mL]**
2	$NaNO_3$	2.55
3	$MgCl_2 . 6 H_2O$	1.216
4	$CaCl_2 . 2 H_2O$	0.441
5	$MgSO_4 . 7 H_2O$	1.46
6	K_2HPO_4	0.1044
7	$NaHCO_3$	1.05

Test conditions:
The definitive test will be conducted in an incubator, to maintain constant temperature and lighting conditions. The test vessels in the incubator will be arranged at random, mechanically shaken throughout exposure and rearranged daily. The temperature will be kept at the range of 21 - 24 °C constant within 2°C.

Results and discussions

Observations of algal cell morphology at exposure termination, definitive test

Nominal test item concentration [mg/L]	Observations
Control	Normal colour, shape and size of the algae cells
0.093	No changes
0.30	No changes
0.95	No changes
3.05	No changes
9.77	No changes
31.25	Irregular shape
100	Irregular shape

Inhibition of growth rate and yield, definitive test

Nominal test item concentration [mg/L]	% inhibition after 72h of exposure (growth rate)	% inhibition after 72h of exposure (yield)
Control	0.0	0.0
0.093	-0.1*	-0.2*
0.30	2.7	12.3
0.95	6.5	27.7
3.05	13.6	49.1
9.77	32.4	79.9
31.25	57.5	94.7
100	86.8	99.3

*: inhibition is lower than 0.0% which means that the algal cell density at exposure termination was higher than in the control

Analytical analysis:

At exposure initiation, the determined concentrations of rimsulfuron were in the range of 80.7 – 85.5% of the nominal concentration, the determined concentrations of nicosulfuron were in the range of 112.0–119.4% of the nominal concentration, the determined concentrations of mesotrione were in the range of 80.1 – 91.0% of the nominal concentration.
After 24, 48 and 72h of exposure, the determined concentrations of rimsulfuron were in the ranges of 22.7 – 72.3%, 28.7 – 73.7% and 17.2 – 73.2% of the nominal concentration, respectively.
After 24, 48 and 72h of exposure, the determined concentrations of mesotrione were in the ranges of 66.5 – 77.4%, 55.7 – 80.7% and 63.3 – 98.6% of the nominal concentration, respectively.
Therefore, the concentrations of rimsulfuron and mesotrione were not stable under test conditions during exposure.
After 24, 48 and 72h of exposure, the determined concentrations of nicosulfuron were in the ranges of 100.5 – 113.6%, 103.6 – 119.2% and 111.9 – 119.6% of the nominal concentration, respectively.
Therefore, the concentrations of nicosulfuron were stable under test conditions during exposure.
Concentrations of rimsulfuron, nicosulfuron and mesotrione in the control, were below LoD during each analysis. Since the concentrations of rimsulfuron and mesotrione were not stable under test conditions during exposure, the geometric mean of the determined concentrations of rimsulfuron and mesotrione were calculated.

The concentration of rimsulfuron, nicosulfuron and mesotrione durin the study.

Time of analysis	Nominal test item concentration [mg/L]	Average determined concentration of rimsulfuron [mg/L]	% of the nominal concentration	Average determined concentration of nicosulfuron [mg/L]	% of the nominal concentration	Average determined concentration of mesotrione [mg/L]	% of the nominal concentration
Exposure initiation	0.093	0.00231	82.5	0.013	118.2	0.0300	91.0
	0.3	0.00736	81.8	0.043	119.4	0.0908	84.1
	0.95	0.0235	81.0	0.133	116.7	0.274	80.1
	3.05	0.0787	85.5	0.437	119.4	0.887	80.6
	9.77	0.234	80.7	1.396	119.3	2.902	82.4
	31.25	0.780	83.0	4.462	119.0	9.424	83.8
	100	2.559	85.3	13.440	112.0	28.913	80.3
24 h of exposure	0.093	0.00102	36.4	0.0116	105.5	0.02198	66.6
	0.3	0.00265	29.4	0.037	102.8	0.0741	68.6
	0.95	0.0087	30.0	0.121	106.1	0.2536	74.2
	3.05	0.0209	22.7	0.368	100.5	0.732	66.5
	9.77	0.080	27.6	1.188	101.5	2.507	71.2
	31.25	0.680	72.3	4.259	113.6	8.705	77.4
	100	1.955	65.2	12.730	106.1	26.070	72.4
48 h of exposure	0.093	0.00111	39.6	0.0114	103.6	0.01839	55.7
	0.3	0.00258	28.7	0.038	105.6	0.06026	55.8
	0.95	0.0090	31.0	0.124	108.8	0.220	64.3
	3.05	0.0286	31.1	0.395	107.9	0.687	62.5
	9.77	0.090	31.0	1.243	106.2	2.238	63.6
	31.25	0.693	73.7	4.470	119.2	9.082	80.7
	100	2.078	69.3	13.400	111.7	27.194	75.5
Exposure termination	0.093	0.00104	37.1	0.0131	119.1	0,0209	63.3
	0.3	0.00199	22.1	0.042	116.7	0,0710	65.7
	0.95	0.0050	17.2	0.135	118.4	0,271	79.2
	3.05	0.0247	26.8	0.430	117.5	1,085	98.6
	9.77	0.074	25.5	1.337	114.3	2,945	83.7
	31.25	0.688	73.2	4.484	119.6	9,537	84.8
	100	2.061	68.7	13.430	111.9	28,608	79.5

· not calculated, LoQ = 0.001 mg/L, LoD = 0.0003 mg/L

Calculated geometric mean s of determined concentration of rimsulfuron and mesotrione.

Nominal test item concentration [mg/L]	Geometric mean of determinaed concentrations of rimsulfuron [mg/L]	Geometric mean of determinaed concentrations of mesotrione [mg/L]
0.093	0.0012	0.022
0.30	0.0030	0.071
0.95	0.0095	0.248
3.05	0.064	0.79
9.77	0.098	2.541
31.25	0.702	9.084
100	2.105	27.319

Conclusion

<u>The endpoint values determined based on the nominal test item concentrations:</u>
The concentration causing a 50% inhibition of the average specific **growth rate** of *Raphidocelis subcapitata*, i.e. the E_rC_{50}/72 h value is 20.211 mg/L (95% confidence interval: 18.799 – 21.745).
The concentration causing a 50% inhibition of **yield** of *Raphidocelis subcapitata*, i.e. the E_yC_{50}/72 h value is 2.657 mg/L (95% confidence interval: 2.205 – 3.201).

<u>The endpoint values determined based on the nominal concentrations of Rimsulfuron:</u>
The concentration causing a 50% inhibition of the average specific **growth rate** of *Raphidocelis subcapitata*, i.e. the E_rC_{50}/72 h value is 0.6052 mg/L (95% confidence interval: 0.5628 – 0.6511).
The concentration causing a 50% inhibition of **yield** of *Raphidocelis subcapitata*, i.e. the E_yC_{50}/72 h value is 0.0800 mg/L (95% confidence interval: 0.0655 – 0.0964).

<u>The endpoint values determined based on the nominal concentrations of Nicosulfuron:</u>
The concentration causing a 50% inhibition of the average specific **growth rate** of *Raphidocelis subcapitata*, i.e. the E_rC_{50}/72 h value is 2.426 mg/L (95% confidence interval: 2.257 – 2.610).
The concentration causing a 50% inhibition of **yield** of *Raphidocelis subcapitata*, i.e. the E_yC_{50}/72 h value is 0.317 mg/L (95% confidence interval: 0.263 – 0.382).

<u>The endpoint values determined based on the nominal concentrations of Mesotrione:</u>
The concentration causing a 50% inhibition of the average specific **growth rate** of *Raphidocelis subcapitata*, i.e. the E_rC_{50}/72 h value is 7.279 mg/L (95% confidence interval: 6.770 – 7.831).

The concentration causing a 50% inhibition of **yield** of *Raphidocelis subcapitata*, i.e. the E_yC_{50}/72 h value is 0.957 mg/L (95% confidence interval: 0.795 – 1.153).

The endpoint values determined based on the geometric mean of determined concentrations of Rimsulfuron:
The concentration causing a 50% inhibition of the average specific **growth rate** of *Raphidocelis subcapitata*, i.e. the E_rC_{50}/72 h value is 0.3554 mg/L (95% confidence interval: 0.3261 – 0.3877).
The concentration causing a 50% inhibition of **yield** of *Raphidocelis subcapitata*, i.e. the E_yC_{50}/72 h value is 0.0352 mg/L (95% confidence interval: 0.0284 – 0.0435).

The endpoint values determined based on the geometric mean of determined concentrations of Mesotrione:
The concentration causing a 50% inhibition of the average specific **growth rate** of *Raphidocelis subcapitata*, i.e. the E_rC_{50}/72 h value is 5.5226 mg/L (95% confidence interval: 5.1249 – 5.9547).
The concentration causing a 50% inhibition of **yield** of *Raphidocelis subcapitata*, i.e. the E_yC_{50}/72 h value is 0.6897 mg/L (95% confidence interval: 0.5702 – 0.8342).

Study 3

Comments of zRMS:	The study is considered valid. All validity criteria were met. • The immobilisation 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 8.4 – 9.0 mg/L (criterion: not less than 3 mg/L) **Agreed endpoints:** The endpoint values determined based on the nominal test item concentration: The EC_{50}/24 h and EC_{50}/48 h >100 mg/L. The endpoint values determined based on the nominal concentration of Rimsulfuron: The EC_{50}/24 h and EC_{50}/48 h >3.0 mg/L. The endpoint values determined based on the nominal concentration of Nicosulfuron: EC_{50}/24 h and EC_{50}/48 h value is higher than 12.0 mg/L. The endpoint values determined based on the nominal concentration of Mesotrione: The EC_{50}/24 h and EC_{50}/48 h >36.0 mg/L.

Reference:	KCP 10.2.1-03
Report	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG *Daphnia magna*, acute immobilisation test, Pawel Bak, 2018, report No. W/206/17
Guideline(s):	Yes, OECD guideline No. 202
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:
The test item Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% G is light rown powder; Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG; batch No.: SCL-19947, rimsulfuron content is 30 g/Kg (w/w), nicosulfuron content is 120 g/Kg (w/w), mesotrione content is 360 g/Kg (w/w); manufacturing date: 27 June 2016, expiry date: 26 June 2018.

Test organism:
The test organism *Daphnia magna* Straus originates from a standard culture maintained at the Institute of Industrial Organic Chemistry Branch Pszczyna, Department of Ecotoxicology. *Daphnia magna* Straus (< 24 h old at exposure initiation); not first brood progeny. Five individuals of *Daphnia magna* were introduced into each replicate.

Preliminary test:
In the preliminary exposure test, the *Daphnia magna* was exposed to the following test item concentrations of 100, 10, 1.0 mg/L plus the control for 48 hours in a semi-static test design.

Definitive test:
Based on the results of the preliminary tests, the definitive test will be performed with a single test item concentration of 100 mg/L plus the control as a limit test.

Test medium:
The test medium Elendt M7 will be used.
Combined microelements stock solution for Elendt M7 medium:

Combined microelement stock solution			**Volume of each ingredient in the combined microelement stock solution [mL/2L]**
Ingredient		**Concentration [g/100mL]**	
1	H_3BO_3	5.719	0.5
	$MnCl_2 . 4 H_2O$	0.721	0.5
	LiCl	0.612	0.5
	RbCl	0.142	0.5
	$SrCl_2 . 6 H_2O$	0.304	0.5
	NaBr	0.032	0.5
	$Na_2MoO_4 . 2 H_2O$	0.126	0.5
	$CuCl_2 . 2 H_2O$	0.0335	0.5
	Kl	0.0065	2.0
	$CoCl_2 . 6 H_2O$	0.020	2.0
	$ZnCl_2$	0.026	2.0
	Na_2SeO_3	0.00438	2.0
	NH_4VO_3	0.00115	2.0
2	$FeSO_4 . 7 H_2O$	0.1991	10.0
	$Na_2EDTA . 2 H_2O$	0.500	

Macroelements stock solution for Elendt M7 medium:

Macroelement stock solution			**Volume of each ingredient in the Elendt M7 medium [mL/10 L]**
Ingredient		**Concentration [g/100mL]**	
3	$NaNO_3$	0.274	1.0
4	$MgSO_4 . 7 H_2O$	24.660	5.0
5	$CaCl_2 . 2 H_2O$	29.380	10.0
6	KCl	5.800	1.0
7	$NaHCO_3$	6.480	10.0
8	K_2HPO_4	0.184	1.0
9	KH_2PO_4	0.143	1.0
10	$Na_2SiO_3 . 9 H_2O$	5.000	2.0

Test conditions:

During exposure, the *Daphnia magna* will no be fed. The test will be conducted with daily cycle 16 h ligh: 8 h dark. The temperature will be maintained in the range of 18-22°C. Temperature: 20.6 – 21.5oC; pH of the control: 7.62 – 7.75; dissolved oxygen concentration in the control: 8.4 – 8.8 mg/L; fluorescent light source; no feeding; no aeration; medium: Elendt M7.

Results and discussions

Nominal test item concen-tration [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

Analitical metods:
The concentrations of rimsulfuron, nicosulfuron and mesotrione were determined using a validated liquid chromatographic method with DAD detection. Samples of fresh test item concentration and the control collected at exposure initiation and at renewal, and spent test item concentration and the control collected at renewal and at exposure termination were chemically determined. In fresh samples, the determined concentrations of rimsulfuron were 85.2 and 84.1%, the determined concentrations of nicosulfuron were 115.5 and 114.1%, and the determined concentrations ofmesotrione were 82.8 and 88.3% of the nominal concentration, at exposure initiation and at renewal, respectively.
In spent samples, the determined concentrations of rimsulfuron were 80.2 and 80.5%, the determined concentrations of nicosulfuron were 114.0 and 113.3%, and the determined concentrations of mesotrione were 82.5 and 87.3% of the nominal concentration, at renewal and at exposure termination, respectively. Therefore, the concentrations of rimsulfuron, nicosulfuron and mesotrione were stable under test conditions.
The endpoint values were determined based on the nominal test item concentration, nominal concentrations of rimsulfuron, nominal concentrations of nicosulfuron and nominal concentrations of mesotrione.
The linearity of response of the analytical method, its specificity, precision, recovery, limit of quantification and detection were assessed in the process of the analytical method validation.
Linearity
The working solutions of rimsulfuron, nicosulfuron and mezotrione at the concentrations of 0.05, 0.1, 0.5, 1.0, 5.0, 10.0 and 20.0 □g/mL were injected successively to the chromatographic column and the chromatograms were recorded. The standard curve (peak area versus quantity of the standard) are linear with coefficient (r2) of 0.9998269 for rimsulfuron, 0.9995570 for nicosulfuron and 0.9995297 for mezotrione. The range of linearity of the analytical graphs range from 0.05 µg/mL to 20.0 µg/mL.
Specificity
The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control water samples and fortified samples. Considering the results of the analysis, no signal of rimsulfuron, nicosulfuron and mezotrione was overlapping with the matrix signal of the control samples under the experimental conditions. Therefore, the specificity of the method was demonstrated.
Extraction recovery level
In order to study the recovery level, the solutions of the standards were added to non-treated water samples and then analyzed using the method described above.
Precision
Precision of a particular method is defined as its repeatability (RSD – relative standard deviation [%]). The repeatability for rimsulfuron, nicosulfuron and mesotrione analyzed in the test samples are presented.

Limit of Quantification and Detection
The Limit of Quantification was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery is obtained (normally 70 – 110% with a relative standard deviation of preferably ≤ 20%).
The Limit of Detection was estimated as the lowest concentration of a detected substance that the analytical procedure can reliably differentiate from the background noise.
The Limit of Quantification (LOQ) for rimsulfuron, nicosulfuron and mesotrione analyzed in water is 0.01 mg/L and the Limit of Detection (LOD) is 0.0003 mg/L.

Conclusion

The endpoint values determined based on the nominal test item concentration:
The median concentrations causing 50% immobilisation of *Daphnia magna* after 24 and 48 h of exposure, i.e. the EC_{50}/24 h and EC_{50}/48 h value is higher than 100 mg/L.

The endpoint values determined based on the nominal concentration of Rimsulfuron:
The median concentrations causing 50% immobilisation of *Daphnia magna* after 24 and 48 h of exposure, i.e. the EC_{50}/24 h and EC_{50}/48 h value is higher than 3.0 mg/L.

The endpoint values determined based on the nominal concentration of Nicosulfuron:
The median concentrations causing 50% immobilisation of *Daphnia magna* after 24 and 48 h of exposure, i.e. the EC_{50}/24 h and EC_{50}/48 h value is higher than 12.0 mg/L.

The endpoint values determined based on the nominal concentration of Mesotrione:
The median concentrations causing 50% immobilisation of *Daphnia magna* after 24 and 48 h of exposure, i.e. the EC_{50}/24 h and EC_{50}/48 h value is higher than 36.0 mg/L.

A 2.2.1.1.3 Study 3

Reference:	KCP 10.2.1-03
Report	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG *Daphnia magna*, acute immobilisation test, Pawel Bak, 2018, report No. W/206/17
Guideline(s):	Yes, OECD guideline No. 202
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:
The test item Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% G is light rown powder;

Test organism:
The test organism *Daphnia magna* Straus originates from a standard culture maintained at the Institute of Industrial Organic Chemistry Branch Pszczyna, Department of Ecotoxicology.

Preliminary test:
In the preliminary exposure test, the *Daphnia magna* was exposed to the following test item concentrations of 100, 10, 1.0 mg/L plus the control for 48 hours in a semi-static test design.

Definitive test:
Based on the results of the preliminary tests, the definitive test will be performed with a single test item concentration of 100 mg/L plus the control as a limit test.

Test medium:
The test medium Elendt M7 will be used.
Combined microelements stock solution for Elendt M7 medium:

Combined microelement stock solution			**Volume of each ingredient in the combined microelement stock solution [mL/2L]**
Ingredient		**Concentration [g/100mL]**	
1	H_3BO_3	5.719	0.5
	$MnCl_2 . 4 H_2O$	0.721	0.5
	LiCl	0.612	0.5
	RbCl	0.142	0.5
	$SrCl_2 . 6 H_2O$	0.304	0.5
	NaBr	0.032	0.5
	$Na_2MoO_4 . 2 H_2O$	0.126	0.5
	$CuCl_2 . 2 H_2O$	0.0335	0.5
	KI	0.0065	2.0
	$CoCl_2 . 6 H_2O$	0.020	2.0
	$ZnCl_2$	0.026	2.0
	Na_2SeO_3	0.00438	2.0
	NH_4VO_3	0.00115	2.0
2	$FeSO_4 . 7 H_2O$	0.1991	10.0
	$Na_2EDTA . 2 H_2O$	0.500	

Macroelements stock solution for Elendt M7 medium:

Macroelement stock solution			**Volume of each ingredient in the Elendt M7 medium [mL/10 L]**
Ingredient		**Concentration [g/100mL]**	
3	$NaNO_3$	0.274	1.0
4	$MgSO_4 . 7 H_2O$	24.660	5.0
5	$CaCl_2 . 2 H_2O$	29.380	10.0
6	KCl	5.800	1.0
7	$NaHCO_3$	6.480	10.0
8	K_2HPO_4	0.184	1.0
9	KH_2PO_4	0.143	1.0
10	$Na_2SiO_3 . 9 H_2O$	5.000	2.0

Test conditions:
During exposure, the *Daphnia magna* will no be fed. The test will be conducted with daily cycle 16 h ligh: 8 h dark. The temperature will be maintained in the range of 18-22°C.

Results and discussions

Nominal test item concen-	**Number of**	**Number of immobilised *Daphnia magna***		**Total of immobilised *Daphnia magna* [%]**
		24 h	**48 h**	

tration [mg/L]	*Daphnia magna*	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

Conclusion

The endpoint values determined based on the nominal test item concentration:
The median concentrations causing 50% immobilisation of *Daphnia magna* after 24 and 48 h of exposure, i.e. the EC_{50}/24 h and EC_{50}/48 h value is higher than 100 mg/L.

The endpoint values determined based on the nominal concentration of Rimsulfuron:
The median concentrations causing 50% immobilisation of *Daphnia magna* after 24 and 48 h of exposure, i.e. the EC_{50}/24 h and EC_{50}/48 h value is higher than 3.0 mg/L.

The endpoint values determined based on the nominal concentration of Nicosulfuron:
The median concentrations causing 50% immobilisation of *Daphnia magna* after 24 and 48 h of exposure, i.e. the EC_{50}/24 h and EC_{50}/48 h value is higher than 12.0 mg/L.

The endpoint values determined based on the nominal concentration of Mesotrione:
The median concentrations causing 50% immobilisation of *Daphnia magna* after 24 and 48 h of exposure, i.e. the EC_{50}/24 h and EC_{50}/48 h value is higher than 36.0 mg/L.

Comments of zRMS:	The study is considered valid. All validity criteria were met. • The doubling time of frond number in the control was 2.5 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). **Agreed endpoints:** Frond number: E_rC_{50} = 0.0166 mg/L_{nom} E_yC_{50} = 0.0112 mg/L_{nom} Dry weight: E_rC_{50} = 10.9479 mg/L_{nom} E_yC_{50} = 0.0264 mg/L_{nom} The endpoint values determined based on the geometric mean measured test item concentrations: The median concentration causing 50% inhibition of the mean specific **growth rate** of *Lemna gibba* determined on the basis of the frond number E_rC_{50}/7 d value is 0.0149 mg/L. The median concentration causing 50% inhibition of **yield** of *Lemna gibba* determined on the basis of the frond number E_yC_{50}/7 d value is 0.0100 mg/L (95% confidence interval: 0.0103 – 0.0124). The median concentration causing 50% inhibition of the mean specific **growth rate** of *Lemna gibba* determined on the basis of the dry weight E_rC_{50}/7 d value is 9.796 mg/L. The median concentration causing 50% inhibition of **yield** of *Lemna gibba* determined on

	the basis of the dry weight E_yC_{50}/7 d value is 0.0236 mg/L (95% confidence interval: 0.0163 – 0.0407).

Reference:	KCP 10.2.1-04
Report	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG *Lemna gibba* CPCC 310, growth inhibition test, Pawel Bak, 2018, report No. W/207/17
Guideline(s):	Yes, OECD guideline No. 221
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:
The test item, Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG is light brown powder.

Test organism:
The duckweed *Lemna gibba* (Linné) CPCC 310 is cultivated in a standard laboratory culture at the Institute of Industrial Organic Chemistry Branch Pszczyna, Department of Ecotoxicology.

Preliminary exposure test:
In the preliminary exposure test, the duckweed *Lemna gibba* was exposed to the following test item concentrations of 100, 1.0, 0.01, 0.001 and 0.0001 mg/L plus the control for 7 days in a semi-static test design with daily renewals.

Definitive test:
The definitive test will be performed with test item concentrations of 100, 20, 4, 0.8, 0.16, 0.032, 0.0064n 0.0013 mg/L plus the control (with a separator factor of 5). Each test item concentration was split up into three replicates, whereas the control into six. Into each replicate 3 colonies containing 3 fronds per colony were introduced. Transparent lids were used to minimize evaporation and accidental contamination, allowing necessary air exchange. The test vessels were arranged at random and repositioned during renewals.

Test medium:
The 20X AAP medium was used.

Stock solution	Ingredient	Concentration in the stock solution [g/1L]
A1	$NaNO_3$	26
	$MgCl_2 . 6 H_2O$	12
	$CaCl_2 . 2 H_2O$	4.4
A2	$MgSO_4 . 7 H_2O$	15
A3	$K_2HPO_4 . 3 H_2O$	1.4
B	H_3BO_3	0.19
	$MnCl_2 . 4 H_2O$	0.42
	$FeCl_3 . 6 H_2O$	0.16
	$Na_2EDTA . 2 H_2O$	0.30
	$ZnCl_2$	0.0033

	$CoCl_2 . 6 H_2O$	0.0014
	$Na_2MoO_4 . 2 H_2O$	0.0073
	$CuCl_2 . 2 H_2O$	10.10^{-6}
C	$NaHCO_3$	15

Test conditions:

The test will be conducted under constant illumination using fluorescent light source (six 24 W light bulbs), twice during exposure (on day 3 and 5) and at exposure termination with 2π receptor lux-meter. and temperature conditions in an incubator. ~~The temperature will be maintained in the range of 22-26 °C.~~

The recorded temperature was in the range of 22.8 – 25.5°C. During exposure the measured mean light intensity was in the range of 8024 – 8442 lux.

In tests, the pH values were measured in fresh test item concentrations and the control before splitting up into replicates at exposure initiation and at each renewal. The pH values were also measured in spent test item concentrations and the control at each renewal and at exposure termination in pooled replicates. The pH values measured in fresh test item concentrations and the control were in the range of 7.28 –7.66 and in the range of 8.17 – 8.98 in spent test item concentrations and the control.

In order to quantify the test item related effects on vegetative growth over a period of 7 days, the number of fronds in each replicate was counted twice during exposure and at exposure termination. Only visibly distinct fronds were counted. The observations of plant development, i.e. size of fronds, necrosis, chlorosis, colony break-up, gibbosity, changes in the appearance of roots were performed at the same time. The observations of plant development in the test item concentrations were compared to that of the control.

The dry weight of the representative sample of the duckweed culture used as the inoculum was measured after exposure initiation. The dry weight of all plants from each test vessel was measured after exposure termination. All colonies (with roots) were transferred onto previously weighed microscopic slides and dried at approximately 60oC in a laboratory oven until constant weight.

The concentrations of rimsulfuron, nicosulfuron and mesotrione were chemically analysed using a validated chromatographic method with DAD detection.
Samples of fresh test item concentrations of 100, 20, 4.0, 0.8, 0.16 and 0.032 mg/L and the control collected at exposure initiation and at each renewal, and spent test item concentrations of 100, 20, 4.0, 0.8, 0.16 and 0.032 mg/L and the control collected at each renewal and at exposure termination were chemically analysed.

In fresh samples the determined concentration of rimsulfuron was in the range of 80.8 – 105.3% of nominal concentration, the determined concentration of nicosulfuron was in the range of 96.5 – 119.3% of nominal concentration and the determined concentration of mesotrione was in the range of 86.6 – 96.4% of nominal concentration.
In spent samples the determined concentrations of rimsulfuron were in the range of 16.8 – 56.1% of nominal concentration and the determined concentrations of mesotrione were in the range of 69.7 – 91.2% of nominal concentration.

Therefore, the concentrations of rimsulfuron and mesotrione were not stable under test conditions between renewals. In spent samples the determined concentrations of nicosulfuron were in the range of 96.1 – 119.8% of nominal concentration. Therefore, the concentrations of nicosulfuron were stable under test conditions between renewals.
If the concentrations of the detected substance are not in the range of 80 – 120% of the nominal concentration, the effect concentrations should be determined on the basis of the geometric mean of the measured concentrations of the detected substance.

Therefore, the geometric mean of determined concentrations of rimsulfuron and mesotrione were calculated.

Results and discussions

Continuation – frond number and dry weight, definitive test

Nominal test item concentration [mg/L]	**Frond number**			**Dry weight [mg]**
	Day 3	**Day 5**	**Day 7**	**Day 7**
100	9	9	9	4.9
	10	10	10	5.4
	9	9	9	5.3
Mean	**9.3**	**9.3**	**9.3**	**5.2**
Standard deviation	*0.58*	*0.58*	*0.58*	*0.26*
Inoculum	Day 0		9	1.4
			9	1.1
			9	2.0
Mean			**9.0**	**1.5**
Standard deviation			*0.00*	*0.46*

Inhibition of growth rate and yield, definitive test

Nominal test item concentration [mg/L]	**Based on frond number**		**Based on dry weight**	
	% inhibition at exposure termination (growth rate)	**% inhibition at exposure termination (yield)**	**% inhibition at exposure termination (growth rate)**	**% inhibition at exposure termination (yield)**
Control	0.0	0.0	0.0	0.0
0.0013	4.1	8.7	0.1	0.3
0.0064	11.0	22.4	17.5	39.7
0.032	80.2	92.2	38.0	67.5
0.16	85.4	94.6	45.2	74.3
0.8	91.4	97.0	49.3	77.7
4.0	96.4	98.8	45.4	74.3
20	94.8	98.2	49.1	77.5
100	98.2	99.4	50.5	78.7

Study deviations

1. In the definitive test, a spacing factor of 5 was applied. This exceeds the spacing factor of 3.2 recommended in the OECD 221 guideline. Considering that the validity criteria of the study comply with the guideline specifications and given that the spacing factor rule is stated as a recommendation, the applicant considers that this deviation do not affect the acceptability of the whole study.

2. As described in the study summary, the measured concentrations of rimsulfuron and mesotrione were (partly) below 80 % of the nominal concentration. Below is described the endpoint recalculation according to the EFSA Supporting publication 2019:EN-1673, Appendix J.

Expressing endpoints from Tier 1 tests and formulation tests (with one or more active substances) for unstable substances - Procedure for formulation tests with more than one active substance (according to "Outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology" [EFSA Supporting publication 2019:EN-1673].

Case 1: All active substances have been analytically measured
Option B (faster to calculate but associated with more uncertainties) is carried out as follows:

(1) For each active substance, calculate the geometric mean concentration between the start and end of the test for each tested concentration level; calculate the recovery rates at each tested concentration (geomean compared with nominal or initial measured).

Rimsulfuron calculations

[product]	0.032	0.16	0.8	4	20	100
[a.s. nom.]	0.00096	0.0048	0.024	0.12	0.6	3
start	0.001	0.00454	0.02177	0.1072	0.535	2.658
day 1 spent	0.001	0.00182	0.0083	0.0622	0.327	1.596
day 1 fresh	0.001	0.00405	0.0196	0.1055	0.528	2.638
day 2 spent	0.001	0.00255	0.01169	0.0673	0.335	1.666
day 2 fresh	0.001041	0.00512	0.02383	0.1264	0.6278	3.138
day 3 spent	0.001	0.00176	0.00758	0.0385	0.19	0.929
day 3 fresh	0.001	0.00441	0.02072	0.1052	0.52	2.606
day 4 spent	0.0003	0.0003	0.00534	0.0389	0.126	0.616
day 4 fresh	0.001	0.00414	0.02015	0.1041	0.52	2.591
day 5 spent	0.0003	0.0003	0.00567	0.0202	0.101	0.502
day 5 fresh	0.001	0.00404	0.02054	0.1054	0.522	2.613
day 6 spent	0.0003	0.0003	0.00584	0.0278	0.131	0.657
day 6 fresh	0.001	0.00418	0.02179	0.1115	0.558	2.847
day 7 spent	0.0003	0.0003	0.00748	0.0301	0.125	0.635
Calc. Geomean	0.001	0.002	0.012	0.064	0.305	1.520
Recovery rates	**52.083**	**39.583**	**50.000**	**54.167**	**51.667**	**52.000**
Geom. Report	0.0005	0.0019	0.012	0.065	0.31	1.56

Mesotrione calculations

[product]	0.032	0.16	0.8	4	20	100
[a.s. nom.]	0.012	0.058	0.29	1.4	7.2	36
start	0.01086	0.054	0.263	1.306	6.584	33.094
day 1 spent	0.00893	0.0446	0.218	1.067	5.32	26.749
day 1 fresh	0.0111	0.055	0.265	1.307	6.535	32.654
day 2 spent	0.00969	0.0486	0.234	1.175	5.858	29.247
day 2 fresh	0.01039	0.0521	0.252	1.259	6.304	31.482
day 3 spent	0.00924	0.0463	0.222	1.169	5.851	29.253
day 3 fresh	0.0104	0.0527	0.258	1.265	6.304	31.506
day 4 spent	0.01056	0.0516	0.256	1.277	5.975	29.836
day 4 fresh	0.01082	0.054	0.265	1.311	6.554	32.753
day 5 spent	0.01038	0.0519	0.256	1.003	5.02	25.515
day 5 fresh	0.01079	0.054	0.263	1.276	6.38	31.936
day 6 spent	0.01059	0.0529	0.26	1.252	6.219	31.016
day 6 fresh	0.01103	0.0559	0.267	1.3	6.493	32.444
day 7 spent	0.0106	0.0528	0.26	1.254	6.265	31.242
Calc. Geomean	0.010	0.052	0.252	1.226	6.101	30.540
Recovery rates	**83.333**	**89.655**	**86.207**	**85.714**	**84.722**	**85.000**

Geom. Report	0.01	0.052	0.25	1.2	6.1	30.6

Nicosulfuron calculations

[product]	0.032	0.16	0.8	4	20	100
[a.s. nom.]	0.0038	0.019	0.096	0.48	2.4	12
start	0.00432	0.0216	0.1043	0.516	2.58	12.91
day 1 spent	0.00388	0.0188	0.0923	0.472	2.477	12.38
day 1 fresh	0.00427	0.0213	0.1032	0.517	2.581	12.89
day 2 spent	0.00421	0.021	0.1025	0.535	2.683	13.39
day 2 fresh	0.00394	0.0196	0.0961	0.497	2.492	12.47
day 3 spent	0.00455	0.0227	0.1075	0.548	2.739	13.69
day 3 fresh	0.00469	0.0222	0.1145	0.56	2.811	14.11
day 4 spent	0.00452	0.0199	0.1091	0.57	2.854	14.32
day 4 fresh	0.00418	0.0209	0.101	0.519	2.595	12.98
day 5 spent	0.00479	0.0225	0.113	0.503	2.518	12.58
day 5 fresh	0.00399	0.0198	0.114	0.504	2.521	12.61
day 6 spent	0.0046	0.0225	0.1102	0.575	2.875	14.17
day 6 fresh	0.00386	0.0193	0.1022	0.506	2.523	12.63
day 7 spent	0.0046	0.0218	0.113	0.575	2.872	13.87
Calc. Geomean	0.004	0.021	0.106	0.527	2.648	13.198
Recovery rates	**113.249**	**110.287**	**110.115**	**109.881**	**110.316**	**109.980**

(2) For each active substance, calculate the mean recovery rate and standard deviation, by considering the recovery rates for each concentration level as in (1).

Rimsulfuron

[Nom.]	Geomean	Recov (%)
0.00096	0.0005	52.08
0.0048	0.0019	39.58
0.024	0.012	50.00
0.12	0.065	54.17
0.6	0.31	51.67
3	1.56	52.00
	mean	**49.92**
	SD	5.23

Mesotrione

[Nom.]	Geomean	Recov (%)
0.012	0.01	83.33
0.058	0.052	89.66
0.29	0.25	86.21
1.4	1.2	85.71
7.2	6.1	84.72
36	30.6	85.00
	mean	**85.77**
	SD	2.14

Nicosulfuron

[Nom.]	Geomean	Recov (%)
0.0038	0.004	113.25
0.019	0.021	110.29
0.096	0.106	110.12
0.48	0.527	109.88
2.4	2.648	110.32
12	13.198	109.98
	mean	**110.64**
	SD	1.29

(3) For each active substance, recalculate the mean measured concentration, based on the mean recovery rate.
(4) Sum up the new calculated concentration levels for the active substance to derive the mean 'sum of active substance' concentration levels.

Rimsulfuron		**Mesotrione**		**Nicosulfuron**		**Product**		
Nominal	Recovered	Nominal	Recovered	Nominal	Recovered	Nominal	Recalc.	Factor
0.00096	0.0005	0.012	0.010	0.0038	0.004	0.01676	0.015	
0.0048	0.002	0.058	0.050	0.019	0.021	0.0818	0.073	
0.024	0.012	0.29	0.249	0.096	0.106	0.41	0.367	
0.12	0.060	1.4	1.201	0.48	0.531	2	1.792	
0.6	0.300	7.2	6.175	2.4	2.655	10.2	9.130	
3	1.498	36	30.877	12	13.277	51	45.652	0.8948

(5) Recalculate the endpoint based on the recovery rates of the 'sum of active substances'.

	Frond number		Dry weight	
Endpoint recalculation	ErC50	EyC50	ErC50	EyC50
Product endpoint	0.0166 mg/L	0.0112 mg/L	10.9479 mg/L	0.0264 mg/L
Factor	0.8948	0.8948	0.8948	0.8948
Corrected endpoint	**0.0149 mg/L**	**0.0100 mg/L**	**9.796 mg/L**	**0.0236 mg/L**

Conclusion

The endpoint values determined based on the nominal test item concentrations:
The median concentration causing 50% inhibition of the mean specific **growth rate** of *Lemna gibba* determined on the basis of the frond number E_rC_{50}/7 d value is 0.0166 mg/L (95% confidence interval: 0.0155 – 0.0177). The median concentration causing 50% inhibition of **yield** of *Lemna gibba* determined on the basis of the frond number E_yC_{50}/7 d value is 0.0112 mg/L (95% confidence interval: 0.0103 – 0.0124).
The median concentration causing 50% inhibition of the mean specific **growth rate** of *Lemna gibba* determined on the basis of the dry weight E_rC_{50}/7 d value is 10.9479 mg/L (95% confidence interval: 6.6135 – 19.6794). The median concentration causing 50% inhibition of **yield** of *Lemna gibba* determined on the basis of the dry weight E_yC_{50}/7 d value is 0.0264 mg/L (95% confidence interval: 0.0163 – 0.0407).

The endpoint values determined based on the geometric mean measured test item concentrations:
The median concentration causing 50% inhibition of the mean specific **growth rate** of *Lemna gibba* determined on the basis of the frond number E_rC_{50}/7 d value is 0.0149 mg/L. The median concentration causing 50% inhibition of **yield** of *Lemna gibba* determined on the basis of the frond number E_yC_{50}/7 d value is 0.0100 mg/L (95% confidence interval: 0.0103 – 0.0124).
The median concentration causing 50% inhibition of the mean specific **growth rate** of *Lemna gibba* determined on the basis of the dry weight E_rC_{50}/7 d value is 9.796 mg/L. The median concentration causing 50% inhibition of **yield** of *Lemna gibba* determined on the basis of the dry weight E_yC_{50}/7 d value is 0.0236 mg/L (95% confidence interval: 0.0163 – 0.0407).

The endpoint values determined based on the nominal concentrations of Rimsulfuron:
The median concentration causing 50% inhibition of the mean specific **growth rate** of *Lemna gibba* determined on the basis of the frond number E_rC_{50}/7 d value is 0.000496 mg/L (95% confidence interval: 0.000464 – 0.000529). The median concentration causing 50% inhibition of **yield** of *Lemna gibba* determined after 7 days on the basis of the frond number E_yC_{50}/7 d value is 0.000334 mg/L (95% confidence interval: 0.000306 – 0.000369).
The median concentration causing 50% inhibition of the mean specific **growth rate** of *Lemna gibba* de-

termined on the basis of the dry weight E_rC_{50}/7 d value is 0.327820 mg/L (95% confidence interval: 0.197994 – 0.589349). The median concentration causing 50% inhibition of **yield** of *Lemna gibba* determined after 7 days on the basis of the dry weight E_yC_{50}/7 d value is 0.000785 mg/L (95% confidence interval: 0.000485 – 0.001212).

The endpoint values determined based on the nominal concentrations of Nicosulfuron:
The median concentration causing 50% inhibition of the mean specific **growth rate** of *Lemna gibba* determined on the basis of the frond number E_rC_{50}/7 d value is 0.001981 mg/L (95% confidence interval: 0.001856 – 0.002110). The median concentration causing 50% inhibition of **yield** of *Lemna gibba* determined after 7 days on the basis of the frond number E_yC_{50}/7 d value is 0.001344 mg/L (95% confidence interval: 0.001231 – 0.001480).
The median concentration causing 50% inhibition of the mean specific **growth rate** of *Lemna gibba* determined on the basis of the dry weight E_rC_{50}/7 d value is 1.311622 mg/L (95% confidence interval: 0.791795 – 2.359483). The median concentration causing 50% inhibition of **yield** of *Lemna gibba* determined after 7 days on the basis of the dry weight E_yC_{50}/7 d value is 0.003125 mg/L (95% confidence interval: 0.001929 – 0.004829).
The endpoint values determined based on the nominal concentrations of Mesotrione:
The median concentration causing 50% inhibition of the mean specific **growth rate** of *Lemna gibba* determined on the basis of the frond number E_rC_{50}/7 d value is 0.006122 mg/L (95% confidence interval: 0.005723 – 0.006537). The median concentration causing 50% inhibition of **yield** of *Lemna gibba* determined after 7 days on the basis of the frond number E_yC_{50}/7 d value is 0.004095 mg/L (95% confidence interval: 0.003740 – 0.004521).
The median concentration causing 50% inhibition of the mean specific **growth rate** of *Lemna gibba* determined on the basis of the dry weight E_rC_{50}/7 d value is 3.909122 mg/L (95% confidence interval: 2.365171 – 7.012785). The median concentration causing 50% inhibition of **yield** of *Lemna gibba* determined after 7 days on the basis of the dry weight E_yC_{50}/7 d value is 0.009557 mg/L (95% confidence interval: 0.005914 – 0.014733).

The endpoint values determined based on the geometric mean of determined concentrations of Rimsulfuron:
The median concentration causing 50% inhibition of the mean specific **growth rate** of *Lemna gibba* determined on the basis of the frond number E_rC_{50}/7 d value is 0.000640 mg/L (95% confidence interval: 0.000611 – 0.000670). The median concentration causing 50% inhibition of **yield** of *Lemna gibba* determined after 7 days on the basis of the frond number E_yC_{50}/7 d value is 0.000453 mg/L (95% confidence interval: 0.000432 – 0.000475).
The median concentration causing 50% inhibition of the mean specific **growth rate** of *Lemna gibba* determined on the basis of the dry weight E_rC_{50}/7 d value is 0.246215 mg/L (95% confidence interval: 0.146291 – 0.460328) The median concentration causing 50% inhibition of **yield** of *Lemna gibba* determined after 7 days on the basis of the dry weight E_yC_{50}/7 d value is 0.000764 mg/L (95% confidence interval: 0.000464 – 0.001175).

The endpoint values determined based on the geometric mean of determined concentrations of Mesotrione:
The median concentration causing 50% inhibition of the mean specific **growth rate** of *Lemna gibba* determined on the basis of the frond number E_rC_{50}/7 d value is 0.00548 mg/L (95% confidence interval: 0.00516 – 0.00581). The median concentration causing 50% inhibition of **yield** of *Lemna gibba* determined after 7 days on the basis of the frond number E_yC_{50}/7 d value is 0.00384 mg/L (95% confidence interval: 0.00354 – 0.00420).
The median concentration causing 50% inhibition of the mean specific **growth rate** of *Lemna gibba* determined on the basis of the dry weight E_rC_{50}/7 d value is 3.40036 mg/L (95% confidence interval: 2.06434 – 6.08136). The median concentration causing 50% inhibition of **yield** of *Lemna gibba* determined after 7 days on the basis of the dry weight E_yC_{50}/7 d value is 0.00891 mg/L (95% confidence interval: 0.00555 – 0.01364).

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

	• The doubling time (Td) of frond number in the control calculated for each week was between 1.6 and 2.1 days (Td = ln 2 / μ) during the study. According to the test guideline, the validity criterion for the study (Td < 2.5 days corresponding to an average growth rate of 0.275 day-1). **Agreed endpoints:** Exposure 7 day period: 7-day E_yC_{50} = 1.2 µg a.s/L (frond number) **7-day E_rC_{50} = 2.1 µg a.s/L (frond number)** NOEC= 0.28 µg a.s/L LOEC=0.74 µg a.s/L **<u>Recovery period</u>:** Complete recovery of *Lemna gibba* after 7-day exposure to Nicosulfuron technical was demonstrated for the exposure concentration of 0.74 µg/L after 7 and 14 days in test medium free of test item. The plants of the exposure concentrations of 2.1 and 7.1 µg/L were still affected after the recovery period of 14 days. Based on these statistical results, the NOEAC of 0.74 µg nicosulfuron /L (No Observed Ecologically Adverse Concentration) for the growth of *Lemna gibba* was determined.

Reference:	KCP 10.2.1-05
Report	"Toxicity of Nicosulfuron technical to the Aquatic Higher Plant *Lemna gibba* in a 7-Day Growth Inhibition Test, Supplemented With Testing for Recovery of Growth". Bätscher, R, 2008, B75341
Guideline(s):	Yes (OECD 221). This study was supplemented with an additional experimental part in which the recovery of plant growth was monitored.
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods
Materials

Test item:

Description:	Nicosulfuron technical
Batch number:	SI-01
A.i. content:	93.1% min

Test system:

Species:	*Lemna gibba* G3 (family Lemnaceae, Macrophyta.
Strain:	-
Age:	-
Source:	Bayer CropScience AG, 40789 Monheim, Germany in 2007.

Medium: 20X AAP

Experimental conditions:

Temperature: 22.0 – 23.0 °C
pH values: 8.6 – 8.8
Mean light intensity: 6500-8500 lux, illumination constant
Test vessels: glass dishes containing 250 mL of each treatment
Initial frond number: 12

Experimental period: 7 days Static-Renewal conditions

Test design:

The test design included three replicates per test concentration and control. Each replicate consisted of a 250-mL glass dish (diameter of 9.5 cm) filled with 150 mL of test medium, resulting in a water depth of approximately 21 mm. The test vessels were covered with glass dishes. The test vessels were labelled with the study number and all necessary additional information to ensure unique identification.
The test plants were exposed for seven days to the following concentrations of Nicosulfuron technical: 0.10, 0.32, 1.0, 3.2, 10 and 32 µg/L. Additionally, a control (test water without addition of the test item) was run in parallel.
A stock solution of the nominal concentration of 20 mg/L was freshly prepared before the test medium renewals. For the preparation of the stock solutions on Days 0, 2 and 5, the amount of 20.0, 20.1 and 20.2 mg of test item, respectively, was mixed into 1000 mL of test water using ultrasonic treatment (15 minutes) and intense stirring (30 minutes at room temperature). The stock solutions were diluted with test water to prepare the test media of the test concentrations mentioned above.
For the determination of the actual test item concentrations, quadruplicate samples were taken from each treatment at the start and end of each renewal period.
The selection of the test concentrations was based on the results of a range-finding test (non-GLP).
After the 7-day exposure of the plants to the test item, the recovery of growth of the affected plants was monitored during two weeks. Some plants of the test concentrations of 0.74, 2.1 and 7.1 µg/L (nominal 1.0, 3.2 and 10 µg/L, respectively) were transferred to test water free of test item. The growth of the treated plants was compared to parallel running control cultures.

Determination of the Growth Inhibition and Calculation of Results:

During the first week of the study, the *Lemna* colonies were inspected in each test vessel for changes in frond and colony number and appearance (discoloration, sinking, root length, or other visible abnormalities) on Days 2, 5 and 7.

The dry weight of a sample of fronds equivalent to that used to inoculate the test vessels was determined at the start of the test. At Day 7, the dry weight of the plants of each test vessel (minus the plants used to start the recovery period at the nominal concentration of 1.0 µg/L) was determined.

The plants were dried at about 60 °C in a laboratory vacuum oven for 48 hours (sufficient to reach a constant weight).

During the two weeks of recovery, the fronds and colonies were counted twice a week (on Days 12, 14, 19 and 21). The dry weight of the plants was determined after the first and the second week of recovery (Days 14 and 21 of the study; minus the plants used to continue the recovery period).

As on Day 7, 14 and 21 some plants were used to start the following week of the recovery phase, the determined dry weights were corrected for these plants (based on the calculated dry weight per frond). These corrected weights are given in the tables and used for calculation of the growth rates and yield. The dry weight determinations on Day 7 and 14 were further used to calculate the dry weight of the plants in each replicate at the start of each week of recovery.
Inhibition of *Lemna* growth after 7 days was determined by:
a) Average specific growth rates (μ)
b) Yield (Y)

For the exposure period (Day 0-7), the EC10, EC20 and EC50 values for the inhibition of the average growth rate and yield based on frond numbers and dry weight and their 95% confidence limits were calculated as far as possible by Probit Analysis . The 7-day NOEC and LOEC were determined by testing the parameters at the test concentrations on statistically significant differences to the control values by multiple Dunnett's tests.
The assessment of recovery of growth of the test plants was based on the section-by-section growth rates based on frond numbers and on the weekly average growth rates based on frond numbers and dry weight of the plants. The growth rates were compared with the control by Williams' tests.The Williams' test was used as the Dunnett's test could not be applied due to the low number of degrees of freedom of the data. Based on these statistical results, the NOEAC (No Observed Ecologically Adverse Concentration) for the growth of *Lemna gibba* was determined.

Analyses of the Test Item Concentrations

During the exposure period, quadruplicate samples were taken from the test media of all test concentrations and from the control at the start (Day 0, 2 and 5) and at the end (Day 2, 5 and 7) of each test medium renewal period. For the sampling of the aged media, the test media of the three replicates per test concentration were pooled. After the exposure period, no further samples were taken.
Immediately after sampling, acetonitrile (2.5 mL) and ammonium hydroxide 24.5% (47 µL) were added to each sample (10 mL) to stabilize the latter during the storage period. The samples were stored in a refrigerator until analysis.
The concentrations of the test item were analytically measured in two of the quadruplicate samples from the test concentrations of 0.32 to 32 µg/L.
From the control, one sample was analyzed from each sampling time. The samples from the lowest test concentration of 0.10 µg/L were not analyzed as this concentration was below the 7-day NOEC

Analytical results:

In the test media of the nominal concentrations of 0.32 to 32 µg/L, the measured concentrations of the test item at the start of the test medium renewal periods (Day 0, 2 and 5) were between 50 and 130% of the nominal values.
At the end of these periods (Day 2, 5 and 7), 50 to 109% of the nominal concentrations were found. The mean measured concentrations of the test item (calculated as time-weighted means) were between 65 and 89% of the nominal values.

The reported biological results were based on the mean measured concentrations of the test item which were 0.28 µg/L (nominal 0.32 µg/L), 0.74 µg/L (nominal 1.0 µg/L), 2.1 µg/L (nominal 3.2 µg/L), 7.1 µg/L (nominal 10 µg/L) and 24 µg/L (nominal 32 µg/L).

The concentration of 0.74 µg/L was determined to be the 7-day LOEC (lowest concentration tested showing effects) as at this concentration the average growth rate and the yield based on frond numbers and dry weight after the exposure period of 7 days **were statistically significantly lower than in the control.**

At the 7-day NOEC (highest concentration tested without toxic effects) corresponding to the next lower concentration of 0.28 µg/L and up to this concentration, the growth of the plants was not inhibited and no abnormalities in growth and appearance of the plants was determined after the exposure period of 7 days.

The EC10, EC20 and EC50 for inhibition of the average growth rates and yield based on frond numbers and dry weight after 7 days of exposure were calculated as far as possible by Probit Analysis.
No abnormalities in appearance of the test plants were recorded during the 7-days test period in the control and at the nominal test concentrations of 0.10 µg/L (not determined analytically) and the mean measured concentration of 0.28 µg/L (nominal 0.32 µg/L).
At the mean measured concentrations of 0.74 to 7.1 µg/L (nominal 1.0 to 10 µg/L), the newly formed fronds were stunted.
At the mean measured concentration of 24 µg/L (nominal 32 µg/L), some plants showed chlorosis.

The pH of the freshly prepared test media and control was between 7.5 and 7.6. The pH of the aged test media and the control during the exposure period was between 8.6 and 8.8 Thus, the increase of the pH over the exposure period was less than 1.5 units as requested by the test guideline. The pH of the aged media at the end of the first and second week of recovery was between 8.7 and 9.3. The increase of the pH was caused by the CO_2 uptake of the plants due to their growth. The water temperature during the study was in the range of 22-23 °C

The biological results after 7 days of exposure can be summarized as follows (based on mean measured concentrations of the test item):

Results based on 7 days exposure phase

EC values (µg/L)	Parameter based on			
	frond numbers		dry weight of the plants	
	Average growth rate	Yield	Average growth rate	Yield
EC10 95% C.I.	0.49 0.0 – 1.1	0.50 0.28 – 0.67	0.38 n.d. – 1.9	0.13 n.d. – 0.75
EC20 95% C.I.	0.81 0.03 – 1.6	0.67 0.44 – 0.84	2.0 0.0 – 7.1	0.39 n.d. – 1.5
EC50 95% C.I.	2.1 0.85 – 6.5	1.2 0.93 – 1.5	>24 n.d.	3.2 0.24 – >24
NOEC	0.28	0.28	0.28	0.28
LOEC	0.74	0.74	0.74	0.74

95% C.I.: 95% confidence interval
n.d.: could not be determined

7-day E_yC_{50} = 1.2 µg a.s/L (frond number)
7-day E_rC_{50} = 2.1 µg a.s/L (frond number)
NOEC= 0.28 µg a.s/L
LOEC=0.74 µg a.s/L

Recovery of the Plants

After the 7-day exposure period, the recovery of growth of the affected plants at the mean measured concentrations of 0.74, 2.1 and 7.1 µg/L (nominal concentrations of 1.0, 3.2 and 10 µg/L, respectively) was monitored during two weeks.

The frond numbers and dry weights in the two weeks of recovery are listed in Tables 8 to 11.

During the first week of recovery (Day 7-14 of the study), the growth rate based on frond numbers and dry weight was statistically significantly lower compared to the control at the test concentrations of 2.1 and 7.1 µg/L (Tables 12-14). At the test concentration of 0.74 µg/L, the

growth during the first week of recovery was not significantly inhibited and no symptoms of toxicity were determined at the end of the week. Thus, the growth of the plants exposed to 0.74 µg/L was completely recovered after one week. At the higher concentrations of 2.1 and 7.1 µg/L, altered growth and chlorosis were determined after the first week of recovery.

In the second week of recovery (Day 14-21 of the study), the average growth rates based on frond numbers and dry weight were still statistically significantly inhibited at the two highest test concentrations of 2.1 and 7.1 µg/L (Table 15-17) and symptoms of toxicity were still observed. The complete recovery of the plants exposed to 0.74 µg/L was confirmed in the second week.

In conclusion, complete recovery of *Lemna gibba* after 7 days of exposure to Nicosulfuron technical was demonstrated for the exposure concentration of 0.74 µg/L after one and two weeks in test medium free of test item.

Thus, based on the complete recovery of the plants within 7 days, the NOEAC (No Observed Ecologically Adverse Effect Concentration) for the growth of *Lemna gibba* after 7-day exposure to Nicosulfuron technical can be determined to be the concentration of 0.74 µg/L.

~~**Recovery phase**~~

~~Complete recovery of *Lemna gibba* after 7-day exposure to Nicosulfuron technical was demonstrated for the exposure concentration of 0.74 µg/L after 7 and 14 days in test medium free of test item. The plants of the exposure concentrations of 2.1 and 7.1 µg/L were still affected after the recovery period of 14 days.~~

Results of the first week recovery period

Section-by-Section Growth Rates Based on Frond Numbers during the First Week of Recovery

Nominal test item concentration# (µg/L)	Section-by-section growth rates µ (day^{-1}) and inhibition of µ (I_r)			
	Days 7–12		Days 12–14	
	µ	I_r (%)	µ	I_r (%)
Control	0.484	0.0	0.317	0.0
1.0	0.465	4.0	0.332	-4.7
3.2	0.213*	56.1	0.254*	19.8
10	0.110*	77.3	0.175*	44.9

#: concentration of the test item during the seven days of exposure
*: mean value significantly lower than in the control
(according to Williams' tests, one-sided, α = 0.05)

Average Growth Rates Based on Frond Numbers during the First Week of Recovery

Nominal test item concentration# (µg/L)	Average Growth rate µ (day^{-1}) and inhibition of µ (I_r)	
	Days 7–14	
	µ	I_r (%)
Control	0.437	0.0
1.0	0.427	2.2
3.2	0.224*	48.6
10	0.129*	70.5

#: concentration of the test item during the seven days of exposure
*: mean value significantly lower than in the control
(according to Williams' tests, one-sided, α = 0.05)

Average Growth Rates Based on Dry Weights during the First Week of Recovery

Nominal test item concentration# (µg/L)	Average Growth rate µ (day^{-1}) and inhibition of µ (I_r)	
	Days 7–14	
	µ	I_r (%)
Control	0.420	0.0
1.0	0.403	4.0
3.2	0.117*	72.1
10	0.047*	88.9

#: concentration of the test item during the seven days of exposure
*: mean value significantly lower than in the control
(according to a Williams' test, one-sided, α = 0.05)

Results of the second week recovery period

Section-by-Section Growth Rates Based on Frond Numbers during the Second Week of Recovery

Nominal test item concentration#	Section-by-section growth rates μ (day^{-1}) and inhibition of μ (I_r)			
	Days 14–19		Days 19–21	
(µg/L)	μ	I_r (%)	μ	I_r (%)
Control	0.378	0.0	0.356	0.0
1.0	0.363	4.1	0.383	-7.5
3.2	0.335*	11.4	0.304	14.4
10	0.279*	26.4	0.241*	32.2

#: concentration of the test item during the seven days of exposure
*: mean value significantly lower than in the control
(according to Williams' test, one-sided, α = 0.05)

Average Growth Rates Based on Frond Numbers during the Second Week of Recovery

Nominal test item concentration#	Average growth rates μ (day^{-1}) and inhibition of μ (I_r)	
	Days 14–21	
(µg/L)	μ	I_r (%)
Control	0.372	0.0
1.0	0.368	0.9
3.2	0.327*	12.2
10	0.268*	28.0

#: concentration of the test item during the seven days of exposure
*: mean value significantly lower than in the control
(according to a Williams' test, one-sided, α = 0.05)

Average Growth Rates Based on Dry Weights during the Second Week of Recovery

Nominal test item concentration#	Average Growth rate μ (day^{-1}) and inhibition of μ (I_r)	
	Days 14–21	
(µg/L)	μ	I_r (%)
Control	0.395	0.0
1.0	0.397	-0.6
3.2	0.319*	19.3
10	0.176*	55.3

#: concentration of the test item during the seven days of exposure
*: mean value significantly lower than in the control
(according to a Williams' test, one-sided, α = 0.05)

Conclusion

7-day EyC50	1.2 µg a.s/L
7-day ErC50	2.1 µg a.s/L
NOEC	0.28 µg a.s/L
LOEC	0.74 µg a.s/L

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

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

Comments of zRMS:	The study is considered valid. All validity criteria were met. • 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 24h LD_{50}/24 h of the reference item (dimethoate) was 0.11 µg a.i./bee (criterion: 0.10 – 0.30 µg a.i./bee). **Agreed endpoints:** **LD_{50}/24 h and LD_{50}/48 h oral > 400 µg/bee**

Reference:	KCP 10.3.1.1.1
Report	"Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG: Honeybees (*Apis mellifera* L.), Acute Oral Toxicity Test". Monika Stalmach, 2019, B/172/16. Institute of Industrial Organic Chemistry Branch Pszczyna
Guideline(s):	Yes, OECD Guideline for the Testing of Chemicals No. 213 (1998) and the EU Method C.16. (2008)
Deviations:	Yes. According to the Amendment No. 1 to the Study Plan B/172/16 should be finished in February 2019, but it was completed in May 2019. The deviation had no impact on the obtained results.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Yes

Materials and methods

Test item:

Description:	Rimsulfuron 3%+Nicosulfuron 12%+Mesotrione 36% WG
Production batch:	SCL–19947
A.i. content:	30 g/kg (w/w) of rimsulfuron; 120 g/kg (w/w) of nicosulfuron; 360 g/kg (w/w) of mesotrione

Test system:

Species:	*Apis mellifera*
Strain:	carnica
Age:	approximately 3 weeks
Average weight:	-
Average length:	-

Source: An apiary at the Institute of Industrial Organic Chemistry, Branch Pszczyna
Acclimation period: -
Diet: 50% (w/v) aqueous sucrose solution

Experimental conditions:

Temperature: 23 – 24.5°C
Humidity: 60 – 62%
Hardness: -
pH: -
Light and photoperiod: 24h darkness (except during observations).
Loading: 3 replicates per dose, 10 bees per replicate
Test procedure: For the oral toxicity test, the test substance was added to a 50% w/v sucrose solution reaching the concentration of 100 µg a.i./bee. Feeders were filled with the dilution. Bees were kept unfed for approximately 2 hours.

Experimental period: 48h

Test design and treatment

Plastic cages with an opening on each side to allow the feeding with micropipettes. The bees were observed for mortality and behavioural abnormalities after 4, 24 and 48 h of exposure.
A preliminary test was conducted with doses of 8.0, 40.0 and 200.0 µg of a.i./bee. According to the results, the following nominal test item concentrations were used: 25.0, 50.0, 100.0, 200.0, 400.0 µg/bee with 0.1% adjuvant and 400.0 µg/bee without 0.1% adjuvant

Results

On the preliminary test after 24 and 48 hours, there were no dead bees in the control group and group exposed to the test item at doses: 8.0 and 40.0 µg/honeybee. After 24 and 48 hours there was one dead bee in the group with the test item at the rate 200.0 µg/honeybee, which corresponds 10.0% mortality in each day.

On the definitive test mMortality of the bees in the control group without 0.1% adjuvant and control with 0.1 % adjuvant after 24 and 48 hours was 0.0%. After 4, 24 and 48 hours there were no dead bees at the test item doses 25.0, 50.0, 100.0, 200.0 and 400.0 µg/bee (without adjuvant). There were also no dead bees in the group with the highest item dose 400.0 µg/bee without 0.1% adjuvant.
The median lethal doses LD_{50}/24h and LD_{50}/48h are above 400.0 µg/honeybee with 0.1% adjuvant, respectively. Due to the results obtained in the group exposed to the test item at rate 400.0 µg/honeybee without 0.1% adjuvant it could be assumed that the median lethal doses (LD_{50}/24 h and LD_{50}/48 h) is higher than 400.0 µg/honeybee without 0.1% adjuvant.
The reduction in amount of the sucrose solution consumed by the insects during 24 and 48 h ranged from -43.3 to 69.5% as compared to the control. The negative values indicate higher sucrose solution consumption in groups treated with the test item compared to the control group.
Mortality of the bees receiving the reference item registered after 4 and 24 hours and the LD_{50} value after 24 hours are presented in Tables 11 and 12. The median lethal dose of dimethoate (LD_{50}/24 h) after 24 hours determined with the log-probit method, is 0.11 µg a.i./bee.

Oral toxicity test results

Dosage		Number of tested bees [no.]	Mortality after 48 h		LD_{50} after 48 h
			Total		
Test item [µg/bee]	Active ingredients [µg/bee]		[no.]	[%]	[µg/bee]
Control without 0.1% adjuvant		30	0	0.0	Above 400.0
Control with 0.1% adjuvant		30	0	0.0	
25.0*	$0.8^a + 3.0^b + 9.0^c$	30	0	0.0	

50.0*	1.5[a] + 6.0[b] + 18.0[c]	30	0	0.0	
100.0*	3.0[a] + 12.0[b] + 36.0[c]	30	0	0.0	
200.0*	6.0[a] + 24.0[b] + 72.0[c]	30	0	0.0	
400.0*	12.0[a] + 48.0[b] + 144.0[c]	30	0	0.0	
400.0 without 0.1% adjuvant	12.0[a] + 48.0[b] + 144.0[c]	30	0	0.0	Above 400.0[#]

[a]: Rimsulfuron
[b]: Nicosulfuron
[c]: Mesotrione
*: test item doses with 0.1% adjuvant
[#]: Based on the obtained mortality results in the group exposed to the test item at rate 400.0 µg/honeybee without 0.1% adjuvant it could be assumed that the median lethal dose is higher than the used dose.

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 24h LD_{50}/24 h of the reference item (dimethoate) was 0.11 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 used dose, i.e. µg test item/honeybee (µg a.i./honeybee).

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

Comments of zRMS:	The study is considered valid. All validity criteria were met. • The average mortality for the total number of controls were 0.0% after48 h (criteriun: it must not exceed 10%) • The LD50/24 h of the reference item (dimethoate) was 0.28 µg a.i./bee (criterion: 0.1 – 0.3 µg a.i./bee) **Agreed endpoints:** **LD_{50}/24 h, LD_{50}/48 h oral > 400 µg/bee**

Reference:	KCP 10.3.1.1.2
Report	"Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG: Honeybees (*Apis mellifera L.*), Acute Contact Toxicity Test". Monika Stalmach, 2019, B/173/16. Institute of Industrial Organic Chemistry Branch Pszczyna
Guideline(s):	Yes, OECD Guideline No. 214 (1998) and the EU Method C.17. (2008)
Deviations:	According to the Amendment No. 1 to the Study Plan B/173/16, study should be completed in February 2019, but it was completed in May 2019. There were also an editorial error regarding the name of the adjuvant used in the definitive test and it has been corrected. The deviations had no impact on the obtained results.
GLP:	Yes

Acceptability: Yes

Duplication (if vertebrate study) Yes

Materials and methods

Test item:

Description:	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG
Production batch:	SCL - 19947
A.i. content:	30 g/kg (w/w) of rimsulfuron; 120 g/kg (w/w) of nicosulfuron; 360 g/kg (w/w) of mesotrione

Test system:

Species:	*Apis mellifera*
Strain:	Carnica
Age:	3 weeks old
Average weight:	-
Average length:	-
Source:	An apiary at the Institute of Industrial Organic Chemistry, Branch, Pszczyna
Acclimation period:	-
Diet:	50% (v/v) honey and sugar solution

Experimental conditions:

Temperature:	23 – 24.5°C
Humidity:	60 – 62%
Hardness:	-
pH:	-
Light and photoperiod:	24h darkness (except during observations).
Loading:	-
Test procedure:	The honeybees were anaesthetized with carbon dioxide, transferred to plastic trays and dosed on the dorsal side of the thorax with 1 µl of test solution containing the test substance or reference substance.

Experimental period: 48h

Test design and treatment

Plastic cages with an opening which allowed the insects to take food.
A preliminary test was done at the dose of 8.0 40.0 and 200.0 µg test item/bee. According to the results, the following nominal test item concentrations were used: 25.0, 50.0, 100.0, 200.0 and 400.0 µg test item/bee with 0.1% adjuvant, one dose of 400 µg test item/bee without 0.1% adjuvant, a control (0.0 µg/bee) with 0.1% adjuvant and a control (0.0 µg/bee) without 0.1% adjuvant. The honeybees were observed for mortality and behavioural abnormalities after 4, 24 and 48 h of exposure.
Appropriate statistical methods were used to analyse mortality data for the LD_{50} calculation.

Results

In the preliminary test, after 48 hours, there were no dead bees in the control group (without adjuvant) and in groups with the test item at the rates of 8.0, 40.0, and 200.0 µg/honeybee without adjuvant.

In the definitive test, mortality of the control with 0.1% adjuvant and control group without 0.1% adjuvant after 24 and 48 hours of exposure was 0.0%, respectively.
After 4 hours there were no dead bees in the: control with 0.1% adjuvant and control group without adju-

vant as well as in groups exposed to the test item doses without adjuvant and the highest dose of test item (400.0 µg/honeybee) with 0.1% adjuvant.
After 24 and 48 hours of exposition there were no dead bees in groups with the test item at the rates of 25.0, 50.0, 100.0, 200.0 and 400.0 µg/honeybee with 0.1% adjuvant, there were also no dead bees in the group treated with 400 µg of test item/honeybee without 0.1% adjuvant. It corresponds to 0.0% mortality in each group.
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. 400.0 µg test item/honeybee with 0.1% adjuvant. Due to the results obtained in the group exposed to the test item at rate 400.0 µg/honeybee without 0.1% adjuvant it could be assumed that the median lethal doses (LD_{50}/24 h and LD_{50}/48 h) is higher than 400.0 µg/honeybee without 0.1% adjuvant.
There were no signs of toxicity (behavioural abnormalities) such as: excitement (uncoordinated movement, increased activity, intensive cleaning) or paralysis were observed during the 48-hour exposure to the test item.
The median lethal dose of dimethoate (LD_{50}/24 h) determined with the log-probit method is 0.28 µg a.i./bee (95% confidence limits: 0.25 – 0.32 µg a.i./bee).

Contact toxicity test results

Dosage		Number of tested bees [no.]	Mortality after 48 h		LD_{50} after 48 h
			Total		
Test item [µg/bee]	Active ingredients [µg/bee]		[no.]	[%]	[µg/bee]
Control without 0.1% adjuvant		30	0	0.0	Above 400.0
Control with 0.1% adjuvant		30	0	0.0	
25.0*	$0.8^a + 3.0^b + 9.0^c$	30	0	0.0	
50.0*	$1.5^a + 6.0^b + 18.0^c$	30	0	0.0	
100.0*	$3.0^a + 12.0^b + 36.0^c$	30	0	0.0	
200.0*	$6.0^a + 24.0^b + 72.0^c$	30	0	0.0	
400.0*	$12.0^a + 48.0^b + 144.0^c$	30	0	0.0	
400.0 without 0.1% adjuvant	$12.0^a + 48.0^b + 144.0^c$	30	0	0.0	Above 400.0[#]

[a]: Rimsulfuron
[b]: Nicosulfuron
[c]: Mesotrione
*: test item doses with 0.1% adjuvant
[#]: Based on the obtained mortality results in the group exposed to the test item at rate 400.0 µg/honeybee without 0.1% adjuvant it could be assumed that the median lethal dose is higher than the used dose.

Conclusion
The median lethal doses (LD_{50}/24h and LD_{50}/48h) are higher than the maximum used dose, i.e. 400 µg test item/honeybee.

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

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

- The mean mortality in the control was ≤ 15% at the end of the test.
- The mean mortality in the reference item group was ≥50 % at the end of the test

Agreed endpoints:

Test item: Rimsulfuron Technical	
LDD_{50}	> 18.51 [µg a.i./bee/day]
LC_{50} / LDD_{50}	> 840.34 [mg a.i./Kg]
NOEDD	≥ 18.51 [µg a.i./bee/day]
NOEC	≥ 840.34 [mg a.i./Kg]

Reference Report: KCP 10.3.1.2.1
Ansaloni, T., 2018 Rimsulfuron Technical - Chronic Toxicity to the Honey Bee, *Apis mellifera* L. Study code: TRC16-193BA

Source: Trialcamp S.L.U. Poligon Industrial l'Alter. Avda. Antic Regne de Valencia, 25, 46290 Alcasser (Valencia). Spain.
Unpublished report No.: TRC16-193BA. Issued: 2018.

Guidelines: CEB (2012) method, adaptions of OECD Guidelines nº 213 (1998), publications of Decourty et al. (2005) and Suchail et al (2001), recommendations of the German ring test group (2013) and EPPO 170

Deviations to Guidelines: None.

GLP: Yes (certified laboratory).

Study Objective: To determine the effects of Rimsulfuron Technical on the honey bee *Apis mellifera* L. from chronic feeding exposure. To determine the median lethal daily dose / Concentration (LDD_{50} / LC_{50}) and the no observed effect daily dose / concentration (NOEDD / NOEC) values, where possible.

Test item: Rimsulfuron technical; Batch code: SCL-30188; active substance: Rimsulfuron; content of a.s. determined by certificate of analysis: 98% (w/w); expiry date: 21 Feb 2017.

Reference product: BAS 152 11 I; Batch number FRE-001226; active ingredient: Dimethoate; content of a.i. analysed: 420.3 g/L density: 1.072 g/cm^3.

Test organisms:

Test species: *Apis mellifera* L. (Hymenoptera, Apidae)

Life Stage: Young adult worker bees (newly hatched; 1 to 2 days old).

Source: Queen-right, healthy colony from a comercial apiary.

Preparation of test organism: Two days before the beginning of the test, frames with capped cells are transferred from the hive to an incubator, transported to Trialcamp facilities and ubicated on a bioclimatic chamber. One day prior to test start, the bees will be randomly collected directly from the frames, introduced into the test units and kept under test conditions until start of the test. Acclimatisation period lasted since bee collection to the start of the test. During this period bees were fed *ad libitum* with a 50 % w/v sucrose solution.

Test design: Limit test; duration 10 days, one control group, one concentration of the test item, one concentration of the reference item; 5 replicates of 10 bees each per treatment group. Daily assessment of mortality and behavioural abnormalities over the 10 day test period.

Five additional test units without bees but with full food syringes containing pure 50 % (w/v) aqueous sucrose solution for evaluation of the evaporation.

Test concentrations / doses:
Control: C (50 % (w/v) aqueous sucrose solution)
Test Item: 100 µg rimsulfuron/bee/day
Reference Item: R: 0.107 µg Dimethoate/bee/day.

Test conditions:
Temperature: 32.17 – 34.11 °C
Relative humidity: 37.40* – 69.98 %

* Short term deviation (<2 h).

Exposure to light: Constant darkness, except during application and assessments.

Sampling: Two duplicate samples, one shipment and one retain, of Treated Solution from the last day of application will be stored in a freezer at ≤ -18 ºC until shipment and delivery to the analytical laboratory for analytical determination of the actual concentration of the test chemical.

Analytical verification: Analytical data were required to demonstrate the concentration of the active ingredient Rimsulfuron (representative sample) and its solubility in the solvent. Quantification was performed by HPLC. The limit of quantification (LOQ) of the analytical method was 10.76 µg/mL, with a limit of detection (LOD) set at 3.23 µg/mL (30 % of the LOQ).

Analytical study was performed to verify the concentration of the samples taken. For the analytical concentration verification, Rimsulfuron residues were determined.

The measured concentration in the samples was within 20 % of nominal test concentration used, thus the concentrations of the test item were confirmed and the endpoints are based on nominal concentrations.

Analytical recoveries for Rimsulfuron

Sample code	Timing	Matrix	Replicate	Nominal Concentration [µg/g*]	Analysed Concentration [µg/g*]	% of Nominal
TRC16-118BA 2S	D9	50 % (w/v) aqueous sucrose solution	1	840.34	762.1610	90.70
			2	840.34	762.6404	90.75

* Considering a density of the 50% (w/v) sucrose solution of 1.19 g/mL

Statistics: Statistical calculations were made by using the statistical program TOXRAT PROFESSIONAL V. 3.2.1

Parametric pair wise test (Student t-test, one sided smaller; $\alpha = 0.05$) was used to evaluate whether there were significant differences between daily consumption of the control and the test item treatment and to determine the NOEDD / NOEC. No statistical analysis was performed on mortality data.

Findings: Results are shown in the tables below.

In the control C (untreated 50 % (w/v) aqueous sucrose solution) the validity criteria was met (mortality < 15% after 10 days of exposure). There was 8.00% of mortality after 10 days of continuous feeding. In the reference item group the mortality continuously increased during the test period and reached 100.00 % (corrected mortality 100.00 %) after 10 days. Consequently, validity criteria for both control and reference item mortality were met and the test was considered valid.

The overall mean daily consumption of feeding solution over the entire test period of the control group (untreated 50 % (w/v) aqueous sucrose solution) was 19.28 µL/bee/day. The overall mean daily consumption of feeding solution at the test item applied dose of 100.0 µg a.i./bee/day was 18.50 µL/bee/day. In the reference item treatment group, the overall mean daily consumption of feeding solution was 18.03 µL/bee/day.

In the test item group, at the consumed dose of 18.50 µg a.i./bee/day, a cumulative mortality of 4.00% was observed at the final assessment after 10 days.

Overall, three individual bees in the treatment group and one individual in the control group were observed with symptoms of intoxication throughout the study. The symptom observed was lack of coordination. By the end of the study (day 10) at the consumed dose of 18.50 µg a.i./bee/day, the percentage of affected bees based on the surviving individuals was 0.00%.

After 10 days of continuous exposure the accumulated mean uptake of product at the treatment level of 100 µg a.i./bee/day was 185.04 µg a.i./bee.

Treatment	10 day cumulative mortality	Corrected mortality[1]	Overall mean consumption of feeding solution	Daily dietary dose	Accumulated mean uptake
Control:					
	[%]		**[µL/bee/day]**	-	-
C	8.00	-	19.28	-	-
Reference item: BAS 152 11 I [µg a.i./bee/day]					
	[%]		**[µL/bee/day]**	**[µg a.i./bee/day]**	**[µg a.i./bee]**
R (0.107)	100.00	100.00	18.03	0.0193	0.135
Test item: Rimsulfuron Technical [µg a.i./bee/day]					
	[%]		**[µL/bee/day]**	**[µg a.s.bee/day]**	**[µg a.s./bee]**
T (100.0)	4.00	-4.35	18.50	18.50	185.04

[1] mortality corrected with the corresponding control mortality according to SCHNEIDER-ORELLI, O. (1947)

Conclusion: All validity criteria were met and the study was deemed valid. The endpoints determined are shown in the table below.
No statistically significant differences were observed in mean daily consumption between any of the test item treatments and the control group.

Overall, three individual bees in the treatment group and one individual in the control group were observed with symptoms of intoxication throughout the study. The symptom observed was lack of coordination. By the end of the study (day 10) at the consumed dose of 18.50 µg a.i./bee/day, the percentage of affected bees based on the surviving individuals was 0.00%.

The results obtained with the toxic reference substance confirmed the sensitivity of the bees under the conditions of the oral test.

Test item: Rimsulfuron Technical	
LDD_{50}	> 18.51 [µg a.i./bee/day]
LC_{50} / LDD_{50}	> 840.34 [mg a.i./Kg]
NOEDD	≥ 18.51 [µg a.i./bee/day]
NOEC	≥ 840.34 [mg a.i./Kg]

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

- Mortality observed in control treatment was equal or less than 15% for the duration of the test (final cumulated mortality = 0.00% for both the negative and the solvent control).
- Mean mortality in the reference product concentration was ≥ 50% at the end of the test (final cumulated mortality = 100.00%).

Agreed endpoints:

Test item: Nicosulfuron Technical	
LC_{50}	> 336.13 mg a.i./kg food
LDD_{50}	> 7.93 µg a.i./bee/day
NOEC	336.13 mg a.i./kg food
NOEDD	**7.93 µg a.i./bee/day**

Reference: KCP 10.3.1.2.2
Report: Ansaloni, T., 2018 Nicosulfuron Technical - Chronic Toxicity to the Honey Bee, *Apis mellifera* L.
Source: Trialcamp S.L.U. Poligon Industrial l'Alter. Avda. Antic Regne de Valencia, 25, 46290 Alcasser (Valencia). Spain.
Unpublished report No.: TRC16-049BA. Issued: 2018.
Guidelines: CEB (2012) method, adaptions of OECD Guidelines nº 213 (1998), publications of Decourty et al. (2005) and Suchail et al (2001), recommendations of the german ring test group (2013) and EPPO 170
Deviations to Guidelines: None.
GLP: Yes (certified laboratory).

Study Objective:	To determine the chronic oral toxicity of the test item Nicosulfuron technical to *Apis mellifera* L under laboratory conditions.	
Test item:	Nicosulfuron technical, batch SCL-70201, purity for Nicosulfuron 99%, expiry December 14h, 2017.	
Reference product:	BAS 152 11 I; Batch number FRE-001226; active ingredient: Dimethoate; content of a.i. analysed: 420.3 g/L density: 1.072 g/cm^3.	
Test organisms:	**Test species:**	*Apis mellifera* L. (Hymenoptera, Apidae)
	Life Stage:	Young adult worker bees (≤ 24h old).
	Source:	Queen-right, healthy colony from a comercial apiary.
	Preparation of test organism:	Two days before the beginning of the test, frames with capped cells are transferred from the hive to an incubator, transported to Trialcamp facilities and ubicated on a bioclimatic chamber. One day prior to test start, the bees will be randomly collected directly from the frames, introduced into the test units and kept under test conditions until start of the test. Acclimatisation period lasted since bee collection to the start of the test. During this period bees were fed *ad libitum* with a 50 % w/v sucrose solution.
Test design:	A single dose of 40 µg Nicosulfuron/bee/day was assessed.; duration 10 days, two control groups, one with untreated sucrose solution 50% w/v and one with sucrose solution mixed with acetone, and the reference product Dimethoate 40% EC at a daily dose of 0.107 µg a.i./bee/day were concurrently tested. Five replicates per treatment each enclosing at least ten bees, were group fed with one feeder per cage containing 1000 µl of test solution, thus providing 100 µl of test solution per bee per day. Five additional test units without bees but with full food syringes containing pure 50 % (w/v) aqueous sucrose solution for evaluation of the evaporation.	
Test concentrations / doses:	Control 1:	C (50 % (w/v) aqueous sucrose solution)
	Control 2:	Sucrose solution + 5% Acetone
	Test Item:	40.00 µg nicosulfuron/bee/day
	Reference Item:	R: 0.107 µg Dimethoate/bee/day.
Test conditions:	Temperature:	33 ± 2 ºC
	Relative humidity:	48.99 – 74.39%
	* Short term deviation (<2 h).	
	Exposure to light:	Constant darkness, except during application and assessments.
Sampling:	Duplicate samples, one shipment and one retain, of Stock solution and Treated Solution from the last day of application will be stored in a freezer at ≤ -18 ºC until shipment and delivery to the analytical laboratory for analytical determination of the actual concentration of the test chemical.	

Analytical verification:

Analytical data were required to demonstrate the concentration of the active ingredient Nicosulfuron (representative sample) and its solubility in the solvent. Quantification was performed by HPLC. The limit of quantification (LOQ) of the analytical method was 9.91 µg/mL, with a limit of detection (LOD) set at 2.97 µg/mL (30 % of the LOQ).

Analytical study was performed to verify the concentration of the samples taken. For the analytical concentration verification, Nicosulfuron residues were determined.

The measured concentration in the samples was within 20 % of nominal test concentration used, thus the concentrations of the test item were confirmed and the endpoints are based on nominal concentrations.

Analytical recoveries for Nicosulfuron

Sample code	Timing	Matrix	Replicate	Nominal Concentration [µg/g*]		Analysed Concentration [µg/g*]		% of Nominal
				(mg/kg)	mg/L*	(mg/kg)	mg/L*	
TRC16-048BA 3S	D9	50 % (w/v) aqueous sucrose solution	1	336.14	400.00	311.66	370.88	92.72
			2	336.14	400.00	311.76	370.99	92.75

* Considering a density of the 50% (w/v) sucrose solution of 1.19 g/mL

Statistics:

Statistical calculations were made by using the statistical program SPSS 19.0; SPSS©Onc, 1989-2010

Mean daily consumptions of the controls and of the test item were compared amongst them by means of a parametric pair wise test (t- test; $\alpha = 0.05$).

The average mortality given by the test item in all the replicates of each concentration at day 10 was first corrected for the control mortality using the Abbott's formula (1925) modified by Shneider – Orelli (1947).

No statistical analysis was performed on mortality data

Findings:

Consumed Diet

Mean daily consumptions in the water control and the solvent control groups were 18.64 and 19.35 µl/bee of the offered diet, respectively.

Mean daily consumption of the bees exposed to the test item was 19.82 µl/bee of the offered diet.

Mean cumulative consumption (consumption over the ten days dosing period) was 79.27 µg Nicosulfuron/bee. No statistical significant difference in mean daily diet consumption was observed between the control groups and between the treatment group and each of the controls.

Daily mean consumption of the reference product bees was 15.04 µl/bee of the offered diet, which corresponds to a daily consumption of 1.42E-02 µg Dimethoate/bee. Cumulative consumption of the reference bees corresponded to a dose of 0.099 µg Dimethoate/bee.

Mortality

Mean cumulative mortality both in the blank control and in the solvent control after the ten days of exposure was 0.00%. Mean cumulative mortality of the hon-

eybees dosed orally with the test item for ten consecutive days was 0.00%. Mean cumulative mortality of the reference product at ten days was 100.00%.

Treatment	10 day cumulative mortality	Abbotts' transformed mortality (%)	Accumulated mean uptake [1]
Sugar solution:			
	[%]		-
U1	0.00	-	-
Sugar solution + acetone			
	[%]		-
U2	0.00	0.00	-
Test item			
	[%]		**[µg a.s./bee]**
T	0.00	0.00	79.27
Reference product			
	[%]		**[µg a.s./bee]**
R	100.00	100.00	0.104(°)

1 Nicosulfuron for the test item
(°) Cumulative over 7 days of application

Conclusion: All validity criteria were met and the study was deemed valid. The endpoints determined are shown in the table below.

The consumed chronic LDD_{50}-value for Nicosulfuron technical was higher than the mean consumed dose of 7.93 µg Nicosulfuron/bee/day.

Based on the mortality data, the NOEDD (No Observed Effect Dietary Dose) was determined to correspond to a daily consumed dose of 7.93 µg Nicosulfuron/bee/day.

No symptoms of intoxication were observed throughout the test for any of the controls bees and for the bees exposed to the test item.

Test item: Nicosulfuron Technical	
LC_{50}	> 336.13 mg a.i./kg food
LDD_{50}	> 7.93 µg a.i./bee/day
NOEC	336.13 mg a.i./kg food
NOEDD	7.93 µg a.i./bee/day

Comments of zRMS:	The study is considered valid. All validity criteria were met. • The mean mortality in the control was ≤ 15 % at the end of the test (Actual 0.00 % for both control and control solvent), • The mean mortality in the reference item group was ≥50 % at the end of

the test (actual 100.00 %).

Agreed endpoints:

Test item: Mesotrione Technical	
NOEC	≥ 2521.01 mg a.s./kg feeding solution
NOEDD	≥ 57.21 µg a.s./bee/day
LC_{50} [95 % IC]	> 2521.01 mg a.s./kg feeding solution [not determined]
LDD_{50} [95 % IC]	> 57.21 µg a.s./bee/day [not determined]

Reference Report:	KCP 10.3.1.2.3 Gimeno, I., 2019 Mesotrione Technical - Chronic Toxicity to the Honey Bee, *Apis mellifera* L. Study code: TRC17-006BA
Source:	Trialcamp S.L.U. Poligon Industrial l'Alter. Avda. Antic Regne de Valencia, 25, 46290 Alcasser (Valencia). Spain. Unpublished report No.: TRC17-006BA. Issued: 2019.
Guidelines:	OECD test No. 245 Guideline for the Testing of Chemicals: Honey bee (*Apis mellifera* L.), Chronic Oral Toxicity Test – 10 Day Feeding (9 October 2017)
Deviations to Guidelines:	None.
GLP:	Yes (certified laboratory).
Study Objective:	To determine the effects of Mesotrione Technical on the honey bee *Apis mellifera* L. from chronic feeding exposure. To do this, the No Observed Effect Dietary Dose/Concentration (NOEDD/NOEC) and the Median Lethal Dietary Dose/Concentration (LDD_{50}/LC_{50}) for day 10 were estimated as long as the data allowed it.
Test item:	Mesotrione Technical. Batch code: SCL-62581. Active substance: Mesotrione. Content of a.s. determined by certificate of analysis: 98.28 % w/w. Expiry date: 11 Mar 2019.
Reference product:	BAS 152 11 I; Batch number FRE-001578; active ingredient: Dimethoate; content of a.i. analysed: 429.0 g/L density: 1.076 g/cm^3.
Test organisms:	**Test species:** *Apis mellifera* L. (Hymenoptera, Apidae) **Life Stage:** Young adult worker bees (not older than 48 hours). **Source:** Commercial bee hives maintained by Trialcamp S.L.U. **Preparation of test organism:** Two days before the beginning of the test, frames with capped cells were transferred from the hive to an incubator, transported to Trialcamp facilities and put into a bioclimatic chamber. One day prior to test start, the bees were randomly collected directly from the frames, introduced into the test units and kept under test conditions until the start of the test. Acclimatisation period lasted since collection to the start of the test. During this period bees were fed ad libitum with 50% w/v sucrose solution.
Test design:	The study was conducted as a dose-response test with test duration of 10 days. A total of 50 bees were tested, divided in 5 parallel replicates, each containing 10 bees. Five additional test units without bees but with full food syringes containing pure 50 % (w/v) aqueous sucrose solution for evaluation of the evaporation.

Test concentrations / doses:
Control: C (50 % (w/v) aqueous sucrose solution)
Solvent crtl group: 50 % (w/v) aqueous sucrose solution + 5 % acetone
5 test item groups: 18.75, 37.50, 75.00, 150.00 and 300.00 µg a.s./bee/day
Reference Item: R: 0.107 µg Dimethoate/bee/day.

Test conditions:
Temperature: 32.1 / 33.0 °C
Relative humidity: 43.8 % / 70.6 % RH
Exposure to light: Constant darkness except during feeding and assessments.

Sampling:
Duplicate samples (one shipment, S, and one retain, R) of the highest and lowest treated solutions and the stock solution were collected on D0 and D9 immediately after preparation of the test solutions. Samples were stored in a freezer at ≤ -18ºC until shipment and delivery to the analytical laboratory for analytical determination of the actual concentration of the test chemical.

Analytical verification:
Analytical data were required to demonstrate the concentration of the active ingredient Mesotrione (representative sample) and its solubility in the solvent. Quantification was performed by HPLC. The limit of quantification (LOQ) of the analytical method was 2.10 µg/mL, with a limit of detection (LOD) set at 0.63 µg/mL (30 % of the LOQ).

Analytical study was performed to verify the concentration of the samples taken. For the analytical concentration verification, Mesotrione residues were determined.

The measured concentration in the samples was within 20 % of nominal test concentration used, thus the concentrations of the test item were confirmed and the endpoints are based on nominal concentrations.

Analytical recoveries for Mesotrione

Sample code	Timing	Matrix	Replicate	Nominal Conc. (mg a.s./kg)	Mean measured Conc. (µg a.s./g)	% of Nominal
TRC17-006BA 6S	D0	50% aqueous sucrose solution + 5% acetone	T1	157.56	138.7	88.0
TRC17-006BA 7S	D0		T5	2521.01	2233.3	88.6
TRC17-006BA 9S	D9		T1	157.56	137.4	87.2
TRC17-006BA 10R	D9		T5	2521.01	2853.1	113.2

T1, T5: Treatment groups, D0, D9: Application day

Statistics:
Endpoints were empirically estimated due to a lack of concentration – response. Moreover no mortality resulted at the highest tested dose/concentration.

Findings:
Results are shown in the tables below.

The mean mortality in the control was ≤ 15 % at the end of the test (actual 0.00 % for both control and control solvent). The mean mortality in the reference item group was ≥50 % at the end of the test (actual 100.00 %). Consequently, the study is considered valid since the validity criteria were met.

The overall mean daily consumption of feeding solutions (i.e. the average consumption/bee over 10 days) in the test item concentrations of 157.56, 315.13, 630.25, 1260.50 and 2521.01 mg a.s./kg feeding solution was 20.81, 19.63, 18.96, 19.63 and 19.07 µL/bee/day, respectively. For the control and solvent control group 18.67 and 20.02 µL/bee/day, respectively. The values of food consumption were corrected for evaporation.

Treatment	10 day cumulative mortality	Overall mean consumption of feeding solution	Dietary dose (based on actual measured consumption of feeding solution)	Mean accumulated uptake of test item during the test period
	[%]	[µL/bee/day]	[µg a.s./bee/day]	[µg a.s./bee]
Control:				
C (–)	0.00	18.67	-	-
Cs(–)	0.00	20.02	-	-
Reference item: dimethoate [µg a.s./bee]				
R (0.107)*	100.00	19.53	0.02	0.13
Test item: Mesotrione Technial [mg a.s./kg feeding solution]				
T1 (157.56)	2.00	20.81	3.90	39.02
T2 (315.13)	0.00	19.63	7.36	73.62
T3 (630.25)	2.00	18.96	14.22	142.19
T4 (1260.50)	2.00	19.63	29.44	294.38
T5 (2521.01)	0.00	19.07	57.21	572.10

* During 6 days of continuous exposure.

Conclusion:

The chronic toxicity of Mesotrione Technical to honey bees was tested under laboratory conditions over a period of 10 days.

The actual mean concentrations of Mesotrione in test item feeding solutions were in the range of 83.8 to 113.2 % of the nominal concentrations; therefore, results are based on nominal.

The 10-day NOEC was empirically estimated to be greater than or equal to 2521.01 mg a.s./kg feeding solution.

The 10-day NOEDD was empirically estimated to be greater than or equal to 57.21 µg a.s./bee/day.

The 10-day LC_{50} was empirically estimated to be greater than 2521.01 mg a.s./kg feeding solution.

The 10-day LDD_{50} w was empirically estimated to be greater than 57.21 µg a.s./bee/day.

The test was deemed valid since all validity criteria were met.

Test item: Mesotrione Technical	
NOEC	≥ 2521.01 mg a.s./kg feeding solution
NOEDD	≥ 57.21 µg a.s./bee/day
LC_{50} [95 % IC]	> 2521.01 mg a.s./kg feeding solution [not determined]
LDD_{50} [95 % IC]	> 57.21 µg a.s./bee/day [not determined]

Comments of zRMS:	The study is considered valid. All validity criteria were met. - There was no mortality in control group. - The average mortality in the reference substance treated group is 86.67 (≥ 50 % at the end of the test (10 days following start of exposure) **Agreed endpoints:** LDD_{50} =5.08±0.18 µg/bee, which is equivalent to 0.15 µg Rimsulfuron/bee, 0.61 µg Nicosulfuron/bee and 1.83 µg Mesotrione/bee. LC_{50} =293.35 ± 14.1 mg/kg, which is equivalent to 8.80 mg Rimsulfuron/kg, 35.20 mg Nicosulfuron/kg and 105.61mg Mesotrione/kg. The NOEC =103 mg as/kg, which is equivalent to 3.09 mg Rimsulfuron/kg, 12.36 mg Nicosulfuron/kg and 37.08 mg Mesotrione/kgfood. NOEDD =2.49 µg as/bee, which is equivalent to 0.07 µg Rimsulfuron/bee, 0.30 µg Nicosulfuron/bee and 0.90 µg Mesotrione/bee.

Reference:	KCP 10.3.1.2.4
Report	"Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG on adult honey bee (*Apis mellifera L.)*, Chronic Oral Toxicity Test". Dr.S. Radha (2022). Bioscience Research foundation. Report No.7961/2020.
Guideline(s):	OECD test No. 245 Guideline for the Testing of Chemicals: Honeybee (*Apis mellifera* L.), Chronic Oral Toxicity Test – 10 Day Feeding (9 October 2017).
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

A range finding study was conducted for Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG with 0.1, 1, 5, 10 and 100 µg/bee in sucrose solution (50% w/v) was conducted to determine the concentrations for the Dose Response Test. Honey bees were acclimatized in the test unit for 24 hours before treating with test substance at different concentrations. A concurrent control group (50% w/v sucrose solution) was also maintained. After 24 h of treatment, the treated diet was withdrawn from the respective treatment groups and replaced with freshly treated diet.The Cumulative moratlity observed at the end of the 10^{th} day of exposure was 0.00, 0.00, 46.66, 83.33 and 100% in bees exposed to control, 0.1, 1, 5, 10 and 100 µg/bee respectively. Based on the results of range finding study, Dose Response Test was conducted with five doses. The bees were orally treated with control, 1 14, 2 06, 3.7, 6.67 and 10 µg/bee concentrations in 50% w/v sucrose solution. A concurrent control group with 50% w/v sucrose solution was also maintained. A toxic reference standard group exposed to 0.8 mg/kg a.i dimethoate/bee in sucrose solution (50% w/v) was also maintained. The mortality of the 0.8 mg a.i/kg reference standard was 86.67%, which was within the range at 10 days following the test substance exposure.

Materials and methods

Test item:	Rimsulfuron 3%+ Nicosulfuron12% + Mesotrione 36% WG Batch No.: SCL - 78944
Test system:	Species: *Apis mellifera* (L). Hymenoptera, Apoidea) (strain: carnica) Source: Bee hive maintained at BRF test facility Stage: 2 days old young adult bee
Dose response test:	Untreated control The test item: 5 concentrations: 57, 103, 185, 333.5 and 500 mg/kg food, corresponding to daily doses of 1.14, 2.06, 3.7, 6.67 and 10 µg/bee/day (nominal- 0.1 g feeding solution/ bee/day) Consumed dose: 1.36, 2.49, 3.73, 5.20 and 7.90 µg/bee/day.
Number of larvae/ Replication:	10 per replicate 3 replicates
Duration:	22 days

Results and discussions

Based on the results of range finding study, the Dose Response Test was conducted. Honeybees were acclimatized for 24 hours. The bees were orally treated with control, 1.14, 2.06, 3.7, 6.67 and 10 µg/bee concentrations in 50% w/v sucrose solution. A concurrent control group with 50% w/v sucrose solution was also maintained.

The average feed consumption recorded for 10 days of treatment with test substance was 1.36, 2.49, 3.73, 5.20 and 7.90 µg/bee corresponding to the nominal dose 1.14, 2.06, 3.7, 6.67 and 10 µg/bee respectively.

At the end of every 24 hour, (10 days exposure) observation bees treated with control group were appeared normal and no toxic sign was observed.

- From day 1 to day 2 exposure, 0% mortality and no clinical signs were observed in bees treated

with control, 1.14, 2.06, 3.7, 6.67 and 10 µg/bee.

- On day 3, bees were found affected in 10 µg/bee.
- From day 4 and day 5, bees were found affected in 6.67 and 10 µg/bee.
- From day 6 and Day 7, mortality and toxicity signs (affected) was observed in bees exposed to 3.7, 6.67 and 10 µg/bee whereas bees exposed to control, 1.14 and 2.06 µg/ bee appeared normal.
- On day 8, mortality and toxicity signs (affected) was observed in bees exposed to 3.7, 6.67 and 10 µg/bee whereas bees exposed to control, 1.14 and 2.06 µg/bee appeared normal.
- On day 9, mortality and toxicity signs (affected) was observed in bees exposed to 2.06, 3.7, 6.67 and 10 µg/bee whereas bees exposed to control and 1.14 µg/bee appeared normal.
- On day 10, mortality and toxicity signs (affected) was observed in bees exposed to 2.06, 3.7, 6.67 and 10 µg/bee whereas bees exposed to control and 1.14 µg/bee appeared normal

The cumulative moratlity observed at the end of the 10th day of exposure was 0, 3.33, 33.33, 46.67 and 83.33% in bees exposed to 1.14, 2.06, 3.7, 6.67 and 10 µg/bee. Control group appeared normal and 0% mortality was observed. On the completion of the study all live bees were euthanized with CO2 exposure and safely disposed.

Table 1: Dose Response test- Summary of mortality and test item intake.

Initial		**Consumed**		**No. of tested bees**	**Mortality total**		**LC_{50}**	**LDD_{50}**
Concentration [mg/kg of food]	**Dose [µg /20mg/ bee/ day]**	**Concentration [mg/kg of food]**	**Dose [µg /bee/ day]**			**No. of bees[%]**	**[mg/kg]**	**[µg /bee/ day]**
Rimsulfuron 3%+ Nicosulfuron 12%+ Mesotrione 36% WG								
Control				30	0	0	293.35 ± 14.1	5.08 ± 0.18
57	1.14	57	1.36	30	0	0		
103	2.06	103	2.49	30	1	3.33		
185	3.7	185	3.73	30	10	33.33		
333.5	6.67	333.5	5.20	30	14	46.67		
500	10	500	7.90	30	25	83.33		
Dimethoate								
0.8 mg a.i./kg	0.016 kg a.i./bee	0.8 mg a.i./kg	0.0144 µg a.i./bee	30	26	86.67	-	
Rimsulfuron 3%+Nicosulfuron 12%+Mesotrione 36% WG	NOEC [mg/kg]				103			
	NOEDD [µg /bee/ day]				2.49			

Validity criteria

- There was no mortality in control group.
- The average mortality in the reference substance treated group is 86.67 (≥ 50 % at the end of the test (10 days following start of exposure).

Conclusion

Based on the Experimental results, the LDD_{50} was calculated to be 5.08±0.18 µg/bee, which is equivalent to 0.15 µg Rimsulfuron/bee, 0.61 µg Nicosulfuron/bee and 1.83 µg Mesotrione/bee.

LC_{50} was determined as 293.35 ± 14.1 mg/kg, which is equivalent to 8.80 mg Rimsulfuron/kg, 35.20 mg Nicosulfuron/kg and 105.61mg Mesotrione/kg.

The NOEC was determined as 103 mg as/kg, which is equivalent to 3.09 mg Rimsulfuron/kg, 12.36 mg Nicosulfuron/kg and 37.08 mg Mesotrione/kgfood.

NOEDD was determined as 2.49 µg as/bee, which is equivalent to 0.07 µg Rimsulfuron/bee, 0.30 µg Nicosulfuron/bee and 0.90 µg Mesotrione/bee.

Reference substance study: The mortality of reference substance 0.8 mg a.i/kg was found to be 86.67% between the stipulated range of 0.5 - 1.0 mg a.i. /kg for 10 days exposure on *Apis mellifera.*

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

Comments of zRMS:

The study is considered valid. All validity criteria were met.

- The cumulative larval mortality from day 3 (D3) until day 8 (D8) was ≤ 15 % across all replicates (actual 14.58 %)
- On day 22 (D22) the adult emergence rate is ≥ 70% across all replicates (actual 77.08 %)
- Reference Item Mortality: The cumulative larval mortality was ≥ 50 % across all replicates on Day 8 (D8) (actual 64.58 %)

Endpoints for D22

Endpoint	Active ingredient	Test item
LC_{50} (95 % Confidence limits)	> 649.091 mg rimsulfuron/kg diet (Not determined)	> 661.056 mg test item/kg diet (Not determined)
LD_{50} (95 % Confidence limits)	> 100.000 µg rimsulfuron/larva (Not determined)	> 101.843 µg test item/larva (Not determined)
LC_{10}	Not determined	Not determined
LD_{10}	Not determined	Not determined
NOEC	≥ 649.091 mg rimsulfuron/kg diet	≥ 661.056 mg test item/kg diet
NOED	≥ 100.000 µg rimsulfuron/larva	≥ 101.843 µg test item/larva

Reference	KCP 10.3.1.3.1
Report:	Aguilar-Alberola, J.A. & Marín Víllora, M. 2018. Toxicity of Rimsulfuron Technical on honeybee larvae (*Apis mellifera* L.) after repeated exposure under laboratory conditions
Test facility:	Trialcamp S.L.U. Unpublished report No.: TRC16-162BA. Issued: 2018.
Guidelines:	OECD Guidance Document No. 239 (2016)
Deviations to Guidance:	None.
GLP:	Yes (certified laboratory).
Study Objective:	To determine the effects of the test item Rimsulfuron Technical on the honeybee larvae (*Apis mellifera* L.), after a repeated exposure in a 22 day in vitro test and to determine the No Observed Effect Dose/Concentration (NOED/NOEC) and the median Lethal Dose/Concentration (LD_{50}/LC_{50}), where possible.
Test item:	Rimsulfuron Technical; Batch number: SCL-30188; active ingredient: Rimsulfu-

	ron; content of a.i. determined by certificate of analysis: 98.19 % w/w.
Reference item:	Dimethoate Technical; Batch number 305015A161; active ingredient: Dimethoate; content of a.i. analysed: 99.9 % w/w.
Test organisms:	Honey bee (*Apis mellifera* L.), synchronized first instar (L1) larvae not older than 30 hours at grafting time.
Source:	Commercial beehives from the in-house test facility stock, adequately fed, healthy and as far as possible disease-free and queen-right.
Preparation of test organisms and larvae collection:	At D-3, the queens of at least three colonies were confined in their own hive containing a comb with empty cells. At D-2, maximum 30 hours after encaging, the queens were released. Combs containing eggs were left in the excluder cages until hatching (D1). Three combs from different hives, containing the highest number of synchronized larvae, were selected for grafting in the laboratory.
Test design:	Dose response test with duration of 22 days from grafting on day 1 to the final assessment on day 22. From day 3 until day 6 of the test, five different concentrations of Rimsulfuron Technical were applied to the larvae of the test item groups, and one single concentration of the reference item was applied to the larvae of the reference item group. Both, test and reference items, were supplied with diet B or C. The analyzed content of rimsulfuron was considered for calculation of the test item doses and dimethoate for the reference item dose. The daily feeding volume increased from 20 µL to 50 µL diet per larva over the application period. The cumulative feeding volume from day 3 until day 6 of 140 µL diet per larva and the density of the diet (1.1 g/cm3) were considered for the calculation of the cumulative doses per larva. A control group was included in the test and exposed for the same period of time under identical exposure conditions to the untreated artificial diet. Each treatment group consisted of 48 larvae from three different colonies (each colony representing a replicate). Mortality assessments were on days 4, 5, 6, 7, and 8. The presence of uneaten food was qualitatively recorded on day 8. Assessment of mortality during pupation phase on day 15 and assessment of emergence on day 22.
Test concentrations and doses:	Control: One control group (U). Test Item: Five test item groups (T1 – T5) with 27.775, 62.032, 137.026, 300.901 and 661.056 mg test item/kg diet, equivalent to the cumulative doses of 4.269, 9.391, 20.661, 45.455 and 100.000 µg rimsulfuron/larva. Reference item: One reference item group (R) with a cumulative dose of 7.39 µg dimethoate/larva.
Endpoints:	NOEC/NOED and LC_{50}/LD_{50} on day 22.
Test conditions:	Air Temperature: Min / Max: 33.7 °C* / 36.6 °C** Relative humidity: Min / Max: 0.0 %RH / 100.0 % RH (Data recorded during period D15-D22 seems not reliable) Exposure to light: Constant darkness except during feeding and assessments *Short term deviation (<2 hours), ** Deviation (>2 hours).
Sampling:	The sampling was performed on every application day. Samples of each stock solution were collected and located in the freezer at < -18 ºC until shipment.
Analytical verification:	A method was validated and specimens of aqueous solution were analysed for concentration determination of rimsulfuron. Quantification was performed by HPLC. The limit of quantification (LOQ) of the analytical method was 10.76 µg/mL with a limit of detection (LOD) set at 3.23 µg/mL (30 % of the LOQ).

Sample description	Nominal concentration of Rimsulfuron [µg/g]	Analysed concentration of Rimsulfuron [µg/g]	Recovery [%]
Stock solution at D3	7138.41	6741.6	94.4
Stock solution at D4	7138.41	6212.8	87.0
Stock solution at D5	7138.41	6131.8	85.9
Stock solution at D6	7138.41	6725.6	94.2

Statistics: Since in all test item groups the mortality was below 50 % at 22D, the LC_{50}/LD_{50} values could not be calculated.
It was decided not to calculate or estimate the endpoints LC_{10} and LD_{10} due to the lack of statistically significant dose/response.
In order to determine the NOED, a Chi2 2x2 Test with Bonferroni correction (one-sided greater, $\alpha = 0.05$) was used.
Statistical calculations were made with the statistical program ToxRatPro Version 3.2.1.

Findings: In the control group, the cumulative larval mortality from day 3 (D3) until day 8 (D8) was 14.58 %. On day 22 (D22), the adult emergence rate in the control group was 77.08 % of the initial grafted larvae. Therefore the validity criteria for control group was met for both test periods; the D8 mortality was under 15.00 % and the D22 emergence rate was greater than 70.00 %, across all replicates.

In the test item doses of 4.269, 9.391, 20.661, 45.455 and 100.000 µg rimsulfuron/larva the cumulative mean mortality at 22 days (D22) after grafting was 31.25, 18.75, 29.17, 18.75 and 33.33 % respectively, equivalent to the mean emergence rate of 68.75, 81.25, 70.83, 81.25 and 66.67 %. No statistically significant differences in the adult emergence at D22 were determined at any of the test item concentrations compared to the control group.

At day 8 there were no affected larvae. Larvae with presence of uneaten food were recorded at treatment levels T1 and T3 (4.269 and 20.661 µg rimsulfuron/larva, respectively). This behavioural effect represented a 6.52 %, and 2.33 % of alive larvae at T1 and T3 treatments, respectively. At the end of the test, in the final assessment of the emergence on day 22 there was not recorded any affected emerged bee (i.e. malformation).

Cumulative mortality in the Reference Item group was 64.58 % at day 8 and 89.58 % at day 22 across all replicates.

Mortality Results of all Treatment Groups at D22

Treatment Group	Dose [µg a. i./larva] [a]	Cumulative Mortality [%]	Corrected Mortality [%]
Control	-	22.92	-
Test Item Rimsulfuron Technical	4.269	31.25	10.81
	9.391	18.75	-5.41
	20.661	29.17	8.11
	45.455	18.75	-5.41
	100.00	33.33	13.51
Reference Item (dimethoate)	7.39	89.58	86.49

[a] Based on the analysed content of active ingredient (rimsulfuron for the test item, dimethoate for the toxic reference item).

Conclusions: In a repeated exposure larval toxicity test with Rimsulfuron Technical and a duration of 22 days, cumulative mortality in the Control group was 14.58 % on D8 and 22.92 % on D22. In the Reference Item group was 64.58 % on D8 and 89.58 % on D22 across all replicates. The study was deemed valid since all validity criteria were met.

The 22-Day adult emergence No Observed Effect Dose (NOED) was determined to be greater than or equal to 100.000 µg rimsulfuron/larva, equivalent to 101.843 µg test item/larva. Based on the NOED value, the corresponding No Observed Effect Concentration (NOEC) was empirically estimated to be greater than or equal to 649.091 mg rimsulfuron/kg diet, equivalent to 661.056 mg test item/kg diet.

The 22-Day adult emergence-LD_{50} was empirically estimated to be greater than 100.000 µg rimsulfuron/larva, equivalents to 101.843 µg test item/larva. With regard LC_{50} value, was estimated to be greater than 649.091 mg rimsulfuron/kg diet, equivalent to 661.056 mg test item/kg diet.

Endpoints for D22

Endpoint	Active ingredient	Test item
LC_{50} **(95 % Confidence limits)**	> 649.091 mg rimsulfuron/kg diet (Not determined)	> 661.056 mg test item/kg diet (Not determined)
LD_{50} **(95 % Confidence limits)**	> 100.000 µg rimsulfuron/larva (Not determined)	> 101.843 µg test item/larva (Not determined)
LC_{10}	Not determined	Not determined
LD10	Not determined	Not determined
NOEC	≥ 649.091 mg rimsulfuron/kg diet	≥ 661.056 mg test item/kg diet
NOED	≥ 100.000 µg rimsulfuron/larva	≥ 101.843 µg test item/larva

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

- Larval mortality in the controls: ≤15% for larvae across all control replicates (between D3 and D8) - being 0 %
- Adult emergence rate: ≥ 70% for bees across all control replicates (between D3 and D22), being from 72.2 to 75%
- Larval mortality in the reference item: ≥ 50% for larvae exposed to 7.6 µg a.i./larva across all reference replicates (between D3 and D8)- 63.9% mortality (between D3 and D8)

Agreed endpoints:

Treatment	Endpoint: Successful adult emergence	Up to D22
Test item doses	ED_{50} [µg a.i./larva][4] (95% CL)	**14.3** (10.2 – 20.2)
	ED_{20} [µg a.i./larva][4] (95% CL)	4.3 (2.9 – 6.4)
	ED_{10} [µg a.i./larva][4] (95% CL)	2.3 (1.4 – 3.8)
	NOED [µg a.i./larva][3]	1.8
Test item concentra-	EC_{50} [mg a.i./kg food][4] (95% CL)	**91** (64 – 128)
	EC_{20} [mg a.i./kg food][4] (95% CL)	27 (18 – 40)

	tions	EC_{10} [mg a.i./kg food][4] (95% CL)	14 (9-24)
		NOEC [mg a.i./kg food][3]	11
	[3] Step-down Cochran-Armitage Test; alpha=0.05; one sided greater [4] Probit analysis using linear maximum likelihood regression; alpha=0.05; one sided greater		

Reference	KCP 10.3.1.3.2
Report:	Scheller, K., 2018. Mesotrione Technical - Repeated exposure of honey bee (*Apis mellifera* L.) larvae under laboratory conditions (*in vitro*)
Test facility:	BioChem agrar Unpublished report No.: 17 48 BLC 0088. Issued: 2018.
Guidelines:	Guidance Document on Honey Bee Larval Toxicity Test following Repeated Exposure, Series on Testing and Assessment, No. 239, OECD (2016) with adaptations based on SCHMEHL et al. (2016)
Deviations to Guidance:	None.
GLP:	Yes (certified laboratory).
Study Objective:	The purpose of this study was to determine the chronic toxicity (e.g., ED_{50}, EC_{50}, NOED and NOEC, and $ED_{10/20}$ and $EC_{10/20}$ if possible, for adult emergence up to D122) of the test item applied to the honey bee, *Apis mellifera* L., larvae in an in vitro test after repeated oral application.
Test item:	Mesotrione Technical, Batch No.: SCL-62581; Analyzed purity: 98.28% (w/w)
Reference item:	Dimethoate tech. (analysed purity: 98.8% w/w)
Test organisms:	Honey bee – *Apis mellifera iberiensi*s Engel (Hymenoptera, Apoidea): first instar larvae; derived from three healthy and queen-right colonies
Source:	Beekeeper Joaquin Cordero, Paseo de Colón No. 19, 41370 Cazalla (Sevilla), Spain

Preparation of test organisms and larvae collection:

One day old honeybee larvae (D1) of *Apis mellifera* L. were transferred from brood combs to polystyrene grafting cells in 48-well cell culture plates 3 days before start of the treatment. On 4 successive days (D3 to D6) the larvae were repeatedly exposed to Mesotrione Technical diluted in the larval food (aqueous sugar solution mixed with royal jelly). After the applications no additional feedings of the larvae took place.

Test design:

In total, 8 treatment groups were set up: 5 doses of the test item, two untreated control groups and 1 dose of the reference item with 3 replicates per dose and 12 larvae per replicate.

Assessments of cumulative larval mortality were done on D4, D5, D6, D7 and D8. Additionally, other observations such as small body size or large quantities of remaining food on D8 were noted. Pupal mortality was assessed at D15 and emergence of adults was evaluated at D22.
In an analytical phase of the study the concentration of the active substance in the test item stock base solution and in the control was determined.

Test concentrations and doses:

Controls: AC untreated diet B/C (50% aqueous yeast/sugar solution and 50% royal jelly containing 0.5 % v/v water)
BC untreated diet B/C (50% aqueous yeast/sugar solution and 50% royal jelly containing 0.5 % v/v acetone)
Test item: AT treated diet B/C at a concentration of 317 mg a.i./kg food
BT treated diet B/C at a concentration of 105 mg a.i./kg food
CT treated diet B/C at a concentration of 35 mg a.i./kg food

DT treated diet B/C at a concentration of 11 mg a.i./kg food
ET treated diet B/C at a concentration of 4 mg a.i./kg food
Reference: AR treated diet B/C at a concentration of 48 mg a.i./kg food

Endpoints: Successful adult emergence (dose-effect relationship), mortality, qualitative observations: e.g. body size, remaining food

Test conditions:
Air Temperature: 34.0 °C – 34.8 °C
Relative humidity: D1 - D8: 90.1 – 99.9%
D8-D15: 80.1 – 84.4%
D15-D22: 61.4 – 65.9%
Photoperiod: Darkness (except during assessments)
Food: 50% aqueous sugar solution and 50% royal jelly

Sampling: Samples of the stock solution were taken on D3, D4, D5 and D6. Until analysis the samples were stored at ≤-18 °C at all times.

Analytical verification: The purpose of the analytical phase of the study was the verification of the concentration of the active ingredient mesotrione in the test item stock solutions. The determination was conducted by an in-house developed method using high performance liquid chromatography (HPLC) with DAD-detection. The limit of quantification (LOQ) of the analytical method was 408.9 mg/L.

The recoveries of mesotrione in the specimens were between 80% and 108%. No active ingredient was detected in the control specimens. Thus, the concentration of the stock solutions from the biological part was verified.

Statistics: Descriptive statistics; Step-down Cochran-Armitage Test (one-sided greater, alpha = 0.05) for determination of NOED/NOEC. ED/ECx values were determined using the Probit analysis with linear maximum likelihood regression.

Findings: On D8, no larval mortalities (0.0%) were observed in the controls AC and BC, respectively. Pupal mortality (between D8 and D22) was 25.0% in the control AC and 27.8% in the solvent control BC. The control groups showed a total mortality of 25.0% (AC), and 27.8% (BC), respectively, on D22. In the test item groups, larval mortalities on D8 ranged between 0.0% and 33.3% (0.0% and 33.3% as corrected for solvent control). Pupal mortalities ranged between 25.0% and 79.2% (0.0% and 71.2% as corrected for solvent control) in the test item treatment groups. Total mortalities on D22 ranged between 25.0% and 86.1% (0.0% and 80.8% as corrected for solvent control). Mortality in the reference (AR) was above 50% across all replicates on D8, being 63.9% (63.9% as corrected for untreated control).

On D8, other observations (e.g. remaining food, small body size) were not observed in any larvae exposed to the test item.

In the final assessment on D22, adult emergence rates of honeybees in the controls were 75.0% (AC) and 72.2% (BC). Adult emergence rate of honeybees treated with test item during the larval stages ranged from 13.9% to 75.0% following an application of 50.1, 16.5, 5.5, 1.8 and 0.6 µg a.i./larva, respectively (cumulative mortality: 86.1% to 25.0%; corrected for control mortality: 80.8% to 0.0%, respectively). Statistically significant effects on adult emergence occurred

for the treatment groups, where larvae were exposed to 50.1 µg a.i./larva (adult emergence: 13.9%, cumulative mortality: 86.1%, cumulative mortality corrected for control: 80.8%), 16.5 µg a.i./larva (adult emergence: 36.1%, cumulative mortality: 63.9%, cumulative mortality corrected for control: 50.0%) and 5.5 µg a.i./larva (adult emergence: 50.0%, cumulative mortality: 50.0%, cumulative mortality corrected for control: 30.8%).

Because control mortality was ≤ 15% on D8, corrected cumulative mortality in the reference item dose of 7.6 µg a.i./larva was ≥ 50% on D8 and adult emergence in the control was ≥ 70% on D22, the study can be regarded as valid.

Toxicity of Mesotrione Technical to larvae of *Apis mellifera* L.

Treatment group	ID	Dose	Conc.	On D8			On D22				
				Larval mort. D3 - D8		Mean OO	Pupal mort. D8-D22[1]		Total mort. D3-D22		Adult emergence rate[2]
		[µg a.i./larva]	[mg a.i./kg food]	[%]		[%]	[%]		[%]		[%]
				abs.	corr.		abs.	corr.	abs.	corr.	abs.
Ctrl	AC	-	-	0.0	-	0.0	25.0	-	25.0	-	75.0
	BC	-	-	0.0	-	0.0	27.8	-	27.8	-	72.2
Test item	AT	50.1	317	33.3	33.3	0.0	79.2	71.2	86.1	80.8	13.9*
	BT	16.5	105	27.8	27.8	0.0	50.0	30.8	63.9	50.0	36.1*
	CT	5.5	35	0.0	0.0	0.0	50.0	30.8	50.0	30.8	50.0*
	DT	1.8	11	0.0	0.0	0.0	33.3	7.7	33.3	7.7	66.7
	ET	0.6	4	0.0	0.0	0.0	25.0	0.0	25.0	0.0	75.0
Ref. item	AR	7.6	48	63.9	63.9	0.0	61.5	48.7	86.1	81.5	13.9

Results are averages based on 3 replicates, containing 12 larvae each
corr.: corrected mortality (according to SCHNEIDER-ORELLI 1947): reference item was corrected by AC and test item was corrected by BC; negative values are set to "0"; calculations are performed with non-rounded values; CL. confidence limit
* Statistically significant difference in pairwise comparison between treatment and untreated control (Step-down Cochran-Armitage Test; alpha=0.05; one sided greater)
OO: Other observations (*e.g.* remaining food)
[1] Average% of pupal mortality was calculated according to the following formula: Sum of dead between D8 and D22 / Sum of living larvae on D8 x 100%
[2] Adult emergence [%] = 100 [%] – Mortality of D22 [%]

Conclusions:

In a repeated exposure larval toxicity study with Mesotrione Technical, the ED_{50} (successful adult emergence up to D22) was calculated to be 14.3 µg a.i./larva, which is equivalent to an EC_{50} of 91 mg a.i./kg food.
The ED_{20} (D22) was determined to be 4.3 µg a.i./larva, which is equivalent to an EC_{20} of 27 mg a.i./kg food.
The ED_{10} (D22) was determined to be 2.3 µg a.i./larva, which is equivalent to an EC_{10} of 14 mg a.i./kg food.
The NOED was 1.8 µg a.i./larva and the corresponding NOEC was 11 mg a.i./kg food.

Treatment	Endpoint: Successful adult emergence	Up to D22
Test item doses	ED_{50} [µg a.i./larva][4] (95% CL)	**14.3** (10.2 – 20.2)
	ED_{20} [µg a.i./larva][4] (95% CL)	4.3 (2.9 – 6.4)
	ED_{10} [µg a.i./larva][4] (95% CL)	2.3 (1.4 – 3.8)
	NOED [µg a.i./larva][3]	1.8
Test item concentrations	EC_{50} [mg a.i./kg food][4] (95% CL)	**91** (64 – 128)
	EC_{20} [mg a.i./kg food][4] (95% CL)	27 (18 – 40)
	EC_{10} [mg a.i./kg food][4] (95% CL)	14 (9-24)
	NOEC [mg a.i./kg food][3]	11

[3] Step-down Cochran-Armitage Test; alpha=0.05; one sided greater
[4] Probit analysis using linear maximum likelihood regression; alpha=0.05; one sided greater

Comments of zRMS:

The study is considered valid. All validity criteria were met.

- Larval mortality in the control: In control (A1), the cumulative larval mortality from D3 to D8 was 5.56% (Criterion: should be ≤15% across all control replicates).
- Adult emergence rate: In control (A1), the adult emergence rate on D22 was 91.67% respectively. (Criterion: should be ≥ 70% across all control replicates).
- Reference item: The larval mortality in standard reference chemical (Dimethoate) on D8 was 87.88% (Criterion: should be ≥ 50% across all reference replicates).

Agreed endpoints:

ED_{50} =0.68 µg/larva (i.e., 0.02 µg rimsulfuron/larva, <0.08 µg nicosulfuron/larva and 0.24 µg mesotrione/larva), which is equivalent to an EC_{50} of 4.45 mg/kg (i.e. 0.13 mg rimsulfuron/kg food, 0.53 mg nicosulfuron/kg food and 1.60 mg mesotrione/kg food).

The ED_{10} <0.2 µg/larva (i.e., < 0.01µg rimsulfuron/larva, <0.02 µg nicosulfuron/larva and <0.07 µg mesotrine/larva) which is equivalent to an EC_{10} of <1.3mg/kg (i.e. <0.04 mg rimsulfuron/kg food, <0.16 mg nicosulfuron/kg food and <0.47 mg mesotrione/kg food).

The ED_{20} <0.2 µg/larva (i.e. <0.01 µg rimsulfuron/larva, <0.02µg nicosulfuron/larva and <0.07 µg mesotrione /larva) which is equivalent to an EC_{20} of <1.3 mg/kg (i.e. <0.04 mg rimsulfuron/kg food, <0.16 mg nicosulfuron/kg food and <0.47 mg mesotrione /kg food).

The NOED <0.2 µg/larva (i.e. <0.01µg rimsulfuron/larva, <0.02 µg nicosulfuron/larva and <0.07 µg‘mesotrione /larva) and the corresponding NOEC was <1.3 mg/kg food (i.e. <0.04 mg rimsulfuron/kg food, <0.16 mg nicosulfuron/kg food and <0.47 mg mesotrione /kg food).

Reference:	KCP 10.3.1.3.3
Report	"Effect of Rimsulfuron 3%+ Nicosulfuron12% + Mesotrione 36% WG on larvae of honey bee, *Apis mellifera* (L.) following repeated exposure". Dr.S. Radha. 2022. Bioscience Research Foundation. Report No. 7962/2020
Guideline(s):	OECD Guideline for the testing of chemicals, No. 239 (2016): Guidance Document on Honey Bee Larval Toxicity Test following Repeated Exposure, Series or Testing and Assessment.
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:	Rimsulfuron 3%+ Nicosulfuron12% + Mesotrione 36% WG Batch No.: SCL - 78944
Test system:	Species: *Apis mellifera* (L). Hymenoptera, Apoidea) (strain: carnica) Source: Bee hive maintained at BRF test facility Stage: First instar larvae (L1, one day old) during grafting of queen-right colonies in good health conditions.
Dose response test:	Control, 0.2,0.3,0.44,0.67 and 1µg/larvae
Number of larvae/ Replication:	12 per replicate 3 replicates
Duration:	22 days

Results and discussions

On D8, larval mortalities of 5.56% was observed in control group. Pupal mortality (between D8 and D15) was 8.33% in the control group. The control group showed a total mortality of 8.33% in control (A1) on D22.

In the test item group 0.2, 0.3, 0.44, 0.67 and 1µg/larva, larval corrected mortalities on D8 was 5.88, 11.76, 8.82, 14.71 and 20.59% respectively. Pupal mortalities 2.86, 2.86, 8.57, 11.43and 14.29% in 0.2, 0.3, 0.44, 0.67 and 1µg/larva respectively. Total mortalities at D22 was 18.18, 33.33, 36.36, 48.48 and 60.61% observed in 0.2, 0.3, 0.44, 0.67 and 1 µg/larva respectively.

Mortality in the reference (R1) was 50% across all replicates on D8; pupal mortality was D (15) 5.71%. Total mortalities at D22 was 87.88% respectively.

Table 1: Toxicity of Rimsulfuron 3%+ Nicosulfuron12% + Mesotrione 36% to larvae of *Apis mellifera L.*

Treatment group	Test solution (ID)	Dose (µg/larva)	Conc. (mg/kg food)	On D8		On D15	On D 22	
				Larval mortality D3 to D8	Mean (OO)	Pupal stage D8 to D15	Total mortality D3-D22	Adult emergence rate %

				mor (%)	corr (%)	(%)	mor (%)	corr (%)	mor (%)	corr (%)	(%)
Control	A1	control	-	5.56	-	-	2.78	-	8.33	-	91.67
Test item	T1	0.2	1.300	11.11	5.88	0	5.56	2.86	25.00	18.18	75.00
	T2	0.3	1.950	16.67	11.76	0	5.56	2.86	38.89	33.33	61.11
	T3	0.44	2.860	13.89	8.82	0	11.11	8.57	41.67	36.36	58.33
	T4	0.67	4.355	19.44	14.71	0	13.89	11.43	52.78	48.48	47.22
	T5	1	6.500	25.00	20.59	0	16.67	14.29	63.89	60.61	36.11
Ref. item	R1	7.39 µga.i./bee	48 mg a.i./kg	72.22	70.59	0	8.33	5.71	88.89	87.88	11.11

Note: D-Day, Mor- Mortality, corT.-Corrected Mortality, O0-Other observation. Results are averages based on 3 replicates, containing 12 larvae each;
corr.: corrected mortality (according to SCHNEIDER-ORELLI 1947): test item was corrected by A2 and reference item were corrected by A1; negative values are set to "0"; calculations are performed with non-rounded values; CL. confidence limit
OO: Other observations (e. g. remaining food)
1 Average% of pupal mortality was calculated according to the following formula:
Sum of dead between D8 and D22 / Sum of living larvae on D8 x 100%
2 Adult emergence [%] = 100 [%] – Mortality of D22 [%]

		Up to D22			
Test item doses	**Endpoint with 95% CL**	**Value based on Nominal Dose (µg/larva)**	**Value based on Rimsulfuron[a] content (µg a.s./larva)**	**Value based on Nicosulfuron[b] content (µg a.s./larva)**	**Value based on Mesotrione[c] content (µg a.s./larva)**
	ED_{10}	<0.2	<0.01	<0.02	<0.07
	ED_{20}	<0.2	<0.01	<0.02	<0.07
	ED_{50}	0.68	0.02	0.08	0.24
	NOED	<0.2	<0.01	<0.02	<0.07
Test item concentrations	**Endpoint with 95% CL**	**Value based on Nominal Dose (mg/kg food)**	**Value based on Rimsulfuron[a] content (µg a.s./larva)**	**Value based on Nicosulfuron[b] content (µg a.s./larva)**	**Value based on Mesotrione[c] content (µg a.s./larva)**
	EC_{10}	<1.3	<0.04	<0.16	<0.47
	EC_{20}	<1.3	<0.04	<0.16	<0.47
	EC_{50}	4.45	0.13	0.53	1.60
	NOEC	<1.3	<0.04	<0.16	<0.47

a: based on the content of Rimsulfuron in the test the item, i.e., 30 g/kg
b: based on the content of Nicosulfuron in the test the item, i.e., 120 g/kg
c: based on the content of Mesotrione in the test the item, i.e., 360 g/kg

Validity criteria

- Larval mortality in the control: In control (A1), the cumulative larval mortality from D3 to D8 was 5.56% (Criterion: should be ≤15% across all control replicates).
- Adult emergence rate: In control (A1), the adult emergence rate on D22 was 91.67% respectively. (Criterion: should be ≥ 70% across all control replicates).
- Reference item: The larval mortality in standard reference chemical (Dimethoate) on D8 was 87.88% (Criterion: should be ≥ 50% across all reference replicates).

Conclusion

In a repeated exposure larval toxicity study with Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG the ED_{50} was calculated to be 0.68 µg/larva (i.e., 0.02 µg rimsulfuron/larva, <0.08 µg nicosulfuron/larva and 0.24 µg mesotrione/larva), which is equivalent to an EC_{50} of 4.45 mg/kg (i.e. 0.13 mg rimsulfuron/kg food, 0.53 mg nicosulfuron/kg food and 1.60 mg mesotrione/kg food).

The ED_{10} was calculated to be <0.2 µg/larva (i.e., < 0.01µg rimsulfuron/larva, <0.02 µg nicosulfuron/larva and <0.07 µg mesotrine/larva) which is equivalent to an EC_{10} of <1.3mg/kg (i.e. <0.04 mg rimsulfuron/kg food, <0.16 mg nicosulfuron/kg food and <0.47 mg mesotrione/kg food).

The ED_{20} was calculated to be <0.2 µg/larva (i.e. <0.01 µg rimsulfuron/larva, <0.02µg nicosulfuron/larva and <0.07 µg mesotrione /larva) which is equivalent to an EC_{20} of <1.3 mg/kg (i.e. <0.04 mg rimsulfuron/kg food, <0.16 mg nicosulfuron/kg food and <0.47 mg mesotrione /kg food).

The NOED was <0.2 µg/larva (i.e. <0.01µg rimsulfuron/larva, <0.02 µg nicosulfuron/larva and <0.07 µg‘mesotrione /larva) and the corresponding NOEC was <1.3 mg/kg food (i.e. <0.04 mg rimsulfuron/kg food, <0.16 mg nicosulfuron/kg food and <0.47 mg mesotrione /kg food).

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

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

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

Comments of zRMS:	The study is considered valid. All validity criteria were met. • after 48 hours, mortality of the control group was 0.0% (criterion: a maximum of 10.0%), • after 48 hours, mortality of the group treated with the reference item at the rate of 5.0 mL/ha was 80.0% (criterion: a minimum of 50%), • all wasps survived the 24-hour oviposition period (criterion: only wasps that survive oviposition can be examined for fecundity), • the mean number of mummies per female in the control groups were 23.1 and 24.1 (criterion: a minimum of 5.0 mummies/female), • all wasps in the control group gave offspring (criterion: a maximum of 2 females giving no offspring) **Agreed endpoints:** The LR_{50} > 660 g product/ha The $NOER_{mortality}$ ≥ 660 g product /ha The ER_{50} = 564.9 g product/ha The $NOER_{fecundity}$ = 165 g product/ha

Reference:	KCP 10.3.2.1-01
Report	An extended laboratory test for evaluating the effects of Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani – Perez), Elzbieta Kulec-Ploszczyca, 2018, report No. B/174/16
Guideline(s):	Yes, according to the ESCORT 1 (Barrett K.L. *et al.*, 1994) and the ESCORT 2 (Candolfi M.P. *et al.*, 2001) guidance documents and the guidelines

	developed by the IOBC, BART and EPPO Joint Initiative (Mead-Briggs M.A. *et al.*, 2000, Mead-Briggs M.A. *et al.*, 2010)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Aim of the study:
The aim of the study will be to determine the impact of Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36 % WG on mortality and fecundity of the parasitic wasp, *A. rhopalosiphi*.

Test item:
Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36 % WG is in the form of light brown powder with a characteristic odour. It forms a solution with water.

Reference item:
An insecticide, Danadim 400 EC will be used in order to verify the reliability of this test and the susceptibility of mites.

Biological test system:

Species:	The parasitic wasp, *Aphidius rhopalosiphi* (De Stefani – Perez), Hymenoptera: Braconidae
Age:	Imago (24-48 hours after emerging from mummies)
Source of origin:	A laboratory-bred culture obtained from Katz Biotech AG (Baruth, Germany) and cultivated at the Institute of Industrial Organic Chemistry, Branch Pszczyna according to the SOP/B/35
Rearing:	The wasp, *A. rhopalosiphi* is reared on the barley, *Hordeum vulgare* L. infested with the bird cherry-oat aphid, *Rhopalosiphum padi*.

Test method:
The study will be performed according to the test method of Mead-Briggs M.A. *et al.* A test unit will consist of a transparent PMMA cylinder (isolator) with a diameter of 11 cm and a height of 20 cm, put on a plastic pot. At the first stage of the experiment, the pot will contain 7-day old barley seedlings. For the purpose of the fecundity assessment, the pots will contains10-40 seedlings of 7-day old barley infested with the bird cherry-oat aphid. Fine metal netting at the top and on two sides of the cylinders are used to provide ventilation in the isolators. There is a hole in the cylinder to introduce the wasps to the test area. This port will be filled with a cotton wool bung soaked with a 10% fructose solution in water.

Definitive test:
The test item with 0.1% adjuvant Trend 90 EC, the reference item and two control groups (one water control and water control with 0.1% adjuvant Trend 90 EC) will be used in the definitive test. On the basis of the preliminary test results, four rates of the test item will be used. These will be 82.5, 165.0, 330.0 and 660 g/ha. Each group will be divided into six replicates. Three will be five wasps in each replicate.

Results and discussion

Effects of Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG and Danadim 400 EC on mortality of *A. rhopalosiphi* after 48 hours and the LR_{50} – definitive test

Study group [app. Rate]		Tested wasps [no.]	Mortality							
			Dead wasps [no.]						Total	
[g/ha]	[g a.i./ha]		Replicates							
	rim + nico + meso		I	II	III	IV	V	VI	[no.]	[%]
Control [0.0]		**30**	0	0	0	0	0	0	**0**	**0.0**
Control with adjuvant 0.1% Trend 90 EC		**30**	0	0	0	0	0	0	**0**	**0.0**
Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG										
82.5	**2.5+9.9+29.7**	**30**	0	0	0	0	0	0	**0**	**0.0**
165	**5.0+19.8+59.4**	**30**	0	0	0	0	0	0	**0**	**0.0**
330	**9.9+39.6+118.8**	**30**	0	0	0	0	0	0	**0**	**0.0**
660	**19.8+79.2+237.6**	**30**	0	0	0	0	0	0	**0**	**0.0**
LR_{50}		**> 660 g/ha** [> (19.8g of Rimsulfuron + 79.2 g of Nicosulfuron + 237.6 g of Mesotrione/ha)]								
$NOER_{mortality}$		**> 660 g/ha** [> (19.8g of Rimsulfuron + 79.2 g of Nicosulfuron + 237.6 g of Mesotrione/ha)]								
[mL/ha]	**[g a.s./h]**	**Danadim 400 EC**								
	Dimethoate									
5.0	**2.0**	**30**	3	4	4	5	4	4	**24**	**80.0**

Effect of the test item on fecundity of *A. rhopalosiphi* and the ER_{50} – definitive test

Replicated (isolator number)	Mummies per female 12 days after oviposition [no.]					
	Control	Control with 0.1% adjuvant	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG			
	Application rate					
	0.0 [g/ha]	Trend 90 EC	82.5 g/ha	165 g/ha	330 g/ha	660 g/ha
I	19	19	23	30	31	13
II	18	20	25	24	21	11
III	13	34	36	22	18	17
IV	32	37	21	28	19	3
V	27	35	20	22	18	14
VI	16	32	19	24	19	2
VII	31	27	31	13	13	10
VIII	29	11	26	25	12	11
IX	28	11	28	19	16	9
X	33	29	31	30	19	14
XI	20	22	22	26	18	14
XII	22	24	26	27	16	18
XIII	21	25	28	25	18	15
XIV	17	14	30	22	8	9
XV	20	21	29	37	10	14
Mean number of mummies per female ± SD	**23.1 ± 6.4**	**24.1[a] ± 8.4**	**26.3± 4.8**	**24.9 ± 5.5**	**[+]17.1 ± 5.4**	**[+] 11.6 ± 4.5**
Fecundity	**-**	**-**	**(-9.4)****	**(-3.6)****	**29.1**	**51.8**

reduction relative to the control (Pr) [%]						
ER_{50}		**564.9 g/ha** (232.0-1327.0)*		17.0 g of Rimsulfuron (7.0-39.8)*		
				67.8 g of Nicosulfuron (27.8-159.3)*		
				203.4 g of Mesotrione (83.5-477.9)*		
$NOER_{mortality}$		**165 g/ha** (5.0 g of Rimsulfuron + 19.8 g of Nicosulfuron + 59.4 g of Mesotrione/ha)				

+: statistically significant differences
*: the ER_{50} values (with 95%-confidence limits)
**: the negative values indicate that mean number of mummies per female in the groups treated with the test item was higher than in the control
a: a control with an adjuvant 0.1% Trend 90 EC was taken into account in calculations of the fecundity reduction and statistical analysis.

Conclusion

On the basis of the obtained mortality results, the LR_{50} and $NOER_{mortality}$ could not be estimated. It can only be concluded that the LR_{50} of Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG is higher than 660 g/ha. The $NOER_{mortality}$ is higher than or equal to 660 g/ha.
On the basis of the obtained fecundity results, the ER_{50} and $NOER_{fecundity}$ values were determined. The ER_{50} is 564.9 g/ha and the $NOER_{fecundity}$ is 165 g/ha.

Comments of zRMS:

The study is considered valid. All validity criteria were met.

- The mortality of the control without 0.1% adjuvant group was 0.0% and mortality of the control with 0.1% adjuvant group was 1.7% on day 7 of exposure (criterion: a maximum of 20%),
- The corrected mortality of the mites exposed to the reference item at the rate of 9.0 mL/ha was 96.7% on day 7 of exposure (criterion: a minimum of 50%),
- The mean number of eggs per female in the control without 0.1% adjuvant group was 7.4 and the mean number of eggs per female in the control with 0.1% adjuvant group was 6.4 (required: ≥ 4 eggs per female).

Agreed endpoints:

Parameter (endpoints)							
Mortality				**Reproduction**			
Study group [application rate]		**Total [%]**	**LR_{50}**	**Study group [application rate]**	**M ean number of eggs/ female (Rr) [no.]**	**Reproduction reduction Pr [%]**	**ER_{50}**
Test item [g/ha]	**Active ingredients [g/kg]**		**[g/ha]**	**Test item [g/ha]**			**[g/ha]**
Control without 0.1% adjuvant		0.0	147.0	Control without 0.1% adjuvant	7.4	–	Above 20.6
Control with 0.1% adjuvant		1.7		Control with 0.1% adjuvant	6.4	13.0	
1.3	0.04[a] + 0.2[b] + 0.5[c]	3.3		1.3	7.1	3.7	
5.2[+]	0.2[a] + 0.6[b] + 1.9[c]	8.3		5.2	5.6	23.9	
20.6[+]	0.6[a] + 2.5[b] + 7.4[c]	28.3		20.6	6.4	13.5	
82.5[+]	2.5[a] + 9.9[b] + 29.7[c]	53.3		82.5	-		
330.0[+]	9.9[a] + 39.6[b] +	51.7		330.0	-		

		118.8[c]					
	$NOER_{mortality}$ = 1.3 [g/ha]			**$NOER_{reproduction} \geq$ 20.6 [g/ha]**			
	Reference item: Danadim 400 EC						
	Reference item [mL/ha]			9.0			
	Active ingredient [g/ha]			3.6			
	Mortality						
	Total [%]			96.7			
	[a]: Rimsulfuron [b]: Nicosulfuron [c]: Mesotrione [+] statistically significant differences ToxRat Professional 3.2.1. software						

Reference:	KCP 10.3.2.1-02
Report	"An extended laboratory test for evaluating the effects of Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG on the predatory mite, *Typhlodromus pyri* (Sch.)". Monika Stalmach, 2019, Study Code B/175/16. Institute of Industrial Organic Chemistry Branch Pszczyna
Guideline(s):	ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Blümel S. et al., 2000)
Deviations:	According the Amendment No. 1 to the Study Plan B/175/16, study should be completed in December 2018, but it was completed in January 2019, which had no impact on the results.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not relevant

Materials and methods

The aim of the extended laboratory test was to evaluate the effects of the test item, Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG on mortality and reproduction of the predatory mite, *T. pyri* (Sch.).
On the basis of the non-GLP preliminary test results it was decided to use five rates of the test item in the definitive test. These were: 1.3, 5.2, 20.6, 82.5 and 330 g/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 groups (with 0.1% adjuvant and without 0.1% adjuvant) and groups treated with the test item at rates 1.3, 5.2 and 20.6 g/ha were made after 10, 12, and 14 days of the treatment. Group exposed to the test item at rates 82.5 and 330 g/ha did not meet the mortality criteria from the first stage of the experiment.
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.

Results
The effects of Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WGon mortality and reproduction of *Typhlodromus pyri* in the definitive test are summarized below.

Parameter (endpoints)							
Mortality				**Reproduction**			
Study group [application rate]		**Total [%]**	**LR_{50}**	**Study group [application rate]**	**M ean number of eggs/ female (Rr) [no.]**	**Reproduction reduction Pr [%]**	**ER_{50}**
Test item [g/ha]	**Active ingredients [g/kg]**		**[g/ha]**	**Test item [g/ha]**			**[g/ha]**
Control without 0.1% adjuvant		0.0	147.0	Control without 0.1% adjuvant	7.4	–	Above 20.6
Control with 0.1% adjuvant		1.7		Control with 0.1% adjuvant	6.4	13.0	
1.3	$0.04^a + 0.2^b + 0.5^c$	3.3		1.3	7.1	3.7	
5.2^+	$0.2^a + 0.6^b + 1.9^c$	8.3		5.2	5.6	23.9	
20.6^+	$0.6^a + 2.5^b + 7.4^c$	28.3		20.6	6.4	13.5	
82.5^+	$2.5^a + 9.9^b + 29.7^c$	53.3		82.5	-		
330.0^+	$9.9^a + 39.6^b + 118.8^c$	51.7		330.0	-		
$NOER_{mortality}$ = 1.3 [g/ha]				**$NOER_{reproduction} \geq$ 20.6 [g/ha]**			
Reference item: Danadim 400 EC							
Reference item [mL/ha]			9.0				
Active ingredient [g/ha]			3.6				
Mortality							
Total [%]			96.7				

[a]: Rimsulfuron
[b]: Nicosulfuron
[c]: Mesotrione
[+] statistically significant differences ToxRat Professional 3.2.1. software

Findings

- In the preliminary test, mortality of the control group after 7 days of exposure was 5.0%. The percentages of mortality corrected using a formula of Abbott, after 7 days of exposure to Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG at the rates 2.6, 13.2, 66 and 330 g/ha (with 0.1% adjuvant) g/ha were 2.6, 36.8, 55.3 and 68.4%, respectively.
- In the definitive test the control without 0.1% adjuvant was accepted as the control reference group.
- In the definitive test, mortality of the mites in the control without 0.1% adjuvant after 7 days of exposure was 0.0%. In the control with 0.1% adjuvant the mortality of the mites was 1.7%. After 7 days of exposure to Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG the percentages of mortality of *T. pyri* at rates 1.3, 5.2, 20.6, 82.5 and 330 g/ha (with 0.1% adjuvant) g/ha were 3.3, 8.3, 28.3, 53.3 and 51.7%, respectively.
- On the basis of the obtained mortality results, the LR_{50} is 147.0 g/ha (with 0.1% adjuvant). The $NOER_{mortality}$ is 1.3 g/ha (with 0.1% adjuvant)
- At the significance level of 0.05, there were no statistically significant differences in mortality between the group treated with the test item at the rate 1.3 g/ha (with 0.1% adjuvant) and the control without 0.1% adjuvant. At the significance level of 0.05, there were statistically significant differences in mortality between the group treated with the test item at the rate 5.2, 20.6, 82.5 and 330 g/ha (with 0.1% adjuvant) and the control without 0.1% adjuvant (Step-down Cochran-Armitage Test Procedure; p(trend) > alpha).
- 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 after Abbott's correction was 96.7%. 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.
- In regards to the mites that escaped during the study, no statistically significant rate/response was found (p(F) > 0.05; i.e. slope of the relationship is not significantly different from zero) for the test item rates of 1.3 g of Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG/ha. On the other hand statistically significant rate/response was observed for the rates of 5.2, 20.6, 82.5 and 330 g of Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG/ha. Unfortunately, due to the lacking rate/response the

shown LR_x could not be valid. Therefore, the toxic metrics happen to be meaningless for this endpoint. On the basis of obtain results the $NOER_{escape}$ value could be estimated and the ToxRat software suggested it is 1.3 g of Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG/ha.

- The sex ratio was correct. Hence, no corrections were made.
- There were no statistically significant differences between control without adjuvant and control with 0.1% adjuvant (Two-sample t-test Procedure). The control without 0.1% adjuvant was accepted as the control reference group.
- The mean reproduction rate (Rr) in the control group was 7.4 eggs/female. The mean reproduction rates after 14 days 1.3, 5.2 and 20.6 g/ha (with 0.1% adjuvant) were 7.1, 5.6 and 6.4 eggs/female, respectively. The percentages of reproduction reduction (Pr) caused tested item at the rates of 1.3, 5.2 and 20.6 g/h were 3.7, 23.9 and 13.5%, respectively.
- At the significance level of 0.05, there were no statistically significant differences in reproduction between the groups treated with the test item at the rates of 1.3, 5.2 and 20.6 g/ha (with 0.1% adjuvant) and control group without 0.1% adjuvant (Wiliams Multiple Sequential t-test Procedure; $|t| > |t^*|$).
- On the basis of the obtained reproduction results it could be estimated that the ER_{50} is higher than 20.6 g/ha (with 0.1% adjuvant) of Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG. The $NOER_{reproduction}$ value could not be estimated. The $NOER_{reproduction}$ is greater than or equal to 20.6 g/ha (with 0.1% adjuvant) of the test item.

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

- mortality (dead larvae and pupae and adults dying during emergence or not successfully moulted) in the control: 13.33% (a criterion: ≤20 %,
- fecundity (mean number of eggs per female per day) in the control 39.7 (criterion: ≥15),
- fertility (mean hatching rate) in the control: 98.73% (criterion: ≥70%).
- mortality in the reference item treatment was 100% (criterion: >50%).

Agreed endpoints:

Study group (application rate) (g/ha)	Mortality		Reproduction			
	Total (%)	Corrected# (%)	Fecundity (No)	Fecundity reduction (%)	Fertility (%)	Fertility reduction (%)
Control						
0.0	13.33	-	37.70	-	98.573	-
Deltamethrin 5% CS						
50	23.33	11.54	33.8	14.86	98.36	0.37
100	36.67	26.92	28	29.47	96.84	1.91
200	66.67	61.54	23.7	40.30	94.43	4.36
400	76.67	73.08	14.57	63.30	91.52	7.30
800	100.00	100.00	0	100.00	0	100.0
Endpoints	**$LR50_{mortality}$**		**168.20 g/ha** (5.05^a + 20.18^b + 60.55^c g a.s./ha)	**$ER50_{fecundity}$**		**200 g/ha** (6.00^a + 23.99^b + 71.96^c g a.s./ha)
	$NOER_{mortality}$		**800 g/ha** (24^a + 96^b + 288^c g a.s./ha)	**$NOER_{fecundity}$**		**<50 g/ha** ($<1.5^a$ + 6^b + 18^c g a.s./ha)
Reference item – ROGOHIT (DIMETHOATE 30% EC)						
0.65	100	100	-			

Reference:	KCP 10.3.2.1 - 03
Report	"A laboratory test for evaluating the effects of Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG on larvae of the green lacewing *Chrysoperla carnea* (L.).". Dr. M. Mohanraj, 2020, 7554/2020. Bioscience Research Foundation
Guideline(s):	ESCORT 1 (Barrett K.L. *et al.*, 1994) ESCORT 2 (Candolfi M.P. *et al.*, 2000) Guidelines developed by the IOBC, BART and EPPO Joint Initiative (Vogt H. *et al.*, 2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG; Batch Number SCL-78944; active substance: Rimsulfuron 30 g/kg, Nicoslufuron 120 g/kg, Mesotrione 360 g/kg
Test species:	*Chrysoperla carnea* (L.), Neuroptera, Chrysopidae from the BFR insectary. The larvae used in the study were 2 – 3 days old.
Diet:	*ad libitum*
Study design:	Number of replicates: 30 replicates for mortality, 10 replicates for reproduction Number of larvae: 1/replicate Test duration: until pupation The test item was applied with a laboratory track sprayer on bean plants at seven application rates. ROGOHIT (Dimethoate 30%) was used as reference item whereas deionised water was used as control. After treatment, the treated leaves were transferred to a reproduction unit.
Application rates:	Control, 50, 100, 200, 400 and 800 g of the test item/ha
Test conditions:	Temperature: 24.0 – 27.0 ºC; humidity: 62.0 – 80.0%; lighting: 16 h light : 8 h dark; light intensity: 1100 – 1800 lux
Statistical analysis:	LR_{50} and NOER for mortality and ER_{50} and NOER for reproduction were determined by using a Probit analysis in NCSS (Number Cruncher Statistical System) and one-way ANOVA using Graphpad Prism 8.0. The means and standard deviations were calculated using validated Excel sheets.
Endpoints:	LR_{50}, NOER ER_{50}, NOER

Results and Conclusions

The effects of Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG on mortality and fecundity of *Chrysoperla carnea* in the extended laboratory test are summarized below:

Study group (application rate) (g/ha)	Mortality		Reproduction			
	Total (%)	Corrected# (%)	Fecundity (No)	Fecundity reduction (%)	Fertility (%)	Fertility reduction (%)
Control						
0.0	13.33	-	37.70	-	98.573	-
Deltamethrin 5% CS						
50	23.33	11.54	33.8	14.86	98.36	0.37
100	36.67	26.92	28	29.47	96.84	1.91
200	66.67	61.54	23.7	40.30	94.43	4.36
400	76.67	73.08	14.57	63.30	91.52	7.30
800	100.00	100.00	0	100.00	0	100.0
Endpoints	**$LR50_{mortality}$**		**168.20 g/ha** (5.05^a + 20.18^b + 60.55^c g a.s./ha)	**$ER50_{fecundity}$**		**200 g/ha** (6.00^a + 23.99^b + 71.96^c g a.s./ha)
	$NOER_{mortality}$		**800 g/ha** (24^a + 96^b + 288^c g a.s./ha)	**$NOER_{fecundity}$**		**<50 g/ha** ($<1.5^a$ + 6^b + 18^c g a.s./ha)
Reference item – ROGOHIT (DIMETHOATE 30% EC)						
0.65	100	100	-			

#: Mortality corrected according to Abbott's formula:
Corrected mortality [%] = ((Mt – Mc) / (100 – Mc)) x 100; Mt = Mortality treated, Mc = Mortality control
+: statistically significant difference between the control and the treatment group at $p < 0.05$
a: Rimsulfuron, b; Nicosulfuron, c; Mesotrione

The validity criterion for mortality was met, because mortality of the control group after 10 days of exposure was 13.33% (criterion: ≤20%), whereas corrected mortality of *C.carnea* after 10 days of exposure to Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG at rates of 50, 100, 200, 400 and 800 g/ha was 23.33, 36.67, 66.67, 76.67 and 100% respectively.

There were no statistically significant differences in mortality between group treated with the test item and the control group (one-way ANOVA, $p < 0.05$).

On the basis of the obtained mortality results, the LR_{50} value is 168.20 g Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG/ha, i.e. 5.05 g Rimsulfuron/ha + 20.18 g Nicosulfuron/ha + 60.55 g Mesotrione/ha. The $NOER_{mortality}$ value is 800 g Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG /ha, i.e. 24 g Rimsulfuron/ha + 96 g Nicosulfuron/ha + 288 g Mesotrione/ha.

For the reference item Rogohit (Dimethoate 30% EC, w/w), the corrected mortality of *C.carnea* after exposure at the rate of 0.65 L/ha was 100%, hence the criterion (>50%) specified in the method description was met. The results showed that the test organisms were sensitive to dimethoate.

The validity criterion for fecundity was met, because the mean number of eggs per female per day in the control group was 39.7 (criterion: ≥15), whereas in the group treated with Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG at rates of 50, 100, 200, 400 and 800 g/ha was 33.8, 28, 23.7, 14.57 and 0respectively. Fecundity reduction in the group treated with Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG at rates of 50, 100, 200, 400 and 800 g/ha was 14.86, 29.47, 40.30, 63.30 and 100% respectively in comparison with the control group.

There were no statistically significant difference between group treated with the test item at all the rates used and the control group (one-way ANOVA, $p < 0.05$).

On the basis of the obtained fecundity results, the ER_{50} value is 200 g Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG /ha, i.e. 6.00 g Rimsulfuron/ha + 23.99 g Nicosulfuron/ha + 71.96 g Mesotrione/ha. The $NOER_{fecundity}$ value is <50 g Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG /ha, i.e. <1.5 g Rimsulfuron/ha + 6 g Nicosulfuron/ha + 18 g Mesotrione/ha.

The validity criterion for fecundity was met, because the mean hatching rate in the control group was 98.73% (criterion: ≥70%), whereas in the group treated with Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG at rates of 50, 100, 200, 400 and 800 g/ha was 98.36, 96.84, 94.43, 91.52 and 0% respectively. Fertility reduction in the group treated with Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG at rates of 50, 100, 200, 400 and 800 g/ha was 0.37, 1.91, 4.36, 7.30 and 100% respectively in comparison with the control group.

There were ç statistically significant difference between group treated with the test item at rates of 200-800 g/ha and the control group (one-way ANOVA, $p < 0.05$).

On the basis of the obtained results, it can be concluded that Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG had no adverse effects on mortality and fecundity of *C.carnea* at all the rates used, i.e. 50 – 100 g/ha.

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

- The mean number of beetles emerging from the fly pupae in the control was 892.5 (a criterion: >400),
- Reduction in reproduction of beetles in the reference group was 91.17 (a criterion: ≥50%)

Agreed endpoints:

Study group (application rate) (g/ha)	Mortality		Fecundity	
	Total (%)	Corrected# (%)	Offsprings produced (No)	Fecundity reduction (%)
Control				
0.0	1.25	-	892.5	-
Deltamethrin 5% CS				
92	2.5	1.27	883.5	1.01
146	10	8.86	823.0	7.79+
234	16.25	15.19	768.0	13.95+
375	57.5	56.96+	400.5	55.13+
600	76.25	75.95+	207.5	76.75+
Reference item – TAFGOR (DIMETHOATE 30% EC)				
0.65 L/ha	90	89.87	78	91.17
Endpoints	**$LR50_{mortality}$**	**373.75 g/ha** ($11.21^a + 44.85^b + 134.55^c$ g a.i./ha)	**$ER50_{fecundity}$**	**378.77 g/ha** ($11.36^a + 45.45^b + 136.36^c$ g a.i./ha)
	$NOER_{mortality}$	**234 g/ha** ($7.02^a + 28.08^b + 84.24^c$ g a.i./ha)	**$NOER_{fecundity}$**	**92 g/ha** ($2.76^a + 11.04^b + 33.12^c$ g a.i./ha)

#: Mortality corrected according to Abbott's formula:
Corrected mortality [%] = ((Mt – Mc) / (100 – Mc)) x 100; Mt = Mortality treated, Mc = Mortality control
+: statistically significant difference between the control and

Reference:	KCP 10.3.2.1 - 04
Report	"A laboratory test for evaluating the effects of Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG on the rove beetle *Aleochara bilineata* (Gyllenhal).". Ms. G. Sonali, 2020, 7555/2020. Bioscience Research Foundation
Guideline(s):	ESCORT 1 (Barrett K.L. *et al.*, 1994) ESCORT 2 (Candolfi M.P. *et al.*, 2000) Guidelines developed by the IOBC, BART and EPPO Joint Initiative (Vogt H. *et al.*, 2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG; Batch Number SCL-78944; active substance: Rimsulfuron 30 g/kg, Nicoslufuron 120 g/kg, Mesotrione 360 g/kg
Test species:	*Aleochara bilineata* (Gyll), Coleoptera:Staphylinidae from the BFR insectary. The adult bettles used in the study were 1 – 7 days old.
Diet:	*ad libitum*
Study design:	Number of replicates: 4 per treatment Number of bettles: 20 (10 female and 10 male) per replicate / 80 (40 female and 40 male) per treatment Test duration: 77 days The test item was sprayed onto the soil surface using a suitable spraying chamber. TAFGOR (Dimethoate 30%) was used as reference item whereas deionised water was used as control.
Application rates:	Control, 92, 146, 234, 375 and 600 g of the test item/ha
Test conditions:	Temperature: 19.8 – 20.7 ºC; humidity: 64 – 77%; lighting: 16 h light : 8 h dark; light intensity: 1550 – 1620 lux
Statistical analysis:	LR_{50} and NOER for mortality and ER_{50} and NOER for reproduction were determined by using a Probit analysis in NCSS (Number Cruncher Statistical System) and one-way ANOVA using Graphpad Prism 8.0. The means and standard deviations were calculated using validated Excel sheets.
Endpoints:	LR_{50}, NOER ER_{50}, NOER

Results and Conclusions

The effects of Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG on mortality and fecundity of *Aleochara bilineata* in the extended laboratory test are summarized below:

Study group (application rate) (g/ha)	Mortality		Fecundity	
	Total (%)	Corrected# (%)	Offsprings produced (No)	Fecundity reduction (%)
Control				
0.0	1.25	-	892.5	-
Deltamethrin 5% CS				
92	2.5	1.27	883.5	1.01
146	10	8.86	823.0	7.79+

234	16.25	15.19	768.0	13.95+
375	57.5	56.96+	400.5	55.13+
600	76.25	75.95+	207.5	76.75+
Reference item – TAFGOR (DIMETHOATE 30% EC)				
0.65 L/ha	90	89.87	78	91.17
Endpoints	**$LR50_{mortality}$**	**373.75 g/ha** (11.21^a + 44.85^b + 134.55^c g a.i./ha)	**$ER50_{fecundity}$**	**378.77 g/ha** (11.36^a + 45.45^b + 136.36^c g a.i./ha)
	$NOER_{mortality}$	**234 g/ha** (7.02^a + 28.08^b + 84.24^c g a.i./ha)	**$NOER_{fecundity}$**	**92 g/ha** (2.76^a + 11.04^b + 33.12^c g a.i./ha)

#: Mortality corrected according to Abbott's formula:
Corrected mortality [%] = ((Mt – Mc) / (100 – Mc)) x 100; Mt = Mortality treated, Mc = Mortality control
+: statistically significant difference between the control and the treatment group at $p < 0.05$
a: Rimsulfuron, b; Nicosulfuron, c; Mesotrione

Mortality of the control group after 28 days of exposure was 0.0 whereas corrected mortality of beetles after 28 days of exposure to Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG at rates of 92, 142, 234, 375 and 600 g/ha was 1.27, 8.86, 15.19, 56.96 and 75.95% respectively.

There were no statistically significant differences in mortality between group treated with the test item at the rate of 92, 146 and 234 and the control group (one-way ANOVA, $p < 0.05$).

On the basis of the obtained mortality results, the LR_{50} value is 373.75 g Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG/ha, i.e. 1.21 g Rimsulfuron/ha + 44.85 g Nicosulfuron/ha + 134.55 g Mesotrione/ha.

The $NOER_{mortality}$ value is 234 g Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG /ha, i.e. 7.02 g Rimsulfuron/ha + 28.08 g Nicosulfuron/ha + 84.24 g Mesotrione/ha.

For the reference item TAFGOR (Dimethoate 30% EC, w/w), the corrected mortality of beetles after exposure at the rate of 0.65 L/ha was 89.97%. The results showed that the test organisms were sensitive to dimethoate.

The mean number of offsprings produced during the 6-week hatching period in the control group was 996.8 (criterion: >400), whereas in the group treated with Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG at rates of 92, 142, 234, 375 and 600 g/ha was 883.5, 823.0, 768.0, 400.5 and 207.5 respectively. Fecundity reduction in the group treated with Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG at rates of 92, 142, 234, 375 and 600 g/ha was 1.01, 7.79, 13.95, 55.13 and 76.75% respectively in comparison with the control group.

There were statistically significant differences in fecundity between group treated with the test item at the rates of 92 g/ha and the control group (one-way ANOVA, $p < 0.05$).

On the basis of the obtained fecundity results, the ER_{50} value is 378.77 g Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG /ha, i.e. 11.36 g Rimsulfuron/ha + 45.45 g Nicosulfuron/ha + 136.36 g Mesotrione/ha.

The $NOER_{fecundity}$ value is 92 g Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG /ha, i.e. 2.76 g Rimsulfuron/ha + 11.04 g Nicosulfuron/ha + 33.12 g Mesotrione/ha.

On the basis of the obtained results, it can be concluded that Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG had no adverse effects on mortality of the beetles at the rates of 92, 146, 234 g/ha and had no adverse effects on fecundity of the beetles at the rates of 92 g/ha.

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

- Maximum mortality in the control was 12.0 % (exposure of 7 DAA1).
- Minimum mortality (corrected to control) in the toxic reference was 100.00 % (exposure of 0 to 14 DAA1).
- Actual minimum value was 7.69 eggs per female (exposure 7 DAA1).

Agreed endpoints:

Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG Aging residue periods studied: 0, 7 and 14 DAA1 (days after the test item application)	
Rate	0.33 [kg of formulated product/ha] (9.90 g rimsulfuron /ha, 39.60 g nicosulfuron /ha, 118.80 g mesotrione /ha)
Effects less than 50 % (compared to the control)	
7-d Mortality < 50%	From 0 DAA1 (fresh dried spray residues)
7-14 d Reduction Fecundity < 50%	From 0 DAA1 (fresh dried spray residues)
No significant effects (compared to the control)	
Mortality	From 7 DAA1 (aged residue for 7 days)
Fecundity	From 0 DAA1 (fresh dried spray residues)

Reference:	KCP 10.3.2.1-05
Report	"Toxicity to the Predatory Mite, *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) after Exposure to Freshly Applied and Aged Spray Deposits under Extended Laboratory Conditions". Sara Valera. 2021. Study code: S20-07857. Trialcamp S.L.U.
Guideline(s):	IOBC (Blümel *et al.*, 2000) modified, Grimm C. *et al*. (2001), Oomen P.A. (1988) and Pia Ternes *et al*. (2001)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Summary

The objective of the study was to determine the effects of freshly applied and aged spray deposits of the test item Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG on the mortality and reproduction on the predatory mite *Typhlodromus pyri* under extended laboratory conditions on cast iron plants (*Aspidistra elatior* Blume, Asparagaceae), applied at one rate, 0.33 kg of formulated product (FP)/ha (9.90 g rimsulfuron /ha, 39.60 g nicosulfuron /ha, 118.80 g mesotrione /ha)

Material and methods

Test item: Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG

Content: rimsulfuron (30 g/kg); nicosulfuron (120 g/kg); mesotrione (360 g/kg)
Batch No.: SCL-78944
Manufacturing date: 27 August 2019
Expiry date: 26 August 2021

Biological test system: protonymphs of the parasitoid the predatory mite *T. pyri* Scheuten (Acari: Phytoseiidae)
– Age: Not older than 24 hours from moulting
– Source: From an in-house culture started with supplied eggs by Katz Biotech Ag. (Baruth – Germany)

Experimental design:

Code	Treatment	Application rate [g a.s./ha](1)	Application rate(2) [FP /ha]
C	Water (Control)	-	-
T1	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG	9.90 g rimsulfuron + 39.60 g nicosulfuron + 118.80 g mesotrione	0.33
R (A1)*	Dimethoate 40 % w/v EC	186.3 g	0.45 L product/ha (3)

(1): "a.s." = active substance; Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG in the test product.
(2): Rate of Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG in kg of formulated product (FP)/ha
(3): Reference product at the maximum rate for intended use: 0.45 L FP /ha.

Cast iron plants (*Aspidistra elatior* Blume, Asparagaceae) were used for trial purposes. Three plots were used with 8 potted plants per plot: one plot for water treated control, one plot for the test item rate and one plot for the toxic reference. The treated plot size was 10 m2 (10 m x 1 m) for the treatments and the pots were arranged in one crop row (0.5 m between plants).

Application was performed using a compressed air knapsack sprayer and one nozzle "Albuz Hollow Cone Yellow ATR-80" simulating an application in field (volume 400 L/ha), working at pressure of 4 bars and applying the plants outdoors. After application, plants were maintained under outdoor conditions with the use of a translucent roof to cover the crop when it rains to provide natural ageing conditions and to avoid the washing-off by rain. The reference item was applied at the same time as the test item. Moreover, the reference item was applied at each ageing period using a similar method, a compressed air knapsack sprayer; the same 8 pots with cast iron plants on day 0 were applied with the reference item in 7 and 14 DAA1.

At each ageing period, 5 fragments of leaves from different plants per treatment group were sampled in order to assemble the test units. Then, twenty protonymphs of *Typhlodromus pyri* were introduced into each test unit (5 replicates per treatment). Direct treatment effects (mortality) and any change in behaviour, with respect to the control, were assessed after 1, 3 and 7 days at each exposure. Reproduction was assessed on days 9, 11 and 14 for the control group and for test item group at each exposure when mortality was below 50 % (corrected to control).

Statistics

Chi² 2 x 2 Test with mortality (dead + escaped individuals) at 7 d (one-sided greater, $\alpha = 0.05$) was used to detect significant differences between mortality data of the test item and the control group in the exposures (bioassays) of 0, 7 and 14 DAA1.

Reproduction was statistically studied with results in the exposures of 0, 7 and 14 DAA1. Reproduction data met normality (Shapiro-Wilk's Test) and homoscedasticity (Levene's Test). Therefore, STUDENT-t test for Homogeneous Variances with cumulative offspring/female at 14 d (one-sided smaller, $\alpha = 0.05$) was used to detect significant differences between fecundity data of the test item and the control groups.

Endpoints

- To study the mortality at 7 days after exposure (lethal effect) to residues on leaves aged for the following periods: 0, 7 and 14 ± 1 days after application (DAA)
- To study the fecundity of the survivor females during 7 days following exposure to residues on leaves for the aforementioned ageing periods.
- The ageing period of the residue at the tested rates with effects below 50%, relative to the control, was determined

Results

Mortality

The effects in mortality were below the trigger value of 50 % with the tested rate of 0.33 kg of formulated product/ha from the exposure at 0 DAA1 (fresh dried spray residues).

Mortality with fresh and dry residues (0 DAA1) of the test item applied at the rate 0.33 kg of formulated product (FP)/ha were significantly different to the control group with 11.96 % mortality (corrected to control). Mortality with the test item residue aged for 7 and 14 days (exposure or bioassay of 7 and 14 DAA1, respectively) was 16.0 % and 11.0 %, respectively (4.55 % and 6.32 %, respectively, corrected to control) and not significantly different to the control group.

When escaped individuals with the test item groups were studied, compared to the control and compared to the dead individuals, the response as escape individuals is the most important cause of mortality in the bioassays of 0, 7 and 14 DAA1, also in the control groups. The percentages of dead individuals in the test item groups were 2 % in the three bioassays, while the percentages of escape individuals were 17 %, 14 % and 9 % in the bioassays 0, 7 and 14, respectively.

The mites in the test item did not show a delay on development compared to the control group in any of the exposures.

The mortality in the reference item was higher than 50 % (corrected to control) in the three performed exposures; 100 % at 0, 7 and 14 DAA1.

			Exposure					
Code	Treatment	Application rate(2) [FP /ha]	0 DAA1(1)		7 DAA1		14 DAA1	
			% M	[%] Cm	% M	[%]Cm	% M	[%]Cm
C	Control (water)	-	8.0 ± 5.70	-	12.0 ± 2.74	-	5.0 ± 5.0	-
T1	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG	0.33	19.0 ± 19.49 sd	11.96	16.0 ± 6.52	4.55	11.0 ± 11.4	6.32
R	Dimethoate 40 % w/v EC	0.45	100	100	100	100	100	100

(1): DAA1 = Days after application; M [%] = Mortality [%]; Cm [%] = Corrected mortality [%]
(2): Rate of the test item (T) in kg of formulated product (FP) per ha and reference item (R) in L of FP per ha
(3): Negative value indicates a decrease relative to the control
"sd": Statistically significant increase compared to the control (Chi2 2x2 Test, one-sided greater, α = 0.05)

Fecundity

The reduction of reproduction at the tested rate of 0.33 kg of formulated product/ha was lower than the trigger value of 50 % at the exposure with fresh and dried residues at 0 DAA and the consecutive exposures at 7 and 14DAA1; with 35.26 %, 18.53 % and 11.43 % reduction respectively when compared to the control. Not significantly reduction compared to the control was obtained in any exposure. A similar

fecundity rate (eggs per female) was observed in the exposure of 14 DAA1 with 8.67 eggs per female, compared to 9.79 eggs per female in the control group, respectively.
In the exposure at 7 DAA1, a higher fecundity rate between days 7 and 9 was observed in the test item respect to the control group. However, a delay on lying the eggs was observed with the test item in the rest of exposures or bioassays, where a lower fecundity rate between days 7 and 11 was observed. In the exposure at 14 DAA1 the number of eggs per female in the reproductive periods 11-14 days after exposure was greater than in the control group.

			Exposure					
Code	Treatment	Application rate(2) [FP /ha]	0 DAA1(1)		7 DAA1		14 DAA1	
			e/f	[%] R	e/f	[%] R	e/f	[%] R
C	Control (water)	-	10.02 ± 2.14	-	7.69 ± 1.29	-	9.79 ± 0.54	-
T1	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG	0.33	6.48 ± 4.06	35.26	6.26 ± 2.04	18.53	8.67 ± 1.46	11.43

(1): DAA1 = Days after application; "e/f"= eggs per female (mean); [%] R = Reduction [%]
(2): Rates in kg of formulated product (FP) per hectare (ha

Test validity criteria

All mortality and reproduction tests were considered to be valid as:

- Maximum mortality in the control was 12.0 % (exposure of 7 DAA1).
- Minimum mortality (corrected to control) in the toxic reference was 100.00 % (exposure of 0 to 14 DAA1).
- Actual minimum value was 7.69 eggs per female (exposure 7 DAA1).

Conclusion

Table 1:Endpoints after exposure of *Typhlodromus pyri*

Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WG **Aging residue periods studied: 0, 7 and 14 DAA1 (days after the test item application)**	
Rate	0.33 [kg of formulated product/ha] (9.90 g rimsulfuron /ha, 39.60 g nicosulfuron /ha, 118.80 g mesotrione /ha)
Effects less than 50 % (compared to the control)	
7-d Mortality < 50%	From 0 DAA1 (fresh dried spray residues)
7-14 d Reduction Fecundity < 50%	From 0 DAA1 (fresh dried spray residues)
No significant effects (compared to the control)	
Mortality	From 7 DAA1 (aged residue for 7 days)
Fecundity	From 0 DAA1 (fresh dried spray residues)

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

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

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

- each replicate produced 129.1 juveniles (mean) at the end of the experiment – (criterion: ≥ 30 juveniles by the end of the experiment),
- the coefficient of variation of reproduction was 24.2% (criterion: ≤ 30%),
- adult mortality over the initial 4 weeks of the experiment was 1.3% (criterion: ≤ 10%).

Agreed endpoints:

EC_{10}, EC_{20}, EC_{50}, LC_{50}, NOEC and LOEC values

Endpoint	Value [mg test item /kg dry weight of artificial soil]	Value [mg rimsulfuron/kg dry weight of the artificial soil]	Value [mg nicosulfuron/kg dry weight of the artificial soil]	Value [mg mesotrione/kg dry weight of the artificial soil]
EC_{10}	**7.7** (< 5.6 – 17.7)	**0.2** (< 0.2 – 0.5)	**0.9** (< 0.7 – 2.1)	**2.8** (< 2.0 – 6.4)
EC_{20}	**29.8** (11.4 – 52.6)	**0.9** (0.3 – 1.6)	**3.6** (1.4 – 6.3)	**10.7** (4.1 – 18.9)
EC_{50}	**397.1** (247.8 – 791.4)	**11.9** (7.4 – 23.7)	**47.7** (29.7 – 95.0)	**143.0** (89.2 – 284.9)
NOEC (reproduction)	**10.0**	**0.3**	**1.2**	**3.6**
LOEC (reproduction)	**18.0**	**0.5**	**2.2**	**6.5**
LC_{50}	**> 1000**	**> 30.0**	**> 120.0**	**> 360.0**
NOEC (survival)	**56.0**	**1.7**	**6.7**	**20.2**
LOEC (survival)	**56.0**	**1.7**	**6.7**	**20.2**

Reference: KCP 10.4.1.1

Report: "Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WDG. Earthworm Reproduction Test (*Eisenia andrei*)". Weronika Dec, 2019, G/265/17, Institute of Industrial Organic Chemistry Branch Pszczyna

Guideline(s): OECD Guideline No. 222 (2016)

Deviations: Contrary to what had been planned, the study finished in February 2019, and not in November 2018,
The temperature in the test room was between 16.5 – 20.5°C. According to OECD Guideline No. 222 (2016), SOP/G/36, and the Study Plan, it should have ranged from 18 to 22°C. It was a short-term deviation (for about six hours) which did not affect the result of the experiment.
These deviations did not affect the study results.

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

Materials and methods

Test item:	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WDG; Batch Number SCL- 58176; active substance: rimsulfuron – 30 g/kg, nicosulfuron – 120 g/kg, mesotrione – 360 g/kg
Test species:	*Eisenia andrei* obtained from a standard laboratory culture cultivated at the Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicology, Laboratory of Soil Toxicology.
Soil:	10% sphagnum peat, 20% kaolin clay, 70% air-dried quartz sand
Study design:	Number of replicates: 4 replicates / concentration + 8 replicates / control Number of earthworms: 10 earthworms/replicate Test duration: 8 weeks
Application rates:	Control, 5.6, 10, 18, 32, 56, 100, 180, 320, 560, and 1000 mg/kg dry weight of the artificial soil
Test conditions:	Temperature: 16.5 – 20.5℃; humidity: 50.4 – 58.4% WHC; lighting: 16 h light : 8 h dark; light intensity: 492 – 555 lux; pH: 5.80 – 5.98
Statistical analysis:	EC_{50}, EC_{20}, EC_{10}, LC_{50} – probit analysis. NOEC (reproduction, survival) –Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Williams Multiple Sequential t-test Procedure. LOEC: a value suggested by the ToxRat Professional 2.10 statistical computer software.
Endpoints:	LC_{50}, EC_{50}, EC_{20}, EC_{10}, NOEC, LOEC

Results and Conclusions

On the basis of the results, it was concluded that after 4 weeks, at the control group there was mortality of adult earthworm noticed and it was equal to 1.3%. At concentrations ranging from 5.6 to 1000 mg of the test item/kg dry weight of artificial soil, after 4 weeks of exposure to the test item, mortality of the adult earthworms was ranging from 0.0 to 15.0%.
The concentration of the test item causing 50% mortality of the adult earthworms (LC50) is higher than 1000 mg/kg dry weight of artificial soil (30.0 mg rimsulfuron + 120.0 mg nicosulfuron + 360.0 mg mesotrione/kg dry weight of the artificial soil).
No changes in the appearance (morphology) and behaviour of the living earthworms were noticed.
After the application of the test item at the concentrations ranging from 5.6 to 1000.0 mg/kg dry weight of artificial soil, the body weight increase was between 1.0 to 17.7%. As for the control group, the body weight increase was equal to 5.7%.
After 8 weeks of the experiment, the obtained results led to the following conclusions:
After the application of the test item at the concentrations ranging from 5.6 to 1000 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 40.8 – 128.5 per replicate. The mean number of juveniles in the control group was equal to 129.1 per replicate.
After 8 weeks of the experiment, it was concluded that Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WDG had statistically significant impact on reproduction of the earthworms at the concentrations between 18.0 – 1000.0 mg/kg dry weight of artificial soil.
The endpoint values showing the impact of the test item on reproduction and survival of adult earthworms are presented in the table given below.

EC_{10}, EC_{20}, EC_{50}, LC_{50}, NOEC and LOEC values

Endpoint	Value [mg test item /kg dry weight of artificial soil]	Value [mg rimsulfuron/kg dry weight of the artificial soil]	Value [mg nicosulfuron/kg dry weight of the artificial soil]	Value [mg mesotrione/kg dry weight of the artificial soil]
EC_{10}	**7.7** (< 5.6 – 17.7)	**0.2** (< 0.2 – 0.5)	**0.9** (< 0.7 – 2.1)	**2.8** (< 2.0 – 6.4)
EC_{20}	**29.8** (11.4 – 52.6)	**0.9** (0.3 – 1.6)	**3.6** (1.4 – 6.3)	**10.7** (4.1 – 18.9)
EC_{50}	**397.1** (247.8 – 791.4)	**11.9** (7.4 – 23.7)	**47.7** (29.7 – 95.0)	**143.0** (89.2 – 284.9)
NOEC (reproduction)	**10.0**	**0.3**	**1.2**	**3.6**
LOEC (reproduction)	**18.0**	**0.5**	**2.2**	**6.5**
LC_{50}	**> 1000**	**> 30.0**	**> 120.0**	**> 360.0**
NOEC (survival)	**56.0**	**1.7**	**6.7**	**20.2**
LOEC (survival)	**56.0**	**1.7**	**6.7**	**20.2**

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

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

A 2.4.2.1 KCP 10.4.2.1 Species level testing

Comments of zRMS:

The study is considered valid. All validity criteria were met.

- mean adult mortality: 6.3% (criterion: ≤ 20%),
- the mean number of juveniles per vessel at the end of the test: 1098.4 (criterion: ≥ 100 juveniles at the end of the test),
- the coefficient of variation calculated for the number of juveniles: 27.9 (criterion: ≤ 30%)

Agreed endpoints:

LC_{10}, LC_{20}, LC_{50} and NOEC

Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg rimsulfuron/kg dry weight of the artificial soil]	Value [mg nicosulfuron/kg dry weight of the artificial soil]	Value [mg mesotrione/kg dry weight of the artificial soil]
LC_{10}	106.7 (57.2 – 161.4)	3.2 (1.7 – 4.8)	12.8 (6.9 – 19.4)	38.4 (20.6 – 58.1)
LC_{20}	282.1 (190.0 – 418.7)	8.5 (5.7 – 12.6)	33.9 (22.8 – 50.2)	101.6 (68.4 – 150.7)
LC_{50}	> 1000 (887.4 – > 1000)	> 30 (26.6 – > 30)	> 120 (106.5 – > 120)	> 360 (319.5 – > 360)
NOEC	100	3.0	12.0	36.0
LOEC	180	5.4	21.6	64.8

EC_{10}, EC_{20}, EC_{50} and NOEC

Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg rimsulfuron/kg dry weight of the artificial soil]	Value [mg nicosulfuron/kg dry weight of the	Value [mg mesotrione/kg dry weight of the

				artificial soil]	**artificial soil]**
	EC_{10}	44.2 (10.0 – 87.7)	1.3 (0.3 – 2.6)	5.3 (1.2 – 10.5)	15.9 (3.6 – 31.6)
	EC_{20}	111.2 (43.8 – 177.0)	3.3 (1.3 – 5.3)	13.3 (5.3 – 21.2)	40.0 (15.8 – 63.7)
	EC_{50}	447.2 (315.1 – 660.4)	13.4 (9.5 – 19.8)	53.7 (37.8 – 79.2)	161.0 (113.4 – 237.7)
	NOEC	56	1.7	6.7	20.2
	LOEC	100	3.0	12.0	36.0

Reference:	KCP 10.4.2.1 - 01
Report	"Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WDG: Collembolan (*Folsomia candida*) reproduction test". Weronika Dec, 2019, G/266/17. Institute of Industrial Organic Chemistry Branch Pszczyna
Guideline(s):	OECD Guideline No. 232 (2016)
Deviations:	At the end of the test the soil moisture content was determined by drying small sample of the artificial soil in 105°C instead of weighing the test vessels as it is mentioned in OECD Guideline No. 232 (2016) (chapter 3.6.6.), Physiological or pathological symptoms or distinct changes in behavior were not described (chapter 3.6.7.), Contrary to what had been planned, the study finished in January 2019, and not in November 2018. These deviations did not affect the study results.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WDG; Batch Number SCL- 58176; active substance: rimsulfuron 30 g/kg, nicosulfuron 120 g/kg, mesotrione 360 g/kg
Test species:	*Folsomia candida* obtained from a standard laboratory culture at the Institute of Industrial Organic Chemistry, Branch Pszczyna, Laboratory of Soil Toxicology. The collembolans used in the study were 9 – 12 days old.
Soil:	5% sphagnum peat, 20% kaolin clay, and 75% air-dried industrial sand
Study design:	Number of replicates: 4 replicates / concentration + 8 replicates / control Number of collembolans: 10 / replicate Test duration: 28 days
Application rates:	Control, 5.6; 10.0; 18.0, 32.0; 56.0; 100.0; 180.0; 320.0; 560.0; and 1000.0 mg of the test item/kg of dry weight of the artificial soil
Test conditions:	Temperature: 19.0 – 21.0℃; humidity: 12.1 – 13.8% (40.6 – 46.4% of the maximum water holding capacity); lighting: 16 h light : 8 h dark; light intensity: 490 – 530 lux; pH: 5.88 – 6.24
Statistical analysis:	EC_{10}, EC_{20}, EC_{50} – a Weibull analysis LC_{10}, LC_{20}, and LC_{50} - a logit analysis NOEC (number of juveniles): - Shapiro-Wilk's Test on Normal Distribution, - Levene's Test on Variance Homogeneity (with residuals), - Williams Multiple Sequential t-test Procedure

NOEC (survival) – Chi 2x2 Table Test with Bonferroni Correction
LOEC – a value suggested by the program

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

Results and Conclusions

Mortality at the concentrations ranging from 5.6 to 1000 mg/kg dry weight of the artificial soil ranged from 0.0 to 47.5%. As for the control group, it was equal to 6.3%.
The concentration of the test item causing a 50% mortality of adults within the exposure period (LC50) is above 1000 mg/kg dry weight of the artificial soil (30.0 mg rimsulfuron + 120.0 mg nicosulfuron + 360.0 mg mesotrione/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.

LC_{10}, LC_{20}, LC_{50} and NOEC values

Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg rimsulfuron/kg dry weight of the artificial soil]	Value [mg nicosulfuron/kg dry weight of the artificial soil]	Value [mg mesotrione/kg dry weight of the artificial soil]
LC_{10}	106.7 (57.2 – 161.4)	3.2 (1.7 – 4.8)	12.8 (6.9 – 19.4)	38.4 (20.6 – 58.1)
LC_{20}	282.1 (190.0 – 418.7)	8.5 (5.7 – 12.6)	33.9 (22.8 – 50.2)	101.6 (68.4 – 150.7)
LC_{50}	> 1000 (887.4 – > 1000)	> 30 (26.6 – > 30)	> 120 (106.5 – > 120)	> 360 (319.5 – > 360)
NOEC	100	3.0	12.0	36.0
LOEC	180	5.4	21.6	64.8

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 294.0 – 1183.3 per replicate. As for the control group, the number of juveniles was equal to 1098.4 per replicate.
The endpoint values showing the impact of the test item on reproduction of Folsomia candida are presented in the table given below.

EC_{10}, EC_{20}, EC_{50} and NOEC values

Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg rimsulfuron/kg dry weight of the artificial soil]	Value [mg nicosulfuron/kg dry weight of the artificial soil]	Value [mg mesotrione/kg dry weight of the artificial soil]
EC_{10}	44.2 (10.0 – 87.7)	1.3 (0.3 – 2.6)	5.3 (1.2 – 10.5)	15.9 (3.6 – 31.6)
EC_{20}	111.2 (43.8 – 177.0)	3.3 (1.3 – 5.3)	13.3 (5.3 – 21.2)	40.0 (15.8 – 63.7)
EC_{50}	447.2 (315.1 – 660.4)	13.4 (9.5 – 19.8)	53.7 (37.8 – 79.2)	161.0 (113.4 – 237.7)
NOEC	56	1.7	6.7	20.2
LOEC	100	3.0	12.0	36.0

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

A 2.5 KCP 10.5 Effects on soil carbon and nitrogen transformation

Comments of zRMS:	The study is considered valid. All validity criteria were met. • The coefficient of variation in the control group was as follows: 14.0, 6.9, 4.7 and 8.3% on 0, the 7th, 14th and 28th day of soil incubation, respec-

	tively. • The criterion of validity: the variation between replicate samples in the control should be less than ± 15%. **Agreed endpoints:** On the basis of the results, it was concluded that Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WDG at the concentrations corresponding to the PEC: 0.9 mg test item/kg dry soil (i.e. 0.026 mg rimsulfuron/kg dry soil + 0.106 mg of nicosulfuron/kg dry soil + 0.317 mg of mesotrione/kg dry soil) and 5 x PEC: 4.5 mg test item/kg dry soil (i.e. 0.13 mg rimsulfuron/kg dry soil + 0.53 mg of nicosulfuron/kg dry soil + 1.59 mg of mesotrione/kg dry soil) can be perceived as having no long-term influence on carbon transformations in soil.

Reference:	KCP 10.5-01
Report	"Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WDG. Soil Microorganisms: Carbon Transformation Test", Weronika Dec, 2019, G/263/17. Institute of Industrial Organic Chemistry Branch Pszczyna.
Guideline(s):	OECD Guideline No. 217 (2000) / EU Method C.22
Deviations:	The predicted environmental concentration (PEC) is calculated assuming 2.5 cm of the soil depth according to the German conditions for the active substances with the mobility in soil KFoc < 500 mL/g. Thus, the applied soil depth is a deviation from OECD Guideline No. 217 (2000), the EU Method C.22, where the PEC is calculated by using 5 cm of the soil depth (chapter 3.3.). The study was finished in February 2019, not in October 2018 as it was planned before. These deviations did not affect the results of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and methods

Materials

Test item:	
Description:	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WDG
Production batch:	SCL-58176
Active ingredients content:	rimsulfuron – 30 g/kg; nicosulfuron – 120 g/kg; mesotrione – 360 g/kg
Vehicle and control:	Distilled water
Test system:	
Species:	Microorganisms
Source:	Agricultural soil taken from the area belonging to the Institute of Industrial Organic Chemistry, Branch Pszczyna.
Experimental conditions:	
Temperature:	18.0 – 20.0°C
Humidity:	42.2 – 50.0% of MWHC
Air changes:	-

Light and photoperiod: Dark (24/24h)

Study design and methods

Experimental period: 29/08/2018 – 27/09/2018

Test design and treatment: 3 portions of soil weighing 1500 g each: one control group and two groups containing the test item. Every portion was divided into three replicates weighing 500 g each. Test duration: 28 days.
Concentrations of the test material:
control, PEC: 0.9 mg of test item/kg of dry weight soil (i.e. 0.026 mg of rimsulfuron + 0.106 mg of nicosulfuron + 0.317 mg of mesotrione/kg dry weight of soil) and 5xPEC: 4.5 mg of test item/kg of dry weight soil (i.e. 0.12 mg of rimsulfuron + 0.53 mg of nicosulfuron + 1.59 mg of mesotrione/kg dry weight of soil).
The mean respiration rate in the treated soil samples was compared with that in the control, and the percent deviation of the treated from the control was calculated after 0, 7, 14, and 28 days of incubation.

Statistics: In order to determine significance of differences between the control and the treated groups, the Shapiro-Wilk's Test on Normal Distribution, the Levene's Test on Variance Homogeneity, and the Williams Multiple Sequential t-test were used.

Results On day 0, and after 7, 14, and 28 days of incubation, no statistically significant differences in respiration intensity between the control soil and the soil treated with the test item at the concentration corresponding to the PEC: 0.9 mg test item/kg dry soil (i.e. 0.026 mg rimsulfuron/kg dry soil + 0.106 mg of nicosulfuron/kg dry soil + 0.317 mg of mesotrione/kg dry soil) and 5 x PEC: 4.5 mg test item/kg dry soil (i.e. 0.13 mg rimsulfuron/kg dry soil + 0.53 mg of nicosulfuron/kg dry soil + 1.59 mg of mesotrione/kg dry soil) were observed.
The percentage deviations between the control soil and the soil treated with the test item at the concentrations corresponding to the PEC: 0.9 mg test item/kg dry soil (i.e. 0.026 mg rimsulfuron/kg dry soil + 0.106 mg of nicosulfuron/kg dry soil + 0.317 mg of mesotrione/kg dry soil) and 5 x PEC: 4.5 mg test item/kg dry soil (i.e. 0.13 mg rimsulfuron/kg dry soil + 0.53 mg of nicosulfuron/kg dry soil + 1.59 mg of mesotrione/kg dry soil) did not exceed 25% on any day of analysis.

Oxygen (O_2) consumption - deviations from the control [%]:

Day	Control	PEC		5 x PEC	
		Consumption [mg/kg dry weight soil/hour]	Deviation [%]	Consumption [mg/kg dry weight soil/hour]	Deviation [%]
0	24.99 ± 3.50	21.45 ± 0.39	1.8	24.02 ± 3.11	13.0
7	21.25 ± 1.47	20.09 ± 2.92	14.5	24.02 ± 3.11	6.9
14	17.90 ± 0.83	16.89 ± 0.01	0.1	24.02 ± 3.11	14.7
28	9.59 ± 0.80	9.59 ± 0.81	8.4	24.02 ± 3.11	4.3

Conclusion

On the basis of the results, it was concluded that Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WDG at the concentrations corresponding to the PEC: 0.9 mg test item/kg dry soil (i.e. 0.026 mg rimsulfuron/kg dry soil + 0.106 mg of nicosulfuron/kg dry soil + 0.317 mg of mesotrione/kg dry soil) and 5 x PEC: 4.5 mg test item/kg dry soil (i.e. 0.13 mg rimsulfuron/kg dry soil + 0.53 mg of nicosulfuron/kg dry soil + 1.59 mg of mesotrione/kg dry soil) can be perceived as having no long-term influence on carbon transformations in soil.

Comments of zRMS:	The study is considered valid. All validity criteria were met. • The coefficients of variation (CV) in the control group were 5.6, 3.6, 1.4, 0.7, and 3.3%, after 0, 7, 14, 28, and 42 days of incubation. The validity criterion was met, because the variation between replicate control samples is less than ± 15%. **Agreed endpoints:** On the basis of the results, it was concluded that Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WDG at the concentration corresponding to the PEC: 0.9 mg test item/kg dry soil (i.e. 0.026 mg rimsulfuron/kg dry soil + 0.106 mg of nicosulfuron/kg dry soil + 0.317 mg of mesotrione/kg dry soil) and 5 x PEC: 4.5 mg test item/kg dry soil (i.e. 0.13 mg rimsulfuron/kg dry soil + 0.53 mg of nicosulfuron/kg dry soil + 1.59 mg of mesotrione/kg dry soil), did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.

	KCP 10.5-02
Reference	
Report	"Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WDG: Soil Microorganisms: Nitrogen Transformation Test". Weronika Dec. 2019. STUDY CODE: G/264/17. Institute of Industrial Organic Chemistry Branch Pszczyna
Guideline(s)	OECD Guideline No. 216 (2000) / EU Method C.21
Deviations	The study was finished in February 2019, not in October 2018 as it was planned before. According the Guideline, the soil extraction should be conducted at 150 rpm for 60 min. However, in this study, the extraction was performed at 90 rpm for 24 hours. The modification resulted from the optimization of the nitrate extraction which showed that the extraction was more effective when the shaking rate was lower and the extraction lasted longer (chapter 3.4.4.4.). The predicted environmental concentration (PEC) is calculated assuming 2.5 cm of the soil depth according to the German conditions for the active substances with the mobility in soil KFoc < 500 mL/g. Thus, the applied soil depth is a deviation from OECD Guideline No. 216 (2000), EU Method C.21, where the PEC is calculated by using 5 cm of the soil depth (chapter 3.3.). According to OECD Guideline No. 216 and EU Method C.21. the substrate chosen for the test (the powdered Lucerne meal) should have a favourable carbon to nitrogen ratio (usually between 12/1 and 16/1). In this study a C/N ratio was lower than the one mentioned in OECD Guideline No. 216 and EU Method C.21 (chapter 3.4.1.). However, it was not a validity criterion or a critical point in the study and it had no influence on the results obtained during the test. These deviations did not affect the results of the study.
GLP	Yes
Acceptability	Yes

Duplication (if vertebrate study)	No

Material and methods

Test material	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WDG
Soil	Agricultural soil collected from a place belonging to the Institute of Industrial Organic Chemistry, Branch Pszczyna
Test design	Three portions of soil (3 x 1500 g), i.e. one control group and two treated groups. Every portion was divided into three replicates (3 x 500 g). The soil was enriched with the organic substrate, i.e. lucerne at dose of 5 g/kg dry weight of soil. Test duration: 28 days.
Concentrations of the test material	control, PEC: 0.9 mg test item/kg dry soil (i.e. 0.026 mg rimsulfuron/kg dry soil + 0.106 mg of nicosulfuron/kg dry soil + 0.317 mg of mesotrione/kg dry soil), 5 x PEC: 4.5 mg test item/kg dry soil (i.e. 0.13 mg rimsulfuron/kg dry soil + 0.53 mg of nicosulfuron/kg dry soil + 1.59 mg of mesotrione/kg dry soil).
Test conditions	Temperature: 18.0 – 22.0°C, soil moisture: 41.7% – 48.1% of the maximum water holding capacity, incubation in darkness.
Endpoints	The concentration of nitrate [mg/kg dry soil] after 0, 7, 14, 28, and 42 days of incubation The nitrate formation rate [mg/kg dry weight of soil/day] for selected time intervals of soil incubation, i.e. 0 - 7, 0 – 14, 0 – 28, 0 – 42 days. Percent deviation from the control in nitrate formation rate calculated for selected time intervals i.e. 0 - 7, 0 – 14, 0 – 28, 0 – 42 days.
Statistical analysis	- Shapiro-Wilk's test on Normal Distribution, - Levene's Test on Variance Homogeneity (with Residuals) - William's Multiple Sequential t-test Procedure

Study design

The aim of the study was to detect long-term adverse effects of Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WDG on the processes of nitrogen transformation in aerobic surface soils.
Agricultural soil was used. It was manually cleared of large objects and sieved to a particle size of 2 mm.
The concentrations of the test item were 0.9 mg test item/kg dry soil (i.e. 0.026 mg rimsulfuron/kg dry soil + 0.106 mg of nicosulfuron/kg dry soil + 0.317 mg of mesotrione/kg dry soil) and 4.5 mg test item/kg dry soil (i.e. 0.13 mg rimsulfuron/kg dry soil + 0.53 mg of nicosulfuron/kg dry soil + 1.59 mg of mesotrione/kg dry soil). The first concentration is the maximum predicted environmental concentration (PEC). The upper tested concentration is the single application rate multiplied by five (5 x PEC).
The treated and the control soils were divided into three replicates.
On days 0, 7, 14, 28, and 42 of incubation, soil samples were collected to determine the quantities of nitrate.
The method involves a measurement of the nitrates ions concentration in a soil extract obtained by using 0.1 M KCl. The pH/ION 7320 digital meter and the NO 800 nitrate electrode were used.
The nitrate formation rate in each treated group was compared with that in the control, and the percent deviation of the treated from the control was calculated.

Results

The difference in the nitrate formation rate between the control soil and the one treated with the test item at the concentration corresponding to the PEC: 0.9 mg test item/kg dry soil (i.e. 0.026 mg rimsulfuron/kg dry soil + 0.106 mg of nicosulfuron/kg dry soil + 0.317 mg of mesotrione/kg dry soil) and 5 x PEC: 4.5 mg test item/kg dry soil (i.e. 0.13 mg rimsulfuron/kg dry soil + 0.53 mg of nicosulfuron/kg dry soil + 1.59 mg of mesotrione/kg dry soil) did not exceed 25% on 42 day of analysis.

Conclusions

On the basis of the results, it was concluded that Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WDG at the concentration corresponding to the PEC: 0.9 mg test item/kg dry soil (i.e. 0.026 mg rimsulfuron/kg dry soil + 0.106 mg of nicosulfuron/kg dry soil + 0.317 mg of mesotrione/kg dry soil) and 5 x PEC: 4.5 mg test item/kg dry soil (i.e. 0.13 mg rimsulfuron/kg dry soil + 0.53 mg of nicosulfuron/kg dry soil + 1.59 mg of mesotrione/kg dry soil), did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.

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

A 2.6.1 KCP 10.6.1 Summary of screening data

A 2.6.2 KCP 10.6.2 Testing on non-target plants

Comments of zRMS:

The study is considered valid. All validity criteria were met.

- the seedling emergence (validity criterion: at least 70%) was as follows:

85.7 – 92.9% – sunflower,

90.5 – 92.9% – cabbage,

81.0 – 95.2% – pea,

85.0 – 92.5% – carrot,

80.0 – 92.5% – onion,

87.5 – 95.0% – oats,

- the mean survival of the emerged control seedlings was 100% for all tested species (validity criterion: at least 90%),
- the control seedlings did not exhibit any visible phytotoxic symptoms,
- environmental conditions for all plants belonging to the same species were identical.

Agreed endpoins:

ER_{50} and NOER values (g/ha).

	Sunflower *Helianthus annuus*	**Cabbage** *Brassica oleracea var. capitata*	**Pea** *Pisum sativum*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	271.8 (148.3 - >330.0**)	>330.0	>330.0	>330.0	50.0 (<0.5 - >330.0**)	>330.0
NOER	36.7	>330.0*	≥330.0	>330.0*	36.7	>330.0*
Shoot length (plants without roots)						
ER_{50}	55.2 (19.2 – 133.0)	79.6 (26.4 – 232.1)	112.9 (76.6 – 174.6)	119.6 (61.3 – 272.1)	22.5 (8.8 – 238.2)	327.8 (208.5 - >330.0**)
NOER	12.2	4.1	12.2	1.4	1.4	12.2
Plant dry weight (plants without roots)						
ER_{50}	12.6 (2.0 – 45.5)	**6.6** (3.8 – 10.6)	25.8 (12.5 – 48.9)	125.4 (66.6 – 280.8)	17.5 (n.d.)	254.1 (169.8 - >330.0**)
NOER	1.4	1.4	12.2	4.1	1.4	36.7

*- the value could not be determined; it can be probably higher than the highest used rate, i.e. 330 g test item/ha.
** - value determined above the tested range of rates.

	n.d. – not determined.

Reference:	KCP 10.6.2-01
Report	"Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WDG. Terrestrial Plant Test: Vegetative Vigour Test". Anna Wróbel. 2020. Study code: G/269/17. Institute of Industrial Organic Chemistry Branch Pszczyna
Guideline(s):	OECD Guideline No. 227 (2006)
Deviations:	Yes. 1. 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 82.9 – 148.7 µE/m2/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:	Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WDG; Batch Number SCL- 58176; active substance: rimsulfuron – 30 g/kg; nicosulfuron – 120 g/kg; mesotrione – 360 g/kg
Test species:	sunflower (*Helianthus annuus*), cabbage (*Brassica oleracea var. capitata*), pea (*Pisum sativum*), carrot (*Daucus carrota*), onion (*Allium cepa*), oats (*Avena sativa*)
Soil:	sandy loam
Study design:	number of rates: seven application rates + control; number of replicates: 4 or 7 replicates/rate; the total number of plants per application rate – 20 or 21; test termination: 21 days after the spraying
Application rates:	Control; 330, 110, 36.7, 12.2, 4.1, 1.4, and 0.5 g test item/ha (i.e. 9.90 + 39.60 + 118.80, 3.30 + 13.20 + 39.60, 1.10 + 4.40 + 13.20, 0.37 + 1.47 + 4.40, 0.12 + 0.49 + 1.47, 0.04 + 0.16 + 0.49, and 0.01 + 0.05 + 0.16 g of rimsulfuron + nicosulfuron + mesotrione/ha). Volume of deionised water used to prepare the highest rate: 300 L/ha
Test conditions:	temperature: 19.0 – 24.4°C, humidity: 48.9 – 93.4%, controlled light – dark cycles (16h:8h), light intensity: 82.9 – 148.7 µE/m^2/s, carbon dioxide concentration: 374 - 380 ppm.
Statistical analysis:	ER_{10}, ER_{25}, ER_{50} – probit analysis (plants number) or Weibull analysis (shoot length and dry weight) NOER: For the plant number: - Fisher's Exact Binomial Test with Bonferroni Correction For the shoot length and the plant dry weight after 21 days of the exposure: - 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 or Multiple Sequentially-rejective U-test After Bonferroni-Holm.

Endpoints: ER_{10}, ER_{25}, ER_{50}, NOER

Results and Conclusions

The test item did not cause mortality of cabbage, carrot and oats. Mortality of plants occurred in cultivation of sunflower, pea and onion.

On the basis of NOER, ER_{10}, ER_{25} and ER_{50} values determined from the shoot length and dry shoot weight it was proved that the test item inhibited the process of growth of sunflower, pea, cabbage, carrot, onion and oats.

The phytotoxic symptoms in cultivation of all tested species were noticed.

They were stunted growth (sunflower, cabbage, pea, carrot, onion, oats), necrosis (sunflower, onion) chlorosis (sunflower, cabbage, pea), spots (sunflower, cabbage, pea, carrot), plant mortality (sunflower, onion, pea). The detailed % mean effects / application rate at day 21 and the corresponding ER_{50} after dose-response assessment are specified in the table below:

Dose (g/ha)	Sunflower		Cabbage		Pea		Carrot		Onion		Oats	
0 (ctrl)	0	nc	0	nc	0	nc	0	nc	0	nc	0	nc
0.5	0	nc	0	nc	0	nc	0	nc	0	nc	0	nc
1.4	0	nc	0	nc	0	nc	0	nc	0	nc	0	nc
4.1	5	s	0	nc	0	nc	0	nc	30	sg	0	nc
12.2	5	s	5	s	5	s/chl	5	s	40	sg	0	nc
36.7	32.9	s/chl/sg/d	30	s/sg	5	s/chl	20	s/sg	50	sg	20	sg
110	54.3	s/chl/sg/n/d	50	s/chl/sg	28.6	s/chl/d	40	s/sg	100	d	30	sg
330	70	s/chl/sg/n/d	60	s/chl/sg	30	s/chl/sg	40	s/sg	100	d	40	sg
ER_{50}	**80.2 g/ha**		**95.6 g/ha**		**>330 g/ha**		**>330 g/ha**		**21.4 g/ha**		**>330 g/ha**	

nc – no changes, sg – stunted growth, chl – chlorosis, s – spots, n – necrosis, d - dead plant

ER_{50} and NOER values (g/ha).

	Sunflower *Helianthus annuus*	**Cabbage** *Brassica oleracea var. capitata*	**Pea** *Pisum sativum*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	271.8 (148.3 - >330.0**)	>330.0	>330.0	>330.0	50.0 (<0.5 - >330.0**)	>330.0
NOER	36.7	>330.0*	≥330.0	>330.0*	36.7	>330.0*
Shoot length (plants without roots)						
ER_{50}	55.2 (19.2 – 133.0)	79.6 (26.4 – 232.1)	112.9 (76.6 – 174.6)	119.6 (61.3 – 272.1)	22.5 (8.8 – 238.2)	327.8 (208.5 ->330.0**)
NOER	12.2	4.1	12.2	1.4	1.4	12.2
Plant dry weight (plants without roots)						
ER_{50}	12.6 (2.0 – 45.5)	6.6 (3.8 – 10.6)	25.8 (12.5 – 48.9)	125.4 (66.6 – 280.8)	17.5 (n.d.)	254.1 (169.8 ->330.0**)
NOER	1.4	1.4	12.2	4.1	1.4	36.7

*- the value could not be determined; it can be probably higher than the highest used rate, i.e. 330 g test item/ha.
** - value determined above the tested range of rates.
n.d. – not determined.

ER_{50} and NOER values (g rimsulfuron/ha).

	Sunflower *Helianthus annuus*	**Cabbage** *Brassica oleracea var. capitata*	**Pea** *Pisum sativum*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	8.15 (4.45 - >9.90**)	>9.90	>9.90	>9.90	1.50 (<0.02 - >9.90**)	>9.90
NOER	1.10	>9.90*	≥9.90	>9.90*	1.10	>9.90*

Shoot length (plants without roots)						
ER_{50}	1.66 (0.58 – 3.99)	2.39 (0.79 – 6.96)	3.39 (2.30 – 5.24)	3.59 (1.84 – 8.16)	0.68 (0.26 – 7.15)	9.83 (6.26 ->9.90**)
NOER	0.37	0.12	0.37	0.04	0.04	0.37
Plant dry weight (plants without roots)						
ER_{50}	0.38 (0.06 – 1.37)	0.20 (0.11 – 0.32)	0.77 (0.38 – 1.47)	3.76 (2.00 – 8.42)	0.53 (n.d.)	7.62 (5.09 ->9.90**)
NOER	0.04	0.04	0.37	0.12	0.04	1.10

*- the value could not be determined; it can be probably higher than the highest used rate, i.e. 30 g of rimsulfuron/ha.
** - value determined above the tested range of rates.
n.d. – not determined

ER_{50} and NOER values (g nicosulfuron/ha).

	Sunflower *Helianthus annuus*	**Cabbage** *Brassica oleracea var. capitata*	**Pea** *Pisum sativum*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	32.62 (17.80 - >39.60**)	>39.60	>39.60	>39.60	6.00 (<0.06 - >39.60**)	>39.60
NOER	4.40	>39.60*	≥ 39.60	>39.60*	4.40	>39.60*
Shoot length (plants without roots)						
ER_{50}	6.62 (2.30 – 15.96)	9.55 (3.17 – 27.85)	13.55 (9.19 – 20.95)	14.35 (7.36 – 32.65)	2.70 (1.06 – 28.58)	39.34 (25.02 ->39.60**)
NOER	1.46	0.49	1.46	0.17	0.17	1.46
Plant dry weight (plants without roots)						
ER_{50}	1.51 (0.24 – 5.46)	0.79 (0.46 – 1.27)	3.10 (1.50 – 5.87)	15.05 (7.99 – 33.70)	2.10 (n.d.)	30.49 (20.39 ->39.60**)
NOER	0.17	0.17	1.46	0.49	0.17	4.40

*- the value could not be determined; it can be probably higher than the highest used rate, i.e. 120 g of nicosulfuron/ha
** - value determined above the tested range of rates
n.d. – not determined.

ER_{50} and NOER values (g mesotrione/ha).

	Sunflower *Helianthus annuus*	**Cabbage** *Brassica oleracea var. capitata*	**Pea** *Pisum sativum*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	97.85 (53.39 - >118.8**)	>118.80	>118.0	>118.80	18.00 (<0.18 - >118.8**)	>118.80
NOER	13.21	>118.80*	≥118.0	>118.80*	13.21	>118.80*
Shoot length (plants without roots)						
ER_{50}	19.87 (6.91 –47.88)	28.66 (9.50 – 83.56)	40.64 (27.58 – 62.86)	43.06 (22.07 – 97.96)	8.10 (3.17 – 85.75)	118.01 (75.06 ->118.8**)
NOER	4.39	1.48	4.39	0.50	0.50	4.39
Plant dry weight (plants without roots)						
ER_{50}	4.54 (0.72 – 16.38)	2.38 (1.37 – 3.82)	9.29 (4.50 – 17.60)	45.14 (23.98 – 101.09)	6.30 (n.d.)	91.48 (61.13 ->118.8**)
NOER	0.50	0.50	4.39	1.48	0.50	13.21

*- the value could not be determined; it can be probably higher than the highest used rate, i.e. 120 g of nicosulfuron/ha
** - value determined above the tested range of rates
n.d. – not determined.

Comments of zRMS:	The study is considered valid. All validity criteria were met. • the seedling emergence in the control (validity criterion: at least 70%) was as follows: 95.2% – pea, 81.0% – cabbage, 85.0% – carrot, 100.0% – sunflower, 70.0% – onion, 95.0% – oats,

- the mean survival of the emerged control seedlings was 100% for all test plant species (validity criterion: at least 90%);
- the control seedlings did not exhibit any visible phytotoxic effects;.
- environmental conditions for all plants of the same species were identical.

Agreed endpoints:

ER_{50} and NOER values (g/ha).

	Pea *Pisum sativum*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Sunflower** *Helianthus annuus*	**Onion** *Allium cepa*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	>330	>330	>330	>330	109.9 (39.9 – 302.6)	>330
NOER	≥330	≥330	110	≥330	110	≥330
Shoot length (plants without roots)						
ER_{50}	141.6 (69.2 – >330*)	49.4 (29.1 – 79.5)	218.5 (154.9 - >330*)	>330	41.8 (12.1 – 193.4)	>330
NOER	36.7	12.2	12.2	36.7	12.2	110
Plant dry weight (plants without roots)						
ER_{50}	112.9 (51.7 – >330*)	43.0 (25.8 – 67.9)	94.7 (47.2 – 186.4)	>330 (115.1 - >330*)	41.2 (19.7 – 96.4)	>330
NOER	36.7	12.2	12.2	36.7	4.1	110

*value determined above the tested range of rates

Visual phytotoxicity:

ER_{50}=16.7 g/ha (cabbage), the most sensitive species

Reference:	KCP 10.6.2-02
Report	"Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WDG. Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test". Anna Wróbel, 2020. Study code: G/268/17. Institute of Industrial Organic Chemistry Branch Pszczyna
Guideline(s):	OECD Guideline No. 208 (2006)
Deviations:	Yes. 1. 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 76.7 and 117.2 µE/m2/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item: Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WDG; Batch Number

	SCL- 58176; active substance: rimsulfuron – 30 g/kg; nicosulfuron – 120 g/kg; mesotrione – 360 g/kg
Test species:	pea (*Pisum sativum*), cabbage (*Brassica oleracea var. capitata*), carrot (*Daucus carota*), sunflower (*Helianthus annuus*), onion (*Allium cepa*), oats (*Avena sativa*).
Soil:	Sandy loam
Study design:	Number of rates: 7 + control; number of replicates/rate: 4 (carrot, onion, oats) or 7 (sunflower, cabbage, pea). The total number of seeds per application rate – 20 (carrot, onion, oats) or 21 (cabbage, pea, sunflower) Test termination: 14 days after the emergence of 50% of the control seedlings
Application rates:	Control, 0.5, 1.4, 4.1, 12.2, 36.7, 110 and 330 g test item/ha (i.e. 0.01 + 0.05 + 0.16, 0.04 + 0.16 + 0.49, 0.12 + 0.49 + 1.47, 0.37 + 1.47 + 4.40, 1.10 + 4.40 + 13.20, 3.30 + 13.20 + 39.60, 9.90 + 39.60 + 118.80 g of rimsulfuron + nicosulfuron + mesotrione/ha), Volume of deionized water used to prepare the highest rate corresponded 300 L water/ha
Test conditions:	Temperature: 19.0 – 24.4°C, humidity: 48.9 – 93.4%, lighting: 16 h light : 8 h dark; light intensity: 76.7 – 117.2 µE/m2/s; carbon dioxide concentration: 323 – 361 ppm
Statistical analysis:	ER_{10}, ER_{25}, ER_{50} – Weibull analysis, NOER: In order to determine the NOER values for the emergence the following statistical tests were used: Fisher's Exact Binomial Test with Bonferroni Correction. In order to determine the NOER values for the shoot length at the end of the experiment (shoots cut down above the ground) and for the plant weight at the end of the experiment (shoots cut down above the ground), the following statistical tests were used: 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
Endpoints:	ER_{10}, ER_{25}, ER_{50}, NOER

Results and Conclusions

The test item i.e. Rimsulfuron 3% + Nicosulfuron 12% + Mesotrione 36% WDG had a varied impact on the growth and seedling emergence of the test plant species. The impact depended on the application rate and species.

Seedling emergence of pea, cabbage, carrot, sunflower and onion was delayed in comparison to the control.

On the basis of ER_{10}, ER_{25}, ER_{50} and NOER values determined from final number of plants it was proved that the test item inhibited seedling emergence of carrot and onion. Moreover, seedling emergence of cabbage was slightly inhibited.

On the basis of ER_{10}, ER_{25}, ER_{50} and NOER values determined from the shoot length it was proved that the test item inhibited the process of growth of all tested species.

On the basis of ER_{10}, ER_{25}, ER_{50} and NOER determined from the dry shoot weight it was proved that the test item inhibited the process of growth of all tested species.

Significant mortality of plants related with the test item was observed only in case of onion.

Among phytotoxic symptoms spots (cabbage, sunflower), chlorosis (pea, cabbage, carrot, sunflower), wilting (carrot, onion), stunted growth (all tested species) and plants mortality (onion, cabbage, oats) were

observed. The detailed % mean effects / application rate at day 14 and the corresponding ER_{50} after dose-response assessment are specified in the table below:

Dose (g/ha)	Sunflower		Cabbage		Pea		Carrot		Onion		Oats	
0 (ctrl)	0	nc	0	nc	0	nc	0	nc	0	nc	0	nc
0.5	0	nc	0	nc	0	nc	0	nc	0	nc	0	nc
1.4	0	nc	0	nc	0	nc	0	nc	0	nc	0	nc
4.1	0	nc	20	sg/chl/s	0	nc	0	nc	10	sg	0	nc
12.2	0	nc	40	sg/chl/s	0	nc	10	sg	10	sg	0	nc
36.7	0	nc	70	sg/chl/s	0	nc	20	sg	60	sg/w	5	d/nc
110	60	chl/s/sg	80	sg/chl/s	50	sg/chl	50	sg/w	60	sg/w/d	10	sg
330	65.7	chl/s/sg	90	sg/chl/s	90	sg/chl	70	sg/w/chl	100	d	37.5	sg
ER_{50}	**97.3 g/ha**		**16.7 g/ha**		**110 g/ha**		**114.6 g/ha**		**47.7 g/ha**		**>330 g/ha**	

nc – no changes, sg – stunted growth, w – wilting, chl – chlorosis, s – spots, n – necrosis, d - dead plant

The following order of the test plant sensitivity was noticed:
onion > carrot > cabbage > sunflower, pea > oats .

ER_{50} and NOER values (g/ha).

	Pea *Pisum sativum*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Sunflower** *Helianthus annuus*	**Onion** *Allium cepa*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	>330	>330	>330	>330	109.9 (39.9 – 302.6)	>330
NOER	≥330	≥330	110	≥330	110	≥330
Shoot length (plants without roots)						
ER_{50}	141.6 (69.2 – >330*)	49.4 (29.1 – 79.5)	218.5 (154.9 - >330*)	>330	41.8 (12.1 – 193.4)	>330
NOER	36.7	12.2	12.2	36.7	12.2	110
Plant dry weight (plants without roots)						
ER_{50}	112.9 (51.7 – >330*)	43.0 (25.8 – 67.9)	94.7 (47.2 – 186.4)	>330 (115.1 - >330*)	41.2 (19.7 – 96.4)	>330
NOER	36.7	12.2	12.2	36.7	4.1	110

*value determined above the tested range of rates

ER_{50} and NOER values (g rimsulfuron/ha).

	Pea *Pisum sativum*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Sunflower** *Helianthus annuus*	**Onion** *Allium cepa*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	>9.90	>9.90	>9.90	>9.90	3.30 (1.20 – 9.08)	>9.90
NOER	≥9.90	≥9.90	≥9.90	≥9.90	3.30	≥9.90
Shoot length (plants without roots)						
ER_{50}	4.25 (2.08 – >9.90*)	1.48 (0.87 – 2.39)	6.56 (4.65 - >9.90*)	>9.90	3.30 (1.20 – 9.08)	>9.90
NOER	1.10	0.37	0.37	1.10	0.37	3.30
Plant dry weight (plants without roots)						
ER_{50}	3.39 (1.55 – >9.90*)	1.29 (0.77 – 2.04)	2.84 (1.42 – 5.59)	>9.90 (3.45 - >9.90*)	1.24 (0.59 – 2.89)	>9.90
NOER	1.10	0.37	0.37	1.10	0.12	3.30

*value determined above the tested range of rates

ER_{50} and NOER values (g nicosulfuron/ha).

	Pea *Pisum sativum*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Sunflower** *Helianthus annuus*	**Onion** *Allium cepa*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	>39.60	>39.60	>39.60	>39.60	13.19 (4.79 – 36.31)	>39.60
NOER	≥39.60	≥39.60	13.20	≥39.60	13.20	≥39.60
Shoot length (plants without roots)						
ER_{50}	16.99	5.93	26.22	>39.60	5.02	>39.60

	(8.30 – >39.60*)	(3.49 – 9.54)	(18.59 - >39.60*)		(1.45 – 23.21)	
NOER	4.40	1.46	1.46	4.40	1.46	13.20
Plant dry weight (plants without roots)						
ER_{50}	13.55 (6.20 – >39.60*)	5.16 (3.10 – 8.15)	11.36 (5.66 – 22.37)	>39.60 (13.81 - >39.60*)	4.94 (2.36 – 11.57)	>39.60
NOER	4.40	1.46	1.46	4.40	0.49	13.20

*value determined above the tested range of rates

ER_{50} and NOER values (g mesotrione/ha).

	Pea *Pisum sativum*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Sunflower** *Helianthus annuus*	**Onion** *Allium cepa*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	>118.80	>118.80	>118.80	>118.80	39.56 (14.36 – 108.94)	>118.80
NOER	≥118.80	≥118.80	39.60	≥118.80	39.60	≥118.80
Shoot length (plants without roots)						
ER_{50}	50.98 (24.91 – >118.80*)	17.78 (10.48 – 28.62)	78.66 (55.76 - >118.80*)	>118.80	15.05 (4.36 – 69.62)	>118.80
NOER	13.21	4.39	4.39	13.21	4.39	39.60
Plant dry weight (plants without roots)						
ER_{50}	40.64 (18.61 – >118.80*)	15.48 (9.29 – 24.44)	34.09 (16.99 – 67.10)	>118.80 (41.44 - >118.80*)	14.83 (7.09 – 34.70)	>118.80
NOER	13.21	4.39	4.39	13.21	1.48	39.60

*value determined above the tested range of rates

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

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

A 2.8 KCP 10.8 Monitoring data