FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: GLOB289H / SAP63H

Product name(s): Zeppos

Chemical active substances:
Iodosulfuron-methyl-sodium, 6 g/kg
Mesosulfuron-methyl, 30 g/kg
Safener: Mefenpyr-diethyl, 90 g/kg

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Globachem N.V. / Ascenza Agro S.A.

Submission date: December 2019

MS Finalisation date: February 2021, September 2021, January 2022, February 2023

Version history

When	What
December 2019	V0 - Original version from applicant for submission to zRMS POLAND in the frame of new PPP registration
February 2021	The assessment of dRR by ZRMS -Poland
July 2021	Additional calculations provided by the applicant
September 2021	Finalisation of the assessment by zRMS
January 2022	Final version of the RR after Commenting period
February 2023	Final version of RR after Commenting period with regard DE's comments and remarks of Ministry of Agriculture.

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9 Ecotoxicology (KCP 10)

This document reviews the ecotoxicological studies and risk assessments for the product Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (0.6+3+9)%WG (also referred to as SAP63H, GLOB289H, Iodosulfuron + Mesosulfuron (0.6% + 3%) WG and Zeppos in the dossier). The product contains two active substances iodosulfuron-methyl-sodium and mesosulfuron-methyl-sodium, and safener mefenpyr-diethyl.
The product can be mixed in the tank with a non-ionic surfactant or a non-esterified rapeseed oil. In order to address the effect on the non-target organisms, the combination of the plant protection product with a non-ionic surfactant (Pottok) and a non-esterified rapeseed oil (Actirob) was also tested in separate ecotoxicological studies. A full risk assessment according to uniform principles is provided, which demonstrates that the product is safe for the environment.

Where appropriate, this document refers to the conclusions of the EU reviews of the active substances. This will be where:

- The active substance data is relied upon in the risk assessment of the formulation; or
- The EU review concluded that additional data/information should be considered at national registration.

Note: This Part B document only reviews data (Annex II and/or Annex III) and additional information that has not previously been considered within the EU review process, as part of the Annex I inclusion decision. New Annex II or Annex III data were included if they are considered essential for the evaluation and in this case a full study summary is provided. In the case where studies have been previously evaluated at European level, detailed summaries have not been provided.

The product Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (0.6% + 3% + 9%) WG was not the representative formulation during the Annex I inclusion of Iodosulfuron-methyl-sodium or Mesosulfuron-methyl and has thus not yet been evaluated.

Iodosulfuron-methyl-sodium
Iodosulfuron-methyl-sodium was included into Annex I of Directive 91/414/EEC in 2003 (Directive 2003/84/EC) and re-evaluated in accordance with Regulation (EC) No 1107/2009 and Commission Implementing Regulation (EU) No 844/2012, leading to the renewal of the approval of the active substance iodosulfuron-methyl-sodium (Commission Implementing Regulation (EU) 2017/407 of 8 March 2017, entry into force 1st of April 2017).

For the implementation of the Uniform Principles of Annex VI, the conclusions of the Renewal Report on iodosulfuron-methyl-sodium, as finalised in the Standing Committee on Plants, Animals, Food and Feed at its meeting on 7 December 2016 shall be taken into account.

In this overall assessment Member States should pay attention to:

- The protection of consumers,
- The protection of non-target terrestrial plants,
- The protection of aquatic plants

The Renewal Report (SANTE/2016/11167 Rev 3, 7/12/2016) for iodosulfuron-methyl-sodium provides a summary of the relevant scientific information from the EU review.

Mesosulfuron-methyl
Mesosulfuron-methyl was included in Annex I of Directive 91/414/EEC in 2003 (Directive 2003/119/EEC) and re-evaluated in accordance with Regulation (EC) No 1107/2009 and Commission Implementing Regulation (EU) No 844/2012, leading to the renewal of the approval of the active substance mesosulfuron-methyl (Commission Implementing Regulation (EU) 2017/755 of 28 April 2017, entry into force 1st of July 2017).

For the implementation of the Uniform Principles of Annex VI, the conclusions of the Renewal Report on mesosulfuron-methyl, as finalised in the Standing Committee on Plants, Animals, Food and Feed at is meeting on 23 March 2017 shall be taken into account.

In this overall assessment Member States should pay attention to:

- The protection of aquatic organisms and non-target terrestrial plants;
- The protection of groundwater

The Renewal Report (SANTE/11827/2016 Rev 2, 23/03/2017) for mesosulfuron-methyl provides a summary of the relevant scientific information from the EU review.

Safener mefenpyr-diethyl

Mefenpyr-diethyl is a safener used in combination with herbicides and was not reviewed under Directive 91/414/EEC or Regulation (EC) No 1107/2009. In order to facilitate the assessment of products containing mefenpyr-diethyl, France and Austria in a work-sharing project prepared an assessment report for this substance in the format of a DAR. France was responsible for the sections “Phys-Chem Properties” (B.1-B.5), Environmental Fate and Ecotoxicology (B.8-B.9) and Austria for sections Toxicology and Residue Data (B.6-B.7). A bilateral peer-review in the form of comments took place between the two rapporteurs; the respective reporting tables were made available to all MS. In September 2011 the assessment report was “peer-reviewed” (in an unscheduled procedure on voluntary basis) by all MS. The revised assessment report can be found on CIRCA (Archive individual substances – Mefenpyr-diethyl (safener)).

All exposure and risk assessments presented will be based on agreed endpoints, if not otherwise stated.

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

GAP rev. 4, date: 2019/08/13

PPP (product name/code):	0.6% Iodo + 3% meso + 9% mefenpyr WG	Formulation type:	WG
Active substance 1:	Iodosulfuron	Conc. of as 1:	6 g/kg
Active substance 2:	Mesosulfuron	Conc. of as 2:	30 g/kg
Safener:	Mefenpyr	Conc. of safener:	90 g/kg
Applicant:	Globachem N.V. / Ascenza Agro S.A.	Professional use:	☒
Zone(s):	Central	Non professional use:	☐
Verified by MS:	--		
Field of use:	Herbicide		

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use-No. (e)	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			**PHI** (days)	**Remarks:**	**Conclusion**						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product / ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		e.g. g safener/synergist per ha (f)	Birds	Mammals	Aquatic organisms	Bees	Non-target	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	PL	Cereals (winter/spring soft wheat, winter/spring durum wheat, triticale, spelt and winter rye)	F	Annual grassy weeds and Annual dicotyledonous weeds: CAPBP	Downwards spraying	BBCH 21-32	a) 1 b) 1	/	a) 0.1 b) 0.1	a) 0.6 + 3 b) 0.6 + 3	100-400	NA	Mefenpyr (safener): 9 g/ha Applied with 0.2 L/ha oil/wetting agent							
2	PL	Cereals (winter/spring soft	F	Annual grassy weeds and Annual dicoty-	Downwards spraying	BBCH 21-32	a) 1 b) 1	/	a) 0.2 b) 0.2	a) 1.2 + 6 b) 1.2 + 6	100-400	NA	Mefenpyr (safener): 18 g/ha							

		wheat, winter/spring durum wheat, triticale, spelt and winter rye)		ledonous weeds: VERPE CAPBP MATCH									Applied with 0.4 L/ha oil/wetting agent							
3	PL	Cereals (winter/spring soft wheat, winter/spring durum wheat, triticale, spelt and winter rye)	F	Annual grassy weeds and Annual dicotyledonous weeds: APESV GALAP MATIN STEME CABP POAAN	Downwards spraying	BBCH 21-32	a) 1 b) 1	/	a) 0.3 b) 0.3	a) 1.8 + 9 b) 1.8 + 9	100-400	NA	Mefenpyr (safener): 27 g/ha Applied with 0.6 L/ha oil/wetting agent							
4	PL	Cereals (winter soft wheat, winter durum wheat, triticale, spelt and winter rye)	F	Annual grassy weeds and Annual dicotyledonous weeds: ALOMY AVEFA CHEAL PAPRH VIOAR	Downwards spraying	BBCH 21-32	a) 1 b) 1	/	a) 0.4 b) 0.4	a) 2.4 + 12 b) 2.4 + 12	100-400	NA	Mefenpyr (safener): 36 g/ha Applied with 0.8 L/ha oil/wetting agent							
5	BE, NL, DE, CZ	Cereals (winter/spring soft wheat, winter/spring durum wheat, triticale, spelt and winter rye)	F	Annual grassy weeds and Annual dicotyledonous weeds: POAAN PAPRH LAMPU APESV CHEAL MATIN STEME	Downwards spraying	BBCH 21-32	a) 1 b) 1	/	a) 0.3 b) 0.3	a) 1.8 + 9 b) 1.8 + 9	100-400	NA	Mefenpyr (safener): 27 g/ha Optionally with 0.6 L/ha oil/wetting agent							
6	BE, NL, DE, CZ	Cereals (winter soft wheat, winter durum wheat, triticale,	F	Annual grassy weeds and Annual dicotyledonous	Downwards spraying	BBCH 21-32	a) 1 b) 1	/	a) 0.4 b) 0.4	a) 2.4 + 12 b) 2.4 + 12	100-400	NA	Mefenpyr (safener): 36 g/ha Applied with 0.8 L/ha							

		spelt and winter rye)		weeds: MATCH MATIN STEME									oil/wetting agent							
7	BE, NL, DE, CZ	Cereals (winter soft wheat, winter durum wheat, triticale, spelt and winter rye)	F	Annual grassy weeds and Annual dicoty-ledonous weeds: ALOMY STEME MATIN GALAP VIOAR	Downwards spraying	BBCH 21-32	a) 1 b) 1	/	a) 0.5 b) 0.5	a) 3 + 15 b) 3 + 15	100-400	NA	Mefenpyr (safener): 45 g/ha Applied with 1 L/ha oil/wetting agent							

* 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

*** IMS: Iodosulfuron-methyl sodium
MSM: Mesosulruon-methyl

Explanation for column 15 – 21 “Conclusion”

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

Remarks table:

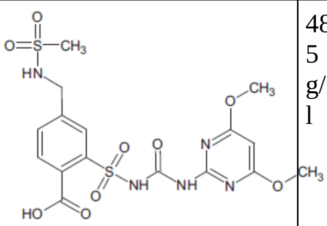
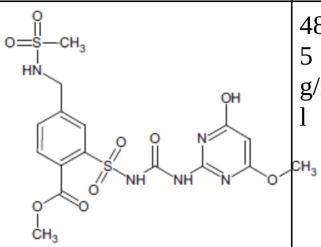
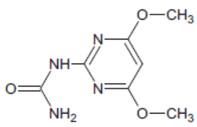
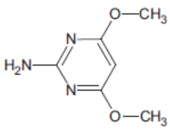
(1) Numeration necessary to allow references
(2) Use official codes/nomenclatures of EU
(3) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure)
(4) F: professional field use, Fn: non-professional field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

9.1.1 Overall conclusions

ZRMS comments:

Since report in dRR format is prepared by the Applicant, all remarks, comments, additional calculations and assessment done by the ZRMS **are included in the commenting boxes or highlighted in the text in blue**.

9.1.2 Table 9.1-3 Metabolites of mesosulfuron-methyl

9.1.2.1 Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
Mesosulfuron		489.5 g/mol	Soil: max. 16.2% of a.s. Water: max. 4.9% of a.s.	Yes, soil organisms and aquatic organisms
AE F160459		489.5 g/mol	Soil: > 5% of a.s. in 2 sequential measurements Water: max. 21.6% of a.s.	Yes, soil organisms and aquatic organisms
AE F099095		198.2 g/mol	Soil: max. 16.2% of a.s. Water: max. 4.9% of a.s.	Yes, soil organisms and aquatic organisms
AE F092944		155.2 g/mol	Soil: > 5 % of as in 2 sequential measurements Water: max. 21.6% of a.s.	Yes, soil organisms and aquatic organisms

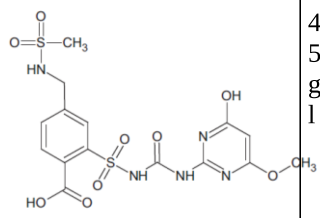
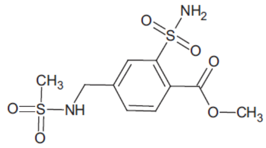
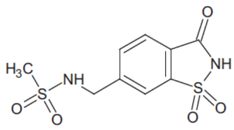
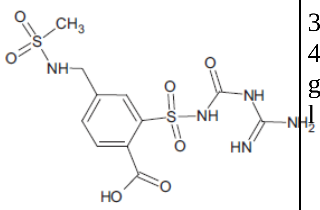
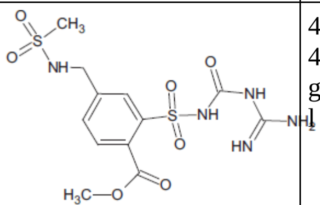
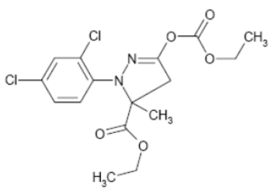
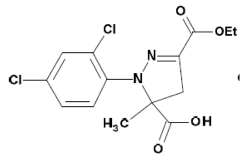
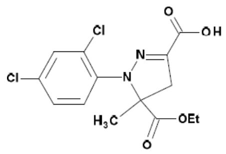
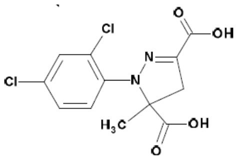
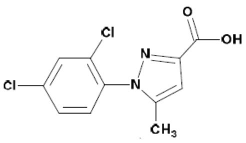
9.1.2.1 Metabolite	**Chemical structure**	**Molar mass**	**Maximum occurrence in compartments**	**Risk assessment required?**
AE F160460		475.5 g/mol	Soil: max. 29.2% of a.s. Water: max. 0.9% of a.s.	Yes, soil organisms and aquatic organisms
AE F140584		322.4 g/mol	Soil: max. 10.1% of a.s. Water: max. 3.2% of a.s.	Yes, soil organisms and aquatic organisms
AE F147447		290.3 g/mol	Soil: > 5 % of as in 2 sequential measurements Water: max. 8.4% of a.s.	Yes, soil organisms and aquatic organisms
BCS-CV14885		393.4 g/mol	Soil: > 5 % of as in 2 sequential measurements Water: max. 1.9% of a.s.	Yes, aquatic organisms
BCS-CO60720		407.4 g/mol	Soil: > 5 % of as in 2 sequential measurements Water: max. 10.9% of a.s.	Yes, aquatic organisms

Table 9.1-4 Metabolites of mefenpyr-diethyl

Metabolite	**Chemical structure**	**Molar mass**	**Maximum occurrence in compartments**	**Risk assessment required?**
AE 2211046		391.26 g/mol	Soil: max. 11.5 % of a.s.	Yes, soil organisms

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
AE F114952		345.18 g/mol	Water: max. 18.6 % of a.s.	No
AE F113225		345.2 g/mol	Soil: max. 44.1 % of a.s. Water: max. 82.8 % of a.s	Yes, soil organisms and aquatic organisms
AE F109453		317.13 g/mol	Water: max. 46.5 % of a.s.	Yes, aquatic organisms
AE F094270		271.1 g/mol	Soil: max. 72.2 % of a.s. Water: max. 62.4 % of a.s.	Yes, soil organisms and aquatic organisms

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

The TER_a and TER_{lt} values exceed the triggers of 10 and 5 for the acute and long-term exposure respectively at the screening step, indicating that iodosulfuron-methyl-sodium, mesosulfuron-methyl and mefenpyr-diethyl do not pose an acute or long-term risk to wild birds and mammals. In addition, the combined effect of simultaneous exposure was considered. Also for the combined exposure, the trigger of 10 for the acute exposure was exceeded, indicating an acceptable risk for the use of GLOB289H according to the intended GAP.

The ratio of the effective application rate to the acute and long term toxicity endpoint is less than 50 for the less sorptive substances iodosulfuron-methyl-sodium and mesosulfuron-methyl. For the more sorptive substance mefenpyr-diethyl, the trigger of 3000 is not exceeded. Therefore it is considered that there is low risk of acute/long term toxicity to birds and mammals from the uptake of contaminated drinking water and no further assessment is required.

Iodosulfuron-methyl-sodium and mesosulfuron-methyl both have a log P_{OW} value < 3. Therefore no assessment of secondary poisoning was considered necessary. Mefenpyr-diethyl has a log Pow value > 3, a risk assessment for secondary poisoning was therefore considered necessary.

Earthworm-eating birds

With a TER of 311.8 which largely exceeds the trigger of 5, there is a large safety margin so the risk for earthworm-eating birds due to exposure to mefenpyr-diethyl via bioaccumulation in earthworms is considered acceptable.

Fish-eating birds

The TER of 212 largely exceeds the trigger value of 5, so the risk for fish-eating birds due to exposure to mefenpyr-diethyl via bioaccumulation in fish is considered acceptable.

Earthworm-eating mammals

With a TER of 216.6 which largely exceeds the trigger of 5, there is a large safety margin so the risk for earthworm-eating mammals due to exposure to mefenpyr-diethyl via bioaccumulation in earthworms is considered acceptable.

Fish-eating mammals

The TER of 198.7 largely exceeds the trigger value of 5, so the risk for fish-eating mammals due to exposure to mefenpyr-diethyl via bioaccumulation in fish is considered acceptable.

9.1.2.3 Effects on aquatic organisms (KCP 10.2)

An acceptable acute and long-term risk to aquatic organisms is identified for the intended use of GLOB289H (+ adjuvant) in winter and spring cereals, considering country-specific risk mitigation measures according to following overview:

Winter cereals (0.5 kg PPP/ha)		
Scenario	**Water body**	**Mitigation measure requested**
D1	Ditch	No
D1	Stream	No
D2	Ditch	Yes
D2	Stream	Yes
D3	Ditch	No
D4	Pond	No
D4	Stream	No
D5	Pond	No
D5	Stream	No
R1	Pond	No
R1	Stream	No
R3	Stream	No
R4	Stream	No
Winter cereals (0.3 kg PPP/ha)		
Scenario	**Water body**	**Mitigation measure requested**
D1	Ditch	No
D1	Stream	No
D2	Ditch	Yes
D2	Stream	Yes
D3	Ditch	No
D4	Pond	No
D4	Stream	No
D5	Pond	No
D5	Stream	No
R1	Pond	No
R1	Stream	No
R3	Stream	No
R4	Stream	No
Spring cereals (0.3 kg PPP/ha)		
Scenario	**Water body**	**Mitigation measure requested**
D1	Ditch	No
D1	Stream	No
D3	Ditch	No
D4	Pond	No
D4	Stream	No
D5	Pond	No
D5	Stream	No
R4	Stream	No

Risk assessment for the product GLOB289H:

SPe3: To protect aquatic organism 5 meter buffer zone to surface water bodies should be applied.

Based on the Tier 1 study with RAC=0.129 µg a.s./L (for the most sensitive species) and FOCUS STEP 1-4 calculations for application winter cereals at rate 15 g a.s./L (covering the risk for applicationrate of 9 g a.s./ha) and for spring cereals the following conclusion for aquatic risk assessment can be provided:

Tier 1 data (RAC =0.129 µg a.s./L)

The risk for winter cereals at rate 15 g a.s./ha is considered acceptable when:

- A vegetative filter strip of 10 m for R3 scenario and a vegetative buffer strip of 20 m for R4 scenario will be applied to surface water bodies
- For D1 scenarios – further refinement is required.
- For member states where D2 is relevant, a national specific risk mitigation measures should be applied.

The risk for winter cereals at rate 9 g a.s./ha is considered acceptable when:

- A vegetative filter strip of 10 m for R3 scenario and a vegetative buffer strip of 20 m for R4 scenario will be applied to surface water bodies
- For member states where D2 is relevant, a national specific risk mitigation measure should be applied.

The risk for spring cereals at rate 9 g a.s./ha is considered acceptable except For D1 scenario – further refinement is required.

Refinment based on geomean approach Tier 2A (RAC=0.196 µg a.s./L)

Refinement of the Lemna Tier 1 was based on the geomean of the growth rate endpoints (Tier 2A) based on frond number from the Lemna EU-reviewed studies (1.71, 1.61 µg a.s./L) and the applicant's study (2.72 µg a.s./L). This is a justified approach as frond number is the primary measurement variable according to the OECD 221-guideline and the same measured variable was used for a geomean calculation.
This results in a geomean ErC_{50} of 1.96 µg a.s./L. Using an AF of 10, RAC for risk assessment of 0.196 µg/L was consided.

Based on this approach the following risk mitigation measures can be concluded:

Winter cereals 15 g a.s./ha:

- A risk mitigation of 10m + DVP 10m for R3 and R4 scenario.
- For D1 (stream) scenario the risk is acceptable with STEP 3. For D1 (ditch) further re-finement is required)
- For member states where D2 is relevant, a national specific risk mitigation measure should beapplied

Winter cereals at rate 9 g a.s./ha

- A risk mitigation of 10m + DVP 10m for R3 and R4 scenario
- For D1 scenarios the risk is considered acceptable with FOCUS Step 3.
- For member states where D2 is relevant, a national specific risk mitigation measure should be applied

Spring cereals:

- Acceptable risk for all scenarios

Refinment based on geomean approach Tier 2A based on the value agreed at EU level (RAC=0.149 µg a.s./L)

During commenting period process further refinement based on Tier 2A based on the value agreed at EU level was considered by the applicant.

EU agreed ErC_{50} endpoints for Lemna were considered as a weight of evidence (i.e, 1.717-frond number, 1.29-frond area) the resulting RAC of 0.149 µg a.s. /L from the geomean approach (Tier 2A).

The following risk mitigation measures are proposed:

Winter cereals at rate 15 g a.s./ha:

- A risk mitigation of 10m + DVP 10m for R3 and R4 scenario.
- For D1 (stream) scenario the risk is acceptable with STEP 3. For D1 (ditch further refine-ment is required)
- For member states where D2 is relevant, a national specific risk mitigation measure should be applied

Winter cereals at rate 9 g a.s./ha

- A risk mitigation of 10m + DVP 10m for R3 and R4 scenario
- For D1 scenarios the risk is considered acceptable with FOCUS Step 3.
- For member states where D2 is relevant, a national specific risk mitigation measure should beap-plied

Spring cereals:

- For D1 scenarios the risk further needs further refinement

Refinment based on SSD approach :

The one of the refiment option was SSD approach with HC_5 of 0.90 µg a.s./L value for mesosulforon methyl obtained from the new data for the active substance .

Winter cereals (0.5 kg PPP/ha)		
Scenario	**Water body**	**Mitigation measure requested**
D1	Ditch	No
D1	Stream	No
D2	Ditch	Yes
D2	Stream	Yes
D3	Ditch	No
D4	Pond	No
D4	Stream	No
D5	Pond	No
D5	Stream	No
R1	Pond	No
R1	Stream	No
R3	Stream	No
R4	Stream	No
Winter cereals (0.3 kg PPP/ha)		
Scenario	**Water body**	**Mitigation measure requested**
D1	Ditch	No
D1	Stream	No
D2	Ditch	Yes
D2	Stream	Yes
D3	Ditch	No
D4	Pond	No
D4	Stream	No
D5	Pond	No
D5	Stream	No
R1	Pond	No
R1	Stream	No
R3	Stream	No
R4	Stream	No
Spring cereals (0.3 kg PPP/ha)		
Scenario	**Water body**	**Mitigation measure requested**
D1	Ditch	No
D1	Stream	No
D3	Ditch	No
D4	Pond	No
D4	Stream	No
D5	Pond	No
D5	Stream	No
R4	Stream	No

The HC_5 of 0.90 µg a.s./L value was not considered by some MSs for the the risk assessment.

Therefore, the applicant provided the justification of using the additional data for the a.s. and provided the alternative approach which may be considered at MSs level, if relevant.

Taking into account that the new active substance data submitted has been already assessed under Data Matching process resulting in a non-change of agreed EU endpoints of toxicological refer-ence values, residue definitions or relevance of metabolites, the applicant proposes to use an SSD-approach (Tier 2B) using the Lemna Tier 1 endpoint from the EU-review together with the end-points of the 9 additional aquatic species tested by the applicant according to “Guidance on tiered risk assessment for edge-of-field surface waters, (EFSA Journal 2013;11(7):3290).

The HC_5 calculated can then be used to refine the Tier 1 Lemna endpoint from the EU-review. This SSD-approach leads to an HC5 of 0.83 µg/L. Using an AF of 3, as advised in the EFSA Jour-nal 2013; 11(7):3290 for the risk assessment for primary producers, the RAC will be 0.28 µg a.s./L.
This would result in an acceptable risk for aquatic organisms when respecting a risk mitigation measure of 10m + DVP 10m.

The final decision of the refinement for scenarios: D1, R3 , R4, D2 should be decided at MSs level.

Formulation GLOB389H/SAP63H:

For acute risk assessment of the formulation, PEC_{sw} based on drift events were calculated using the SWASH Drift Calculator tool in ditch, pond and stream surface waters. The resulting PEC_{sw} were used for comparison with the measured mixture toxicity for aquatic plants. The risk for aquatic invertebrates and algae is covered by the risk assessment for aquatic plants, as *lemna gibba* is by far the most sensitive species (more than factor 10 difference).
The PEC/RAC ratio is <1 for product if a 5m buffer zone is considered. An acceptable risk for the formulated product GLOB289H can be concluded based on the proposed use pattern, further consideration is not required.

9.1.2.4 Overall conclusions

9.1.2.5

9.1.2.6 Effects on bees (KCP 10.3.1)

Although the guidance document is not yet approved, the evaluation of the risk for honeybees was performed in accordance with the recommendations of the "EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera, Bombis* spp. and solitary bees)", EFSA Journal 2013; 11(7):3295.
The hazard quotient after acute contact exposure was 2.5, which is below the trigger value of 42. It can therefore be concluded that the intended use of GLOB289H gives a low acute contact risk to honey bees.
For the acute oral exposure, an ETR of 0.02 was calculated. This is below the trigger of 0.2, indicating an acceptable risk.
The chronic ETRs for adult honeybees and honeybee larvae are 0.033 and 0.07 respectively, exceeding the respective trigger values of 0.03 and 0.2, indicating an acceptable risk.
In general, there is no concern for honeybees when the product (+ adjuvant) is applied according the GAP. No mitigation measures are necessary.

9.1.2.7 Effects on arthropods other than bees (KCP 10.3.2)

Considering the acceptable risk to *Aphidius rhopalosiphi*, *Typhlodromus pyri*, *Crysoperla carnea* and *Aleochara bilineata* from the glass plate and extended laboratory studies, the product complies with the trigger values recommended by ESCORT 2. It is therefore concluded that the risk to non-target arthropods following the recommended uses of the product (+adjuvant) will be negligible. No mitigation measures are necessary.

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

microbial activity (KCP 10.5)

All the long-term TER values calculated in the earthworms and other non-target soil organisms risk assessment largely exceed the trigger value of 5. Based on these results can be concluded that GLOB289H (+ adjuvant) poses low long-term risk to earthworms, collembola and predatory mites.

The maximum concentrations with an effect ≤ 25% are higher than the maximum PEC_{soil} of iodosulfuron-methyl-sodium, mesosulfuron-methyl, their relevant metabolites and GLOB289H (+ adjuvant) from the intended use so the risk is acceptable.
The use of GLOB289H according to the proposed use pattern in cereals will not have unacceptable effects on soil micro-organisms. No mitigation measures necessary.

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

Based on propabilistic risk assessment with HR50 value and AF of 1, the use of GLOB289H according to the proposed use pattern in cereals will not have unacceptable effects on considering risk mitigation measures are used. Following combinations offer an acceptable risk:
Winter cereals – 0.5 kg/ha

- 1m buffer zone combined with 90% drift reducing nozzles
- 5m buffer zone combined with 50% drift reducing nozzles
- 10m buffer zone

Winter and spring cereals – 0.3 kg/ha

- 1m buffer zone combined with 90% drift reducing nozzles
- 5 m buffer zone.

The final risk mitigation measures should be provided at MSs level.

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

Not required

9.1.3 Grouping of intended uses for risk assessment

The only intended uses are in winter and spring cereals with different doses depending on the weed addressed. For the risk assessment, both winter and spring cereals are considered (if necessary) starting with the highest dose (worst-case scenario), and including lower doses if necessary for refinements.

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

Table 9.1-2: Critical use pattern of GLOB289H grouped according to application pattern

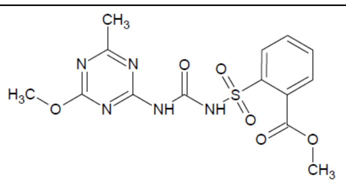
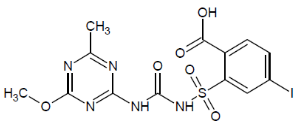
Grouping according to crop group of crop group and application pattern			
Group	**Intended uses**	**Relevant use parameters for grouping**	**Relevant parameter or value for sorting**
Effects on birds and mammals (9.2 and 9.3)			
Cereals	winter wheat (durum and soft), triticale, spelt, winter rye, spring wheat (durum and soft)	Application rate	0.5 kg/ha
Effects on aquatic organisms (9.5)			

Grouping according to crop group of crop group and application pattern			
Group	**Intended uses**	**Relevant use parameters for grouping**	**Relevant parameter or value for sorting**
As the use of risk mitigation measures is required to get an acceptable risk, all uses were included in the risk assessment.			
Effects on bees and arthropods other than bees (9.6 and 9.7)			
Cereals	winter wheat (durum and soft), triticale, spelt, winter rye, spring wheat (durum and soft)	Application rate	0.5 kg/ha
Effects on non-target soil meso- and macrofauna (9.8)			
Cereals	winter wheat (durum and soft), triticale, spelt, winter rye, spring wheat (durum and soft)	Application rate	0.5 kg/ha
Effects on soil microbial activity (9.9)			
Cereals	winter wheat (durum and soft), triticale, spelt, winter rye, spring wheat (durum and soft)	Application rate	0.5 kg/ha
Effects on non-target terrestrial plants (9.10)			
Cereals	winter wheat (durum and soft), triticale, spelt, winter rye, spring wheat (durum and soft)	Application rate	0.5 kg/ha 0.3 kg/ha (representative for both winter and spring cereals)

9.1.4 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 GLOB289H is indicated in the table.

Table 9.1-3 Metabolites of iodosulfuron-methyl sodium

Metabolite	**Chemical structure**	**Molar mass**	**Maximum occurrence in compartments**	**Risk assessment required?**
Metsulfuron-methyl (AE F075736)		381.4 g/mol	Soil: max. 88.5 % of a.s. Water: max. 67.8 % of a.s.	Yes, soil organisms and aquatic organisms
AE F145740		493.2 g/mol	Water: max. 12.6 % of a.s. Soil: max. 8.7 % of a.s.	Yes, soil organisms and aquatic organisms

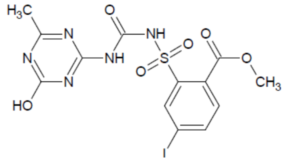
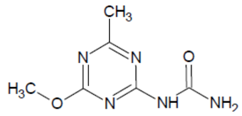
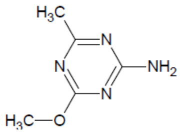
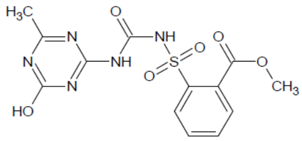
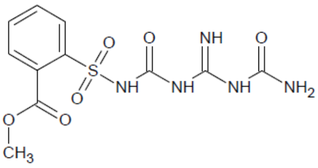
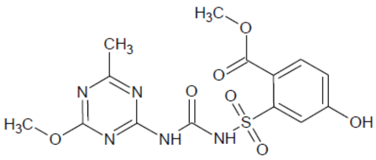
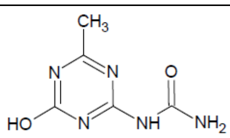
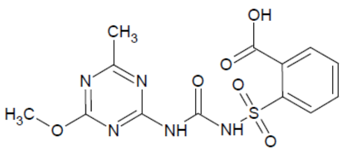
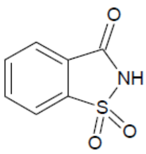
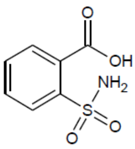
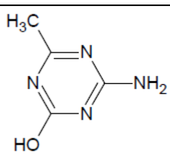
Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
AE F145741		493.2 g/mol	Soil: > 5 % of as in 2 sequential measure-ments, max. 6.9 % of a.s. Water: max. 8.7 % of a.s.	Yes, soil organisms and aquatic organisms
AE 0000119		183.2 g/mol	Water: max.24.9 % of a.s. Soil: max. 19.9 % of a.s.	Yes, soil organisms and aquatic organisms
AE F059411		140.1 g/mol	Soil: max. 40.9 % of a.s. Water: max. 27.5 % of a.s.	Yes, soil organisms and aquatic organisms
AE F161778		367.3 g/mol	Soil: max. 14.5 % of a.s. Water: max. 2.6 % of a.s.	Yes, soil organisms and aquatic organisms
BCS-CW81253		343.2 g/mol	Soil: max. 35.1 % of a.s.	Yes, soil organisms and aquatic organisms
AE 0002166		397,4 g/mol	Soil: max. 20.0 % of a.s. Water: max. 25.1 % of a.s.	Yes, soil organisms and aquatic organisms
AE 0034855		169.1 g/mol	Water: max. 24.2 % of a.s.	Yes, aquatic organisms
AE 0014966		367.3 g/mol	Water: max. 15.5 % of a.s.	Yes, aquatic organisms
AE F159737		183.2 g/mol	Water: > 5 % of as in 2 sequential measure-ments, max. 7.8 % of a.s.	Yes, aquatic organisms
AE 1234964		201.2 g/mol	Water: > 5 % of as in 2 sequential measure-ments, max. 7.4 % of a.s.	Yes, aquatic organisms
AE F154781		126.1 g/mol	Water: > 5 % of as in 2 sequential measure-ments, max. 8.7 % of a.s.	Yes, aquatic organisms

Table 9.1-3 Metabolites of mesosulfuron-methyl

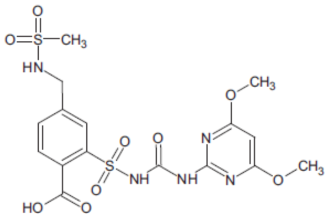
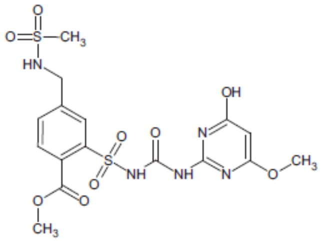
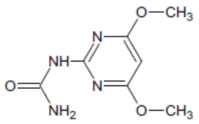
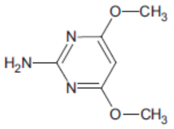
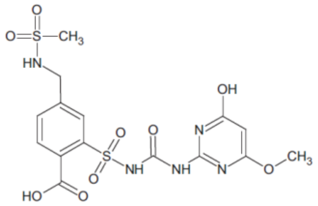
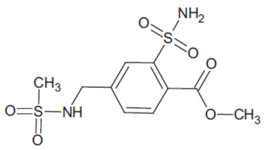
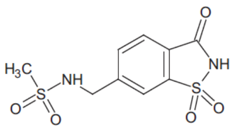
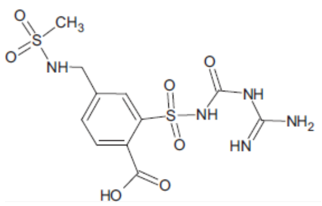
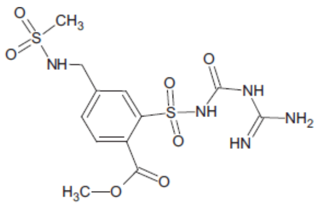
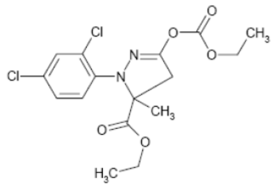
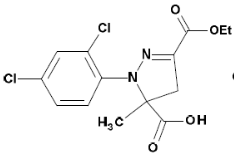
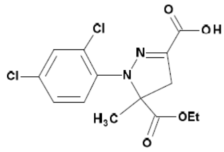
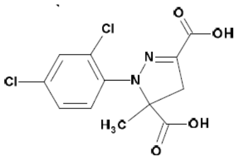
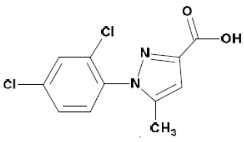
Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
Mesosulfuron		489.5 g/mol	Soil: max. 16.2% of a.s. Water: max. 4.9% of a.s.	Yes, soil organisms and aquatic organisms
AE F160459		489.5 g/mol	Soil: > 5% of a.s. in 2 sequential measurements Water: max. 21.6% of a.s.	Yes, soil organisms and aquatic organisms
AE F099095		198.2 g/mol	Soil: max. 16.2% of a.s. Water: max. 4.9% of a.s.	Yes, soil organisms and aquatic organisms
AE F092944		155.2 g/mol	Soil: > 5 % of as in 2 sequential measurements Water: max. 21.6% of a.s.	Yes, soil organisms and aquatic organisms
AE F160460		475.5 g/mol	Soil: max. 29.2% of a.s. Water: max. 0.9% of a.s.	Yes, soil organisms and aquatic organisms
AE F140584		322.4 g/mol	Soil: max. 10.1% of a.s. Water: max. 3.2% of a.s.	Yes, soil organisms and aquatic organisms
AE F147447		290.3 g/mol	Soil: > 5 % of as in 2 sequential measurements Water: max. 8.4% of a.s.	Yes, soil organisms and aquatic organisms
BCS-CV14885		393.4 g/mol	Soil: > 5 % of as in 2 sequential measurements Water: max. 1.9% of a.s.	Yes, aquatic organisms
BCS-CO60720		407.4 g/mol	Soil: > 5 % of as in 2 sequential measurements Water: max. 10.9% of a.s.	Yes, aquatic organisms

Table 9.1-4 Metabolites of mefenpyr-diethyl

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
AE 2211046		391.26 g/mol	Soil: max. 11.5 % of a.s.	Yes, soil organisms
AE F114952		345.18 g/mol	Water: max. 18.6 % of a.s.	No
AE F113225		345.2 g/mol	Soil: max. 44.1 % of a.s. Water: max. 82.8 % of a.s	Yes, soil organisms and aquatic organisms
AE F109453		317.13 g/mol	Water: max. 46.5 % of a.s.	Yes, aquatic organisms
AE F094270		271.1 g/mol	Soil: max. 72.2 % of a.s. Water: max. 62.4 % of a.s.	Yes, soil organisms and aquatic organisms

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with iodosulfuron-methyl-sodium, mesosulfuron-methyl and safener mefenpyr-diethyl. Full details of these studies are provided in the respective EU RAR and related documents.

Effects on birds of GLOB289H were not evaluated as part of the EU assessment of iodosulfuron-methyl-sodium or mesosulfuron-methyl. However, further data on GLOB289H are not considered essential. Birds are typically exposed to dry residues on their food items following spraying of the formulated product. During this process, much of the formulation constituents, as well as the adjuvants, are likely to be lost by volatilisation. Since oral exposure is the main route of exposure, toxicity data for the active substances are used in preference to data from the formulated product.
The risk to birds from the proposed use of GLOB289H will be assessed using active substance data.
Avian acute oral and long-term reproduction studies have been carried out with iodosulfuron-methyl-sodium, mesosulfuron-methyl and mefenpyr-diethyl. A summary of the relevant acute and long-term endpoints is provided in Table 9.2-1.

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

Species	Substance	Exposure System	Results	Reference
Coturnix coturnix japonica Japanese quail	Iodosulfuron-methyl-sodium	Acute (oral)	LD_{50} > 2000 mg/kg bw	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
Colinus virgianus Bobwhite quail	Iodosulfuron-methyl-sodium	Acute (oral)	**LD_{50} > 2000 mg/kg**	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
Anas platyrhynchos Mallard duck	Iodosulfuron-methyl-sodium	Acute (oral)	LD_{50} > 2000 mg/kg	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
Colinus virginianus Bobwhite quail	Iodosulfuron-methyl-sodium	Long-term	**NOEL= 78 mg/kg bw/day**	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
Anas platyrhynchos Mallard duck	Iodosulfuron-methyl-sodium	Long-term	NOEL = 125 mg/kg bw/day	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
Colinus virgianus Bobwhite quail	Mesosulfuron-methyl	Acute	**LD_{50} > 2000 mg/kg bw/day**	EFSA Conclusion Mesosulfuron-methyl, 2016
Anas platyrhynchos Mallard duck	Mesosulfuron-methyl	Acute	LD_{50} > 2000 mg/kg bw/day	EFSA Conclusion Mesosulfuron-methyl, 2016
Anas platyrhynchos Mallard duck	Mesosulfuron-methyl	Long-term	LD_{50}/10 > 200 mg/kg bw/day	EFSA Conclusion Mesosulfuron-methyl, 2016
Colinus virgianus Bobwhite quail	Mesosulfuron-methyl	Long-term	**NOEL = 93 mg/kg bw/day**	EFSA Conclusion Mesosulfuron-methyl, 2016
Anas platyrhynchos Mallard duck	Mesosulfuron-methyl	Long-term	NOEL = 126 mg/kg bw/day	EFSA Conclusion Mesosulfuron-methyl, 2016
Bobwhite quail	Mefenpyr-diethyl	Acute	**LD_{50}>3776 mg/kg bw**	DAR, 2011
Bobwhite quail	Mefenpyr-diethyl	Acute (oral)	**NOLED > 2000 mg/kg bw/d**	DAR Mefenpyr-diethyl, 2011
Bobwhite quail	Mefenpyr-diethyl	Long-term	**NOAEL = 106 mg/kg bw/day**	DAR Mefenpyr-diethyl, 2011

Bold values will be used in the risk assessment

9.2.1.1 Justification for new endpoints

EU agreed endpoints were used in the risk assessment. No deviations were made.

9.2.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).
To achieve a concise risk assessment, the risk envelope approach is applied. The critical GAP used for the risk assessment in presented in Table 9.2-2.

Table 9.2-2: Critical GAP used in the risk assessment on birds

Crops Included	Rate (No. of applns x max rate, kg a.i./ha)	Interval (days)
All winter and spring cereals	Iodosulfuron-methyl-sodium: 1 x 0.003 Mesosulfuron-methyl: 1 x 0.015 Mefenpyr-diethyl: 1 x 0.045 GLOB289H: 1 x 0.5 kg product/ha	-

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

In a first screening assessment, an 'indicator species' is used to identify substances that clearly pose a low risk to birds. This 'indicator species' is not a real species but, by virtue of its size and feeding habits is considered to have higher exposure than other species that occur in a particular crop at a particular time. The approach used to select the 'indicator species' is described in Appendix I of the EFSA Guidance.
The DDD (daily dietary dose) was calculated as follows:

DDD_{acute} = Application rate (kg a.s./ha) x short-cut value x MAF
DDD_{repro} = Application rate (kg a.s./ha) x short-cut value x MAF x TWA
Where:

- The shortcut value is given in the EFSA Guidance document
- MAF = 1 as it concerns only one application
- TWA (time-weighted average factor) = 0.53 (default value)

The acute risk to birds was assessed by calculating toxicity exposure ratios (TER_A) using the following equation:

$TER_A = LD_{50}$ (mg/kg bw/day) / DDD (mg/kg bw/day)

Long-term toxicity exposure ratios (TER_{LT}) were calculated using the following equation:

$$TER_{LT} = \frac{\text{NOEL (mg/kg bw/day)}}{\text{Long - term ETE (mg/kg bw/day)}}$$

The results of the acute and reproductive screening risk assessments are summarised in the following tables.

Table 9.2-3: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of GLOB289H in cereals – iodosulfuron-methyl-sodium

Intended use	Cereals				
Active substance/product	Iodosulfuron-methyl-sodium				
Application rate (kg/ha)	1 × 0.003				
Acute toxicity (mg/kg bw)	> 2000				
TER criterion	10				
Crop scenario Growth stage	**Indicator species for screening**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**

-	Small omnivorous birds	158.8	1	0.48	> 4166.7
Reprod. toxicity (mg/kg bw/d)	78				
TER criterion	5				
Crop scenario Growth stage	**Indicator species for screening**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
-	Small omnivorous birds	64.8	1 x 0.53	0.10	780

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 TER_a and the TER_{lt} values exceed the trigger of 10 and 5 for the acute and long-term risk assessment respectively, indicating that iodosulfuron-methyl-sodium does not pose an acute or a long-term risk to wild birds after the use of the PPP according to the proposed GAP.

Table 9.2-4: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of GLOB289H in cereals – mesosulfuron-methyl

Intended use	**Cereals**				
Active substance/product	Mesosulfuron-methyl				
Application rate (g/ha)	1 × 15				
Acute toxicity (mg/kg bw)	> 2000				
TER criterion	10				
Crop scenario Growth stage	**Indicator species for screening**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
-	Small omnivorous birds	158.8	-	2.38	> 840.3
Reprod. toxicity (mg/kg bw/d)	93				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
-	Small omnivorous birds	64.8	1 x 0.53	0.52	178.9

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 TER_a and the TER_{lt} values exceed the trigger of 10 and 5 for the acute and long-term risk assessment respectively, indicating that mesosulfuron-methyl does not pose an acute or a long-term risk to wild birds after the use of the PPP according to the proposed GAP.

Table 9.2-5: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of GLOB289H in cereals – mefenpyr-diethyl

Intended use	**Cereals**				
Active substance/product	Mefenpyr-diethyl				
Application rate (g/ha)	1 × 45				
Acute toxicity (mg/kg bw)	~~> 2000~~ **>3776**				
TER criterion	10				
Crop scenario Growth stage	**Indicator species for screening**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**

-	Small omnivorous birds	158.8	-	7.15	~~> 279.72~~ 528.11
Reprod. toxicity (mg/kg bw/d)	106				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
-	Small omnivorous birds	64.8	1 x 0.53	2.92	36.30

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.
in blue verified by zRMS

The TER_a and the TER_{lt} values exceed the trigger of 10 and 5 for the acute and long-term risk assessment respectively, indicating that mefenpyr-diethyl does not pose an acute or a long-term risk to wild birds after the use of the PPP according to the proposed GAP.

Assessment of acute mixture toxicity

Apart from the individual risk of both active substances, also the combined effect of simultaneous exposure to both active substances and the safener should be considered. According to Appendix B of the Guidance Document on the Risk Assessment for birds and mammals, the basic concept of the risk assessment is that animals are exposed to residues of the active substances in the environment. Thus, the assessment of GLOB289H is not an assessment of the formulation as such, but an assessment of the effects of an exposure to a mixture of active substances in the environment, resulting from the use of the formulation. Toxicity studies for birds with formulated products are typically not available. For the assessment of acute effects, a surrogate LD_{50} is calculated. A model often used to estimate the acute toxicity of mixtures is the assumption of dose/concentration additivity of toxicity (Finney approach of concentration additivity of toxicity (Finney, D.J., 1948 and 1971).

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

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

With:

$X(a.s._i)$ = fraction of active substance [i] in the mixture;
(please note that the sum $\sum X(a.s._i)$ must be 1)

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

As the LD_{50} for both active substances and the safener > 2000 mg/kg bw, the combined LD_{50} will also be > 2000 mg/kg bw.

Table 9.2.6.~~5~~: Acute LD_{50} for the mixture of active substances

Test substance	[Xa.s.] in formulation (g/kg)	X(a.s.i) *	LD_{50} (mg/kg bw)	Fraction of active substance/NOEL for the active substance	LD_{50} mix** (mg/kg bw)	Tox per fraction (a.s.) # (mg/kg bw)	Tox per fraction (mix) ##	Deviation "tox per fraction a.s." and "tox per fraction mix" > 10%?
Iodosulfuron-methyl-sodium	6	0.048	>2000	0.000024	~~2000~~ 3012.04	~~42000~~ 41666.66	2000 (±10%: 1980-2020)	Yes 92.97
Mesosulfuron-methyl	30	0.238	>2000	0.000119		~~8400~~ 8403.4		Yes 64.16
Mefenpyr-diethyl	90	0.714	~~> 2000~~ 3776	0.000189		~~2800~~ 5288.51		Yes 43.05
Total	126	1	-	0.000332		-	-	

ª Concentration of an active substance in the formulation, divided by, the total concentration of all active substances in the formulation.
** LD_{50} (mix) = 1/(∑(Xa.s.i / LD_{50} a.s.i))
\# LD_{50} a.s.i / X a.s.i,
\## LD_{50} mix / ∑ Xa.s.i
in blue verified by zRMS

If one active substance can be identified where the two quotients 'tox per fraction (a.s.)' and 'tox per fraction (mix)' deviate by less than 10%, this indicates that this a.s. will contribute at least 90% to mixture toxicity. This is not the case, therefore a combined risk assessment is performed using the calculated LD_{50} mix.

Table 9.2- 7.~~6~~: Screening assessment of the acute risk for birds due to the use of GLOB289H in cereals – combined risk assessment

Intended use	**Cereals**				
Active substance/product	GLOB289H				
Application rate (kg/ha)	1×0.063 kg a.s./ha (0.003 kg iodosulfuron + 0.015 kg mesosulfuron + 0.045 kg mefenpyr)				
Acute toxicity (mg/kg bw) $LD_{50\ mix}$	~~> 2000~~ >3012.04				
TER criterion	10				
Crop scenario Growth stage	**Indicator species for screening**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
-	Small omnivorous birds	158.8	-	10.0	~~> 200~~ > 301.2

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

The TER_a value exceeds the trigger of 10, indicating that there is not acute risk from the combined exposure of the active substances and the safener to wild birds after the use of the PPP according to the proposed GAP.

zRMS comments:

Safe use of individual active substances included in GLOB289H/SAP63H on winter cereals for birds were confirmed based on TER_A and TER_{LT} values which were above trigger value of 10 and 5, respectively.
Apart from the individual risk of both active substances and safner, also the combined effect of simultaneous exposure to both active substances with safener was considered.
The TER_a value exceeds the trigger of 10, indicating that there is not acute risk from the combined exposure of the active substances and the safener to wild birds after the use of the PPP according to the proposed GAP.
It should be noted that according to B&M GD, 2009, the tong-term combitox for birds and mammals should be also provided.
Therefore, zRMS calculated the combined long-term risk and performed these calculations in the Tables below:

Table 9-2-7. Long-term NOEL for the mixture of active substances for birds.

Test Substance	Concentration of active substance in formulation (g/kg)	Fraction of active substance in the formulation mixture[a]	NOEL toxicity endpoint (mg as/kg bw)	Fraction of active substance/NOEL for the active substance	NOELmix (mg/kg bw)
Iodosulfuron-methyl-sodium	6	0.05	78	0.000641	102.04
Mesosulfuron-methyl	30	0.24	93	0.0025	
Mefenpyr-diethyl	90	0.71	106	0.0067	
Total	126			0.0098	
		1	-		

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

Table 9-2-8. Comparison of the measured and predicted endpoints for GLOB289H/SAP63H using the long-term toxicity data for birds.

Test Substance	Concentration of active substance in formulation (g/kg)	Fraction of active substance in the formulation mixture[a]	Long term toxicity endpoint (mg as/kg bw)	Tox per fraction a.s.	Tox per fraction mix	Deviation (%)
Iodosulfuron-methyl-sodium	6	0.05	78	1560	102.04	93.46
Mesosulfuron-methyl	30	0.24	93	387.5		73.67
Mefenpyr-diethyl	90	0.71	106	149.3		31.66
Total	126					

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

The deviation between the tox per fraction of all substances and mixture is > 10 %.
Consequently, the risk assessment is performed with the surrogate NOEL of 102.04 for the mixture of active substances with safner .

Table 9-2-9. The long-term risk assessment for mixture toxicity for birds.

Intended use	**Winter cereals, 0.5 kg prod./ha**				
Application rate (g/ha)	0.063kg sum of active substances/ha				
Acute toxicity (mg/kg bw)	NOELmix= 102.04				
TER criterion	5				
GAP crop	**Indicator/generic focal species**	**SVm**	**MAF_{90} x F_{twa}**	**DDD_{90} (mg/kg bw/d)**	**TER_{Lt}**
Cereals	Small omnivorous bird	64.8	1.0 x 0.53	2.16	47.24

The TER_{LT}value exceeds the trigger of 5, indicating that there is long-term risk from the combined exposure of the active substances with safener to wild birds after the use of the PPP according to the proposed GAP.

9.2.2.2 Higher-tier risk assessment

No higher-tier assessment is considered 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 GLOB289H 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 < 500, iodosulfuron-methyl-sodium belongs to the group of less sorptive substances. The trigger of 50 is not exceeded, further exposure calculations are therefore not needed.

Application rate (AR) (g/ha)	3			Trigger: > 50
Acute toxicity (mg/kg bw)	> 2000	AR / acute tox =	> 0.0015	<
Reprod. toxicity (mg/kg bw/d)	78	AR / repro tox =	0.0385	<

With a K(f)oc of < 500, mesosulfuron-methyl belongs to the group of less sorptive substances. The trigger of 50 is not exceeded, further exposure calculations are therefore not needed.

Application rate (AR) (g/ha)	15			Trigger: > 50
Acute toxicity (mg/kg bw)	> 2000	AR / acute tox =	> 0.0075	<
Reprod. toxicity (mg/kg bw/d)	840	AR / repro tox =	0.0179	<

With a K(f)oc of > 500, mefenpyr-diethyl belongs to the group of more sorptive substances. The trigger of 3000 is not exceeded, further exposure calculations are therefore not needed.

Application rate (AR) (g/ha)	45			Trigger: > 3000
Acute toxicity (mg/kg bw)	~~> 2000~~ > 3776	AR / acute tox =	> ~~0.0225~~ 0.0119	<
Reprod. toxicity (mg/kg bw/d)	106	AR / repro tox =	0.4245	<

9.2.2.4 Effects of secondary poisoning

The log P_{ow} of iodosulfuron-methyl-sodium amounts to 1.96 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 mesosulfuron-methyl sodium amounts to 1.39 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 mefenpyr-diethyl amounts to 3.83 and thus exceeds the trigger value of 3, indicating a possible risk of secondary poisoning.

Risk assessment for earthworm-eating birds via secondary poisoning

According to EFSA/2009/1438, the risk for vermivorous birds is assessed for a bird of 100 g body weight with a daily food consumption of 104.6 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil.

Table 9.2-11.~~7~~: Assessment of the risk for earthworm-eating birds due to exposure to mefenpyr-diethyl via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals

Parameter	Mefenpyr-diethyl	comments
PEC_{soil} (mg/kg soil)	0.048	dRR Part B8: table 8.7-11
log P_{ow} / P_{ow} (= K_{OW})	3.83/6760.83	
Koc	609.9	
Foc	0.02	Default
BCF_{worm}	6.73	$BCF_{worm/soil} = (0.84 + 0.012 \times K_{ow}) / foc \times Koc$
PEC_{worm}	0.32	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.34	$DDD = PEC_{worm} \times 1.05$
NOAEL (mg/kg bw/d)	106	
TER_{lt}	311.8	

TER values shown in bold fall below the relevant trigger.

No chronic test for the earthworm is available. Therefore, the endpoint of the acute toxicity test was divided by 10. With a TER of 311.8 there is a large safety margin so the risk for earthworm-eating birds due to exposure to mefenpyr-diethyl via bioaccumulation in earthworms is considered acceptable.

Risk assessment for fish-eating birds via secondary poisoning

According to EFSA/2009/1438, the risk for piscivorous birds is assessed for a birds of 1000 g body weight with a daily food consumption of 159 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water.

Table 9.2.12-8: Assessment of the risk for fish-eating birds due to exposure to mefenpyr-diethyl via bioaccumulation in fish (secondary poisoning) for the intended use in cereals

Parameter	Mefenpyr-diethyl	comments
PEC_{sw} (mg/L)	0.00869	Max. (dRR Part B8: table 8.9-59)
BCF_{fish}	362	Max. worst case (DAR)
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	3.15	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.5	$DDD = PEC_{fish} \times 0.159$
NOAEL (mg/kg bw/d)	106	
TER_{lt}	212	

TER values shown in bold fall below the relevant trigger.

The TER of 212 largely exceeds the trigger of 5, the risk for fish-eating birds due to exposure to mefenpyr-diethyl via bioaccumulation in fish is thus considered acceptable.

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.2.4 Overall conclusions

In the screening step, the TER_{acute} and $TER_{long\text{-}term}$ values exceed the triggers of 10 and 5 for the acute and long-term assessments respectively, indicating that iodosulfuron-methyl-sodium, mesosulfuron-methyl and mefenpyr-diethyl do not pose an acute and long-term risk to wild birds after use of GLOB289H according to the critical 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 iodosulfuron-methyl-sodium, mesosulfuron-methyl and the safener mefenpyr-diethyl. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on mammals of GLOB289H were not evaluated as part of the EU assessment of the active substances or safener. However, further data on GLOB289H are not considered essential. Mammals are typically exposed to dry residues on their food items following spraying of the formulated product. During this process, much of the formulation constituents are likely to be lost by volatilisation. Since oral exposure is the main route of exposure, toxicity data for the active substances are used in preference to data from the formulated product.
The risk to mammals from the proposed use of GLOB289H will be assessed using active substance data.
Mammalian acute oral and long-term reproduction studies have been carried out with iodosulfuron-methyl-sodium, mesosulfuron-methyl and mefenpyr-diethyl. A summary of the relevant acute and long-term endpoints is provided in Table 9.2-1.

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

Species	Substance	Exposure System	Results	Reference
Wistar rat	Iodosulfuron-methyl-sodium	Acute	LD_{50} = **2678** mg/kg bw	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Wistar rat	Iodosulfuron-methyl-sodium	Long-term, combined chronic toxicity and oncogenicity study (2 years)	NOAEL = **2.96** mg/kg bw/d	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Rat	Mesosulfuron-methyl	Acute	LD_{50} = **> 5000** mg/kg bw	EFSA Conclusion Mesosulfuron-methyl, 2016
Rat	Mesosulfuron-methyl	Long-term, 2-generation study	NOEL = **840** mg/kg bw/d	EFSA Conclusion Mesosulfuron-methyl, 2016
Rat	Mefenpyr-diethyl	Acute, oral	LD_{50} = **> 5000**	DAR Mefenpyr-diethyl, 2011
Rat	Mefenpyr-diethyl	Reproduction	NOAEL = **88.8**	DAR Mefenpyr-diethyl, 2011

Bold values will be used in the risk assessment

9.3.1.1 Justification for new endpoints

EU agreed endpoints were used in the risk assessment. No deviations were made.

9.3.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Mammals and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).
To achieve a concise risk assessment, the risk envelope approach is applied. The critical GAP used for the risk assessment in presented in Table 9.3-2.

Table 9.3-2: Critical GAP used in the risk assessment on mammals

Crops Included	Rate (No. of applns x max rate, kg a.i./ha)	Interval (days)
All winter and spring cereals	Iodosulfuron-methyl-sodium: 1 x 0.003 Mesosulfuron-methyl: 1 x 0.015 Mefenpyr-diethyl: 1 x 0.045 GLOB289H: 1 x 0.5 kg product/ha or 1 x 0.063 kg a.s./ha (sum of a.i.)	-

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

The results of the acute and reproductive screening risk assessments are summarised in the following tables.

Table 9.3-3: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of GLOB289H in cereals – iodosulfuron-methyl-sodium

Intended use	Cereals				
Active substance/product	Iodosulfuron-methyl sodium				
Application rate (g/ha)	1 x 3				
Acute toxicity (mg/kg bw)	2678				
TER criterion	10				
Crop scenario Growth stage	**Indicator species for screening**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
-	Small herbivorous mammal	118.4	1	0.36	7438.9
Reprod. toxicity (mg/kg bw/d)	2.96				
TER criterion	5				
Crop scenario Growth stage	**Indicator species for screening**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
-	Small herbivorous mammals	48.3	1 x 0.53	0.08	37

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 TER_a and the TER_{lt} values exceed the trigger of 10 and 5 for the acute and long-term risk assessment respectively, indicating that iodosulfuron-methyl-sodium does not pose an acute or a long-term risk to wild mammals after the use of the PPP according to the proposed GAP.

Table 9.3-4: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of GLOB289H in cereals – mesosulfuron-methyl

Intended use	Cereals				
Active substance/product	Mesosulfuron-methyl				
Application rate (g/ha)	1 x 15				
Acute toxicity (mg/kg bw)	> 5000				
TER criterion	10				
Crop scenario Growth stage	**Indicator species for screening**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
-	Small herbivorous mammal	118.4	1	1.78	>2813.7
Reprod. toxicity (mg/kg bw/d)	840				

TER criterion	5				
Crop scenario Growth stage	**Indicator species for screening**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
-	Small herbivorous mammals	48.3	1 x 0.53	0.38	2210.5

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

The TER_a and the TER_{lt} values exceed the trigger of 10 and 5 for the acute and long-term risk assessment respectively, indicating that mesosulfuron-methyl does not pose an acute or a long-term risk to wild mammals after the use of the PPP according to the proposed GAP.

Table 9.3-5: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of GLOB289H in cereals – mefenpyr-diethyl

Intended use	Cereals				
Active substance/product	Mefenpyr-diethyl				
Application rate (g/ha)	1 x 45				
Acute toxicity (mg/kg bw)	> 5000				
TER criterion	10				
Crop scenario Growth stage	**Indicator species for screening**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
-	Small herbivorous mammal	118.4	1	5.33	>938
Reprod. toxicity (mg/kg bw/d)	88.8				
TER criterion	5				
Crop scenario Growth stage	**Indicator species for screening**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
-	Small herbivorous mammals	48.3	1 x 0.53	1.15	77.2

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

The TER_a and the TER_{lt} values exceed the trigger of 10 and 5 for the acute and long-term risk assessment respectively, indicating that mefenpyr-diethyl does not pose an acute or a long-term risk to wild mammals after the use of the PPP according to the proposed GAP.

Assessment of acute mixture toxicity

Apart from the individual risk of both active substances, also the combined effect of simultaneous exposure to both active substances and the safener should be considered. According to Appendix B of the Guidance Document on the Risk Assessment for birds and mammals, the basic concept of the risk assessment is that animals are exposed to residues of the active substances in the environment. Thus, the assessment of GLOB289H is not an assessment of the formulation as such, but an assessment of the effects of an exposure to a mixture of active substances in the environment, resulting from the use of the formulation. Toxicity studies for mammals with formulated products are not available. For the assessment of acute effects, a surrogate LD_{50} is calculated. A model often used to estimate the acute toxicity of mixtures is the assumption of dose/concentration additivity of toxicity (Finney approach of concentration additivity of toxicity (Finney, D.J., 1948 and 1971).

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

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

With:

$X(\text{a.s.}_i)$ = fraction of active substance [*i*] in the mixture; (please note that the sum $\sum X(\text{a.s.}_i)$ must be 1)

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

Table 9.3-5: Acute LD_{50} for the mixture of active substances

Test substance	[Xa.s.] in formulation (g/kg)	$X(\text{a.s.}_i)$ *	LD_{50} (mg/kg bw)	LD_{50} mix** (mg/kg bw)	Tox per fraction (a.s.) # (mg/kg bw)	Tox per fraction (mix) ##	Deviation “tox per fraction a.s.” and “tox per fraction mix" > 10%?
Iodosulfuron-methyl-sodium	6	0.048	2678	4801.74	56238	4801.74 (±10%: 4754-4850)	Yes
Mesosulfuron-methyl	30	0.238	> 5000		21000		Yes
Mefenpyr-diethyl	90	0.714	> 5000		7000		Yes
Total	126	1	-		-	-	

[a] Concentration of an active substance in the formulation, divided by, the total concentration of all active substances in the formulation.
** LD_{50} (mix) = $1/(\sum(\text{Xa.s.}_i / LD_{50}\ \text{a.s.}_i))$
# LD_{50} a.s.$_i$ / X a.s.$_i$,
LD_{50} mix / $\sum$ Xa.s.$_i$

One active substance can be identified where the two quotients ‘tox per fraction (a.s.)’ and ‘tox per fraction (mix)’ deviate by less than 10%, this indicates that this a.s. will contribute at least 90% to mixture toxicity. This is not the case, therefore a combined risk assessment is performed using the calculated LD_{50} mix.

Table 9.33-6: Screening assessment of the acute risk for mammals due to the use of GLOB289H in cereals – combined risk assessment

Intended use	**Cereals**				
Active substance/product	GLOB289H				
Application rate (kg/ha)	1×0.063 g a.s./ha (0.003 g iodosulfuron + 0.015 mesosulfuron + 0.045 mefenpyr)				
Acute toxicity (mg/kg bw) $LD_{50\ mix}$	4801.74				
TER criterion	10				
Crop scenario Growth stage	**Indicator species for screening**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
-	Small herbivorous mammal	118.4	-	7.46	643.66

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

With a TER of 643.66, the trigger of 10 is largely exceeded, indicating that there is not acute risk from the combined exposure of the active substances and the safener to wild mammals after the use of the PPP according to the proposed GAP.

zRMS comments:

Safe use of individual active substances included in GLOB289H/SAP63H on winter cereals for mammals were confirmed based on TER_A and TER_{LT} values which were above trigger value of 10 and 5, respectively.
Apart from the individual risk of both active substances and safner, also the combined effect of simultaneous exposure to both active substances with safener was considered.
The TER_a value exceeds the trigger of 10, indicating that there is not acute risk from the combined exposure of the active substances and the safener to wild mammals after the use of the PPP according to the proposed GAP.
It should be noted that according to B&M GD, 2009 the long-term combitox for birds and mammals should be also provided.
Therefore, zRMS calculated the combined long-term risk and performed these calculations in the Tables below:

Table 9-3-7. Long-term NOEL for the mixture of active substances for mammals.

Test Substance	Concentration of active substance in formulation (g/kg)	Fraction of active substance in the formulation mixture[a]	NOEL toxicity endpoint (mg as/kg bw)	Fraction of active substance/NOEL for the active substance	NOELmix (mg/kg bw)
Iodosulfuron-methyl-sodium	6	0.05	2.96	0.017	39.52
Mesosulfuron-methyl	30	0.24	840	0.0002857	
Mefenpyr-diethyl	90	0.71	88.8	0.008	
Total	126			0.0253	
		1	-		

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

Table 9-3-8. Comparison of the measured and predicted endpoints for GLOB289H/SAP63H using the long-term toxicity data for mammals.

Test Substance	Concentration of active substance in formulation (g/kg)	Fraction of active substance in the formulation mixture[a]	Long term toxicity endpoint (mg as/kg bw)	Tox per fraction a.s.	Tox per fraction mix	Deviation (%)
Iodosulfuron-methyl-sodium	6	0.05	2.96	59.2	39.52	33.2
Mesosulfuron-methyl	30	0.24	840	3500		98.88
Mefenpyr-diethyl	90	0.71	88.8	125.07		68.88
Total	126					

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

The deviation between the tox per fraction of all substances and mixture is > 10 %.
Consequently, the risk assessment is performed with the surrogate NOEL of 39.52 for the mixture of active substances with safner.

Table 9-3-9. The long-term risk assessment for mixture toxicity.

Intended use		**Winter cereals, 0.5 kg prod./ha**			
Application rate (g/ha)		0.063kg sum of active substances/ha			
Acute toxicity (mg/kg bw)		NOELmix= 39.52			
TER criterion		5			
GAP crop	**Indicator/generic focal species**	**SVm**	**MAF_{90} x F_{twa}**	**DDD_{90} (mg/kg bw/d)**	**TER_{Lt}**
Cereals	Small omnivorous mammals	48.3	1.0 x 0.53	1.61	24.54

The TER_{LT} value exceeds the trigger of 5, indicating that there is long-term risk from the combined exposure of the active substances and the safener to wild mammals after the use of the PPP according to the proposed GAP.

9.3.2.2 Higher-tier risk assessment

No higher tier risk assessment is considered necessary

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 < 500, iodosulfuron-methyl-sodium belongs to the group of less sorptive substances. The trigger of 50 is not exceeded, further exposure calculations are therefore not needed.

Application rate (AR) (g/ha)	3			Trigger: > 50
Acute toxicity (mg/kg bw)	2678	AR / acute tox =	> 0.0011	<
Reprod. toxicity (mg/kg bw/d)	2.96	AR / repro tox =	1.0135	<

With a K(f)oc of < 500, mesosulfuron-methyl belongs to the group of less sorptive substances. The trigger of 50 is not exceeded, further exposure calculations are therefore not needed.

Application rate (AR) (g/ha)	15			Trigger: > 50
Acute toxicity (mg/kg bw)	> 5000	AR / acute tox =	> 0.003	<
Reprod. toxicity (mg/kg bw/d)	840	AR / repro tox =	0.018	<

With a K(f)oc of > 500, mefenpyr-diethyl belongs to the group of more sorptive substances. The trigger of 3000 is not exceeded, further exposure calculations are therefore not needed.

Application rate (AR) (g/ha)	45			Trigger: > 3000
Acute toxicity (mg/kg bw)	> 5000	AR / acute tox =	> 0.009	<
Reprod. toxicity (mg/kg bw/d)	88.8	AR / repro tox =	0.507	<

zRMS comments:

Since GLOB289H / SAP63H is not a for spray applications / not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later. Therefore, the leaf scenario does not have to be considered taking onto account the proposed uses (cereals).
Evaluation of exposing for birds through the drinking water Puddle scenario for the active substances solely, demonstrate that the acceptable risk for birds for proposed use pattern GLOB289H / SAP63H in cereals**.**

9.3.2.4 Effects of secondary poisoning

The log P_{ow} of iodosulfuron-methyl sodium amounts to 1.96 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 mesosulfuron-methyl sodium amounts to 1.39 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 mefenpyr-diethyl is 3.83, therefore a bioaccumulation risk assessment is performed.

Risk assessment for earthworm-eating mammals via secondary poisoning

According to EFSA/2009/1438, the risk for vermivorous mammals is assessed for a small mammal of 10 g body weight with a daily food consumption of 12.8 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil.

Table 9.3-7: Assessment of the risk for earthworm-eating mammals due to exposure to mefenpyr-diethyl via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals

Parameter	Mefenpyr-diethyl	comments
PEC_{soil} (mg/kg soil)	0.048	dRR Part B8: table 8.7-11
log P_{ow} / P_{ow} (= K_{ow})	3.83/6760.83	
Koc	609.9	
Foc	0.02	Default
BCF_{worm}	6.73	$BCF_{worm/soil} = (0.84 + 0.12 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.32	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.41	$DDD = PEC_{worm} \times 1.28$

Parameter	Mefenpyr-diethyl	comments
NOEL (mg/kg bw/d)	88.8	
TER_{lt}	216.6	

TER values shown in bold fall below the relevant trigger.

With a TER of 216.6 there is a large safety margin so the risk for earthworm-eating mammals due to exposure to mefenpyr-diethyl via bioaccumulation in earthworms is considered acceptable.

Risk assessment for fish-eating mammals via secondary poisoning

According to EFSA/2009/1438, the risk for piscivorous mammals is assessed for a mammal of 3000 g body weight with a daily food consumption of 425 g. Bioaccumulation in fish is estimated based on predicted concentrations of mefenpyr-diethyl in surface water

Table 9.3-8: Assessment of the risk for fish-eating mammals due to exposure to mefenpyr-diethyl via bioaccumulation in fish (secondary poisoning) for the intended use in cereals

Parameter	Mefenpyr-diethyl	comments
PEC_{sw} (twa = 21 d) (mg/L)	0.00869	Max. (dRR Part B8: table 8.9-59)
BCF_{fish}	362	Max. worst case (DAR)
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	3.15	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.447	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	88.8	
TER_{lt}	198.7	

TER values shown in bold fall below the relevant trigger.

With a TER of 198.7, the trigger of 5 is largely exceeded. The risk for fish-eating mammals due to exposure to mefenpyr-diethyl via bioaccumulation in fish is considered acceptable.

ZRMS comments:

The risk to earthworm – and fish –eating mammals from secondary poising is considered to be low as the log P_{ow} is below 3 for both active substances. However, The log P_{ow} of mefenpyr-diethyl is 3.83, therefore a bioaccumulation risk assessment was required.
The TER_{LT} exceeded the trigger value of 5 indicating that the risk for fish-eating mammals due to exposure to mefenpyr-diethyl via bioaccumulation in fish is considered acceptable.

9.3.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.3.4 Overall conclusions

In the screening step, the TER_{acute} and $TER_{long\text{-}term}$ values exceed the triggers of 10 and 5 for the acute and long-term assessments respectively, indicating that iodosulfuron-methyl-sodium, mesosulfuron-methyl and mefenpyr-diethyl do not pose an acute and long-term risk to mammals after use of GLOB289H according to the critical GAP.

ZRMS comments:

We agree with the conclusion provided above.

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

Not required

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 active substances iodosulfuron-methyl-sodium mesosulfuron-methyl , safener mefenpyr-diethyl and their relevant metabolites. Full details of these studies are provided in the respective EU RAR and related documents.

Effects on aquatic organisms of GLOB289H were not evaluated as part of the EU assessment of the active substances or the safener. 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. The endpoints used for the aquatic risk assessment are presented in Table 9.5-1.

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

Species	Substance	Exposure System	Results	Reference
Fish – acute				
Oncorhynchus mykiss	Iodosulfuron-methyl-sodium	96 h, s	LC_{50} > **100 mg a.s./L** $_{mm}$	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Lepomis marcrochirus		96 h, s	LC_{50} > 100 mg a.s./L $_{mm}$	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Cyprinodon variegatus		96 h, s	LC_{50} > 100 mg a.s./L $_{mm}$	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Oncorhynchus mykiss	AE F075736	-	**LC_{50} > 10 mg a.s./L**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	AE F145740	-	**LC_{50} > 10 mg a.s./L**	Assumption: 10x more

Species	Substance	Exposure System	Results	Reference
				toxic than parent
Oncorhynchus mykiss	AE F145741	-	**LC_{50} > 10 mg a.s./L**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	AE 0000119	-	**LC_{50} > 10 mg a.s./L**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	AE F161778	-	**LC_{50} > 10 mg a.s./L**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	BCS-CW811253	-	**LC_{50} > 10 mg a.s./L**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	AE F059411	-	**LC_{50} > 10 mg a.s./L**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	AE 0014966	-	**LC_{50} > 10 mg a.s./L**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	AE 0043885	-	**LC_{50} > 10 mg a.s./L**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	AE F154781	-	**LC_{50} > 10 mg a.s./L**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	AE 0002166	-	**LC_{50} > 10 mg a.s./L**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	AE1234964	96 h, s	Mortality, LC_{50} > 100 mg a.s./L $_{nom}$	EFSA, 2016
		-	**LC_{50} > 10 mg a.s./L**	**Assumption: 10x more toxic than parent**
Oncorhynchus mykiss	AE F159737	96 h, s	Mortality, LC_{50} > 100 mg a.s./L $_{nom}$	EFSA, 2016
		-	**LC_{50} > 10 mg a.s./L**	**Assumption: 10x more toxic than parent**
		Fish – chronic		
Oncorhynchus mykiss	Iodosulfuron-methyl-sodium	28 d (flow-through)	**Growth NOEC = 7.79 mg a.s./L $_{mm}$**	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Pimephales promelas		35 d (flow-through)	Growth NOEC > 9.8 mg a.s./L	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Oncorhynchus mykiss	AE F075736	-	**Growth NOEC = 0.779 mg a.s./L**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	AE F145740	-	**Growth NOEC = 0.779 mg a.s./L**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	AE F145741	-	**Growth NOEC = 0.779 mg a.s./L**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	AE 0000119	-	**Growth NOEC = 0.779 mg a.s./L**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	AE F161778	-	**Growth NOEC = 0.779 mg a.s./L**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	BCS-CW811253	-	**Growth NOEC = 0.779 mg a.s./L**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	AE F059411	-	**Growth NOEC = 0.779 mg a.s./L**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	AE 0014966	-	**Growth NOEC = 0.779 mg a.s./L**	Assumption: 10x more toxic than parent

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	AE 0043885	-	**Growth NOEC = 0.779 mg a.s./L**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	AE F154781	-	**Growth NOEC = 0.779 mg a.s./L**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	AE 0002166	-	**Growth NOEC = 0.779 mg a.s./L**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	AE1234964	-	**Growth NOEC = 0.779 mg a.s./L**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	AE F159737	-	**Growth NOEC = 0.779 mg a.s./L**	Assumption: 10x more toxic than parent
Aquatic invertebrates – acute				
Daphnia magna	Iodosulfuron-methyl-sodium	48 h, s	**Immobility, EC_{50} > 100 mg a.s./L $_{mm}$**	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Americamysis bahia		96 h, s	Mortality, LC_{50} > 100 mg a.s./L $_{nom}$	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Daphnia magna	AE F075736	-	**EC_{50} > 10 mg a.s./L**	Assumption: 10x more toxic than parent
Daphnia magna	AE F145740	-	**EC_{50} > 10 mg a.s./L**	Assumption: 10x more toxic than parent
Daphnia magna	AE F145741	-	**EC_{50} > 10 mg a.s./L**	Assumption: 10x more toxic than parent
Daphnia magna	AE 0000119	-	**EC_{50} > 10 mg a.s./L**	Assumption: 10x more toxic than parent
Daphnia magna	AE F161778	-	**EC_{50} > 10 mg a.s./L**	Assumption: 10x more toxic than parent
Daphnia magna	BCS-CW811253	-	**EC_{50} > 10 mg a.s./L**	Assumption: 10x more toxic than parent
Daphnia magna	AE F059411	48 h, s	Immobility, EC_{50} > 100 mg metabolite/L	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
		-	**EC_{50} > 10 mg a.s./L**	Assumption: 10x more toxic than parent
Daphnia magna	AE 0014966	-	**EC_{50} > 10 mg a.s./L**	Assumption: 10x more toxic than parent
Daphnia magna	AE 0043885	-	**EC_{50} > 10 mg a.s./L**	Assumption: 10x more toxic than parent
Daphnia magna	AE F154781	-	**EC_{50} > 10 mg a.s./L**	Assumption: 10x more toxic than parent
Daphnia magna	AE 0002166	-	**EC_{50} > 10 mg a.s./L**	Assumption: 10x more toxic than parent
Daphnia magna	AE1234964	48 h, s	Immobility, EC_{50} > 100 mg metabolite/L $_{nom}$	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Daphnia magna	AE F159737	48 h, s	Immobility, EC_{50} > 100 mg/L	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Aquatic invertebrates – chronic				

Species	Substance	Exposure System	Results	Reference
Daphnia magna	Iodosulfuron-methyl-sodium	21 d, ss	**EC_{10} = 7.9 mg a.s./L $_{nom}$**	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Daphnia magna	AE F075736	-	**EC_{10} = 0.79 mg a.s./L**	Assumption: 10x more toxic than parent
Daphnia magna	AE F145740	-	**EC_{10} = 0.79 mg a.s./L**	Assumption: 10x more toxic than parent
Daphnia magna	AE F145741	-	**EC_{10} = 0.79 mg a.s./L**	Assumption: 10x more toxic than parent
Daphnia magna	AE 0000119	-	**EC_{10} = 0.79 mg a.s./L**	Assumption: 10x more toxic than parent
Daphnia magna	AE F161778	-	**EC_{10} = 0.79 mg a.s./L**	Assumption: 10x more toxic than parent
Daphnia magna	BCS-CW811253	-	**EC_{10} = 0.79 mg a.s./L**	Assumption: 10x more toxic than parent
Daphnia magna	AE F059411	-	**EC_{10} = 0.79 mg a.s./L**	Assumption: 10x more toxic than parent
Daphnia magna	AE 0014966	-	**EC_{10} = 0.79 mg a.s./L**	Assumption: 10x more toxic than parent
Daphnia magna	AE 0043885	-	**EC_{10} = 0.79 mg a.s./L**	Assumption: 10x more toxic than parent
Daphnia magna	AE F154781	-	**EC_{10} = 0.79 mg a.s./L**	Assumption: 10x more toxic than parent
Daphnia magna	AE 0002166	-	**EC_{10} = 0.79 mg a.s./L**	Assumption: 10x more toxic than parent
Daphnia magna	AE1234964	-	**EC_{10} = 0.79 mg a.s./L**	Assumption: 10x more toxic than parent
Daphnia magna	AE F159737	-	**EC_{10} = 0.79 mg a.s./L**	Assumption: 10x more toxic than parent
		Algae		
Pseudokirchneriella subcapitata	Iodosulfuron-methyl-sodium	96 h, s	**E_rC_{50} = 0.152 mg a.s./L $_{nom}$** E_bC_{50} = 0.064 mg a.s/L $_{nom}$ NOEC = 0.018 mg a.s./L $_{nom}$	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Navicula pelliculosa		72 h, s	E_rC_{50} > 100 mg a.s./L $_{nom}$ E_bC_{50} > 100 mg a.s./L $_{nom}$ NOEC = 100 mg a.s./L $_{nom}$	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Pseudokirchneriella subcapitata	AE F075736	-	**E_rC_{50} = 0.015 mg a.s./L**	Assumption: 10x more toxic than parent
Pseudokirchneriella subcapitata	AE F145741	72 h, s	E_rC_{50} > 10 mg/L $_{nom}$ NOE_rC < 0.625	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
		-	~~**E_rC_{50} = 0.015 mg a.s./L**~~	~~Assumption: 10x more toxic than parent~~
Pseudokirchneriella subcapitata	AE F145740	72 h, s	E_rC_{50} > 10 mg/L $_{nom}$ NOE_rC = 10 mg/L $_{nom}$	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
		-	~~**E_rC_{50} = 0.015 mg a.s./L**~~	~~Assumption: 10x more toxic than parent~~
Pseudokirchneriella subcapitata	AE 0002166	72 h, s	E_rC_{50} > 10 mg/L $_{nom}$ NOE_rC ~~=~~ < 10 mg/L $_{nom}$	EFSA Conclusion Iodosulfuron-methyl-

Species	Substance	Exposure System	Results	Reference
				sodium, 2016
		-	**E_rC_{50} = 0.015 mg a.s./L**	Assumption: 10x more toxic than parent
Pseudokirchneriella subcapitata	AE F161778	72h, s	E_rC_{50} > 10 mg/L nom NOE_rC < 10 mg/L nom	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
		-	~~**E_rC_{50} = 0.015 mg a.s./L**~~	~~Assumption: 10x more toxic than parent~~
Pseudokirchneriella subcapitata	BCS-CW81253	72 h, s	E_rC_{50} > 10 mg/L nom NOE_rC = 10 mg/L nom	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
		-	~~**E_rC_{50} = 0.015 mg a.s./L**~~	~~Assumption: 10x more toxic than parent~~
Pseudokirchneriella subcapitata	AE F059411	96 h, s	E_rC_{50} > 100 mg/L nom NOEC = 100 mg/L nom	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
		-	~~**E_rC_{50} = 0.015 mg a.s./L**~~	~~Assumption: 10x more toxic than parent~~
Pseudokirchneriella subcapitata	AE 0014966	-	**E_rC_{50} = 0.015 mg a.s./L**	Assumption: 10x more toxic than parent
Pseudokirchneriella subcapitata	AE 0034855	-	**E_rC_{50} = 0.015 mg a.s./L**	Assumption: 10x more toxic than parent
Pseudokirchneriella subcapitata	AE 1234964	-	**E_rC_{50} = 0.015 mg a.s./L**	Assumption: 10x more toxic than parent
Pseudokirchneriella subcapitata	AE F459737	-	**E_rC_{50} = 0.015 mg a.s./L**	Assumption: 10x more toxic than parent
Pseudokirchneriella subcapitata	AE F154781	72 h, s	E_rC_{50} > 10 mg/L nom NOEC = 10 mg/L nom	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
		-	~~**E_rC_{50} = 0.015 mg a.s./L**~~	~~Assumption: 10x more toxic than parent~~
Higher plant				
Lemna gibba	Iodosulfuron-methyl-sodium	14 d, ss	Frond number 7d EC_{50} = 0.00079 mg a.s./L nom 14d EC_{50} = 0.00083 mg a.s./L nom 7d E_rC_{50} = 0.00134 mg a.s./L nom NOEC = 0.00040 mg a.s./L nom	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Lemna gibba (duck weed)		iodosulfuron-methyl-sodium + mefenpyr-diethyl (formulated as OD) 7 d static	frond number 7d E_rC_{50}=0084 mg product/L (nom) correspond to 0.00074 mg a.s./L (nom) biomass 7d E_rC_{50} ≥ 0.100 mg product/L (nom) > 0.00882 mg a.s./L (nom)	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016

Species	Substance	Exposure System	Results	Reference
Myriophyllum spicatum		10 d, s	NOEC = 0.0010 mg a.s./L nom (based on effects on pH and oxygen production)	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Elodea canadensis		10 d,s	NOEC = 0.00022 mg a.s./L nom (based on effects on pH and oxygen production $NOEC_{biomass}$) = 0.00046 mg a.s./L nom	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Myriophyllum spicatum		14 d, s	Shoot length E_yC_{50} = 0.00203 mg a.s./L mm Wet weight E_yC_{50} = 0.00251 mg a.s./L mm Dry weight E_yC_{50} > 0.00845 mg a.s./L mm NOEC = 0.00089 mg a.s./L mm	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Lemna gibba	Iodosulfuron-methyl-sodium + metsulfuron-methyl	6 weeks (semi-static)	Frond number **7d E_rC_{50} = 0.00108 mg a.s./L nom** 6w E_rC_{50} = 0.000679 mg a.s./L nom NOEC = 0.000400 mg a.s./L nom Frond area 7d E_bEC_{50} = 0.00112 mg a.s./L nom 6w E_rC_{50} = 0.000609 mg a.s./L nom NOEC = 0.000400 mg a.s./L nom	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Lemna gibba	AE F075736	7 d, ss	frond number 7d E_rC_{50} = 0.00112 mg/L nom 7d NOEC = 0.00032 mg/L nom Biomass 7d E_bEC_{50} = 0.00131 mg/L nom 7d NOEC = 0.00032 mg/L nom	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Lemna gibba	AE F075736	7 d, s	frond number **7d E_rC_{50} = 0.00057 mg/L nom** 7d NOEC = 0.00025 mg/L nom Biomass 7d E_bEC_{50} = 0.00365 mg/L nom 7d NOEC = 0.00025 mg/L nom	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Lemna gibba	AE F145741	7d, s	frond number 7d E_rC_{50} = 4.69 mg/L nom 7d NOEC = 0.76 mg/L nom Frond area **E_rC_{50} = 3.84 mg/L mm** NOE_rC = 0.76 mg/L mm Biomass 7d E_bEC_{50} > 11.4 mg/L nom 7d NOEC = 1.60 mg/L nom	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Lemna gibba	AE F145740	7 d, s	frond number **E_rC_{50} > 10 mg/L nom** NOE_rC = 10 mg/L nom Frond area E_rC_{50} > 10 mg/L mm NOE_rC = 10 mg/L mm	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Lemna gibba	AE 0002166	7 d, ss	frond number **E_rC_{50} = 0.0230 mg/L twa** Biomass E_bC_{50} = 0.0583 mg/L twa NOEC = 0.00769 mg/L twa	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Lemna gibba	AE F161778	7 d, ss	frond number **E_rC_{50} = 0.0281 mg/L nom** Biomass E_bC_{50} = 0.0305 mg/L	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016

Species	Substance	Exposure System	Results	Reference
			NOEC = 0.10 mg/L	
Lemna gibba	BCS-CW81253	7d, s	frond number **E_rC_{50} > 10 mg/L $_{nom}$** NOE_rC = 10 mg/L $_{nom}$ Frond area E_rC_{50} > 10 mg/L $_{mm}$ NOE_rC = 10 mg/L $_{mm}$	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Lemna gibba	AE 0000119	7 d, ss	frond number **E_rC_{50} > 100 mg/L $_{nom}$** Biomass E_bC_{50} > 100 mg/L $_{nom}$ NOEC = 100 mg/L $_{nom}$	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Lemna gibba	AE F059411	7d, ss	frond number **E_rC_{50} > 100 mg/L $_{nom}$** Biomass E_bC_{50} > 100 mg/L $_{nom}$ NOEC = 56 mg/L $_{nom}$	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Lemna gibba	AE F059411	7 d, ss	frond number E_rC_{50} > 100 mg/L $_{nom}$ Biomass E_bC_{50} > 100 mg/L $_{nom}$ NOEC = 32 mg/L $_{nom}$	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Lemna gibba	AE 0014966	7 d, ss	frond number **E_rC_{50} = 0.575 mg/L $_{nom}$** Biomass E_bC_{50} = 0.380 mg/L $_{nom}$ NOEC = 0.18 mg/L $_{nom}$	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Lemna gibba	AE 0034855	7 d, ss	frond number **E_rC_{50} > 100 mg/L $_{nom}$** Biomass E_bC_{50} > 100 mg/L $_{nom}$ NOEC = 100 mg/L $_{nom}$	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Lemna gibba	AE 1234964	7 d, s	frond number **E_rC_{50} > 100 mg/L $_{nom}$** Biomass E_bC_{50} > 100 mg/L $_{nom}$ NOEC = 0.32 mg/L $_{nom}$	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Lemna gibba	AE F159737	7 d, s	frond number **E_rC_{50} > 100 mg/L $_{nom}$** Biomass E_bC_{50} > 100 mg/L $_{nom}$ NOEC = 0.32 mg/L $_{nom}$	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Lemna gibba	AE F154781	7 d, s	frond number **E_rC_{50} > 10 mg/L $_{nom}$** NOE_rC = 10 mg/L $_{nom}$ Biomass E_bC_{50} > 10 mg/L $_{nom}$ NOE_rC = 10 mg/L $_{nom}$	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Higher-tier studies (micro- or mesocosm studies)				
Outdoor pond study with eight species of aquatic macrophytes exposed for six weeks (Hoberg, 2011, Report No 13798.6259) combined with the 6 week endpoint on *Lemna gibba* from the laboratory study (Bruns, 2013, Report No EBIML025). As no intermediate biological measurements were performed over 6 weeks, it cannot be excluded that a certain recovery might have taken place. Therefore, the only endpoints derived from this study are NOEAECs, based on the assumption that some recovery might have occurred during the 6 weeks. NOEAEC based on measured initial test concentration 0.27 µg a.s./L compared with FOCUS exposure profiles or geomean measured concentration 0.16 µg a.s./L compared with PEC_{max} should beused for the risk assessment, along with an assessment factor of 3.				

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

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

Species	Substance	Exposure System	Results	Reference
Fish – acute				
Oncorhynchus mykiss	Mesosulfuron-methyl	96 h, s	**Mortaility, LC_{50} > 100 mg a.s./L $_{nom}$**	EFSA Conclusion Mesosulfuron-methyl, 2016
Lepomis macrochirus		96 h, s	Mortality, LC_{50} > 100 mg a.s./L $_{nom}$	EFSA Conclusion Mesosulfuron-methyl, 2016
Cyprinodon variegates		96 h, s	Mortality, LC_{50} > 100 mg a.s./L $_{nom}$	EFSA Conclusion Mesosulfuron-methyl, 2016
Oncorhynchus mykiss	BCS-CV14885	-	**LC_{50} > 10 mg a.s./L $_{nom}$**	EFSA Conclusion Mesosulfuron-methyl, 2016
Oncorhynchus mykiss	Mesosulfuron	-	**LC_{50} > 10 mg a.s./L $_{nom}$**	EFSA Conclusion Mesosulfuron-methyl, 2016
Oncorhynchus mykiss	AE F160459	-	**LC_{50} > 10 mg a.s./L $_{nom}$**	EFSA Conclusion Mesosulfuron-methyl, 2016
Oncorhynchus mykiss	AE F099095	96 h, s	Mortality, LC_{50} = 70.7 mg/L $_{nom}$	EFSA Conclusion Mesosulfuron-methyl, 2016
		-	**LC_{50} > 10 mg a.s./L $_{nom}$**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	AE F092944	96 h, s	Mortality, LC_{50} > 97 mg/L $_{mm}$	EFSA Conclusion Mesosulfuron-methyl, 2016
		-	**LC_{50} > 10 mg a.s./L $_{nom}$**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	AE F160460	-	**LC_{50} > 10 mg a.s./L $_{nom}$**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	AE F147447	-	**LC_{50} > 10 mg a.s./L $_{nom}$**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	AE F140584	-	**LC_{50} > 10 mg a.s./L $_{nom}$**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	BCS-CO60720	-	**LC_{50} > 10 mg a.s./L $_{nom}$**	Assumption: 10x more toxic than parent
Fish – chronic				
Oncorhynchus mykiss	Mesosulfuron-methyl	28 d, ss	**NOEC (juvenile fish) = 32 mg a.s./L $_{nom}$**	EFSA Conclusion Mesosulfuron-methyl, 2016
Pimephales promelas		32 d, flow-through	ELS NOEC = 95 mg a.s./L $_{nom}$	EFSA Conclusion Mesosulfuron-methyl, 2016
Oncorhynchus mykiss	BCS-CV14885	-	**NOEC = 3.2 mg a.s./L $_{nom}$**	Assumption: 10x

Species	Substance	Exposure System	Results	Reference
				more toxic than parent
Oncorhynchus mykiss	Mesosulfuron	-	**NOEC = 3.2 mg a.s./L $_{nom}$**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	AE F160459	-	**NOEC = 3.2 mg a.s./L $_{nom}$**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	AE F099095	-	**NOEC = 3.2 mg a.s./L $_{nom}$**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	AE F092944	-	**NOEC = 3.2 mg a.s./L $_{nom}$**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	AE F160460	-	**NOEC = 3.2 mg a.s./L $_{nom}$**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	AE F147447	-	**NOEC = 3.2 mg a.s./L $_{nom}$**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	AE F140584	-	**NOEC = 3.2 mg a.s./L $_{nom}$**	Assumption: 10x more toxic than parent
Oncorhynchus mykiss	BCS-CO60720	-	**NOEC = 3.2 mg a.s./L $_{nom}$**	Assumption: 10x more toxic than parent
Aquatic invertebrates – acute				
Daphnia magna	Mesosulfuron-methyl	48 h, s	**Mortality, EC_{50} > 100 mg a.s./L $_{nom}$**	EFSA Conclusion Mesosulfuron-methyl, 2016
Mysidopsis bahia		48 h, s	Mortality, EC_{50} > 100 mg a.s./L $_{nom}$	EFSA Conclusion Mesosulfuron-methyl, 2016
Crassostrea virginica		96 h, flow-through	Mortality/shell deposition, EC_{50} > 100 mg a.s./L $_{nom}$	EFSA Conclusion Mesosulfuron-methyl, 2016
Daphnia magna	BCS-CV14885	-	**EC_{50} > 10 mg a.s./L**	Assumption: 10x more toxic than parent
Daphnia magna	Mesosulfuron	-	**EC_{50} > 10 mg a.s./L**	Assumption: 10x more toxic than parent
Daphnia magna	AE F160459	-	**EC_{50} > 10 mg a.s./L**	Assumption: 10x more toxic than parent
Daphnia magna	AE F099095	48 h, s	Mortality, EC_{50} > 100 mg/L $_{nom}$	EFSA Conclusion Mesosulfuron-methyl, 2016
		-	**EC_{50} > 10 mg a.s./L**	Assumption: 10x more toxic than parent
Daphnia magna	AE F092944	48 h, s	Mortality, EC_{50} = 223 mg a.s./L $_{nom}$	EFSA Conclusion Mesosulfuron-methyl, 2016
		-	**EC_{50} > 10 mg a.s./L**	Assumption: 10x more toxic than parent
Daphnia magna	AE F092944	48 h, s	Mortality, EC_{50} > 100 mg a.s./L $_{nom}$	EFSA Conclusion Mesosulfuron-methyl, 2016
		-	~~**EC_{50} > 10 mg a.s./L**~~	~~Assumption: 10x more toxic than parent~~
Daphnia magna	AE F160460	-	**EC_{50} > 10 mg a.s./L**	Assumption: 10x more toxic than parent

Species	Substance	Exposure System	Results	Reference
Daphnia magna	AE F147447	-	**EC_{50} > 10 mg a.s./L**	Assumption: 10x more toxic than parent
Daphnia magna	AE F140584	-	**EC_{50} > 10 mg a.s./L**	Assumption: 10x more toxic than parent
Daphnia magna	BCS-CO60720	-	**EC_{50} > 10 mg a.s./L**	Assumption: 10x more toxic than parent
		Aquatic invertebrates - chronic		
Daphnia magna	Mesosulfuron-methyl	21 d, ss	**NOEC = 1.8 mg a.s./L $_{nom}$**	EFSA Conclusion Mesosulfuron-methyl, 2016
Daphnia magna	BCS-CV14885	-	**NOEC = 0.18 mg a.s./L $_{nom}$**	Assumption: 10x more toxic than parent
Daphnia magna	Mesosulfuron	-	**NOEC = 0.18 mg a.s./L $_{nom}$**	Assumption: 10x more toxic than parent
Daphnia magna	AE F160459	-	**NOEC = 0.18 mg a.s./L $_{nom}$**	Assumption: 10x more toxic than parent
Daphnia magna	AE F099095	-	**NOEC = 0.18 mg a.s./L $_{nom}$**	Assumption: 10x more toxic than parent
Daphnia magna	AE F092944	21 d, ss	Reproduction NOEC = 24.9 mg/L $_{mm}$	EFSA Conclusion Mesosulfuron-methyl, 2016
		-	**NOEC = 0.18 mg a.s./L $_{nom}$**	Assumption: 10x more toxic than parent
Daphnia magna	AE F160460	-	**NOEC = 0.18 mg a.s./L $_{nom}$**	Assumption: 10x more toxic than parent
Daphnia magna	AE F147447	-	**NOEC = 0.18 mg a.s./L $_{nom}$**	Assumption: 10x more toxic than parent
Daphnia magna	AE F140584	-	**NOEC = 0.18 mg a.s./L $_{nom}$**	Assumption: 10x more toxic than parent
Daphnia magna	BCS-CO60720	-	**NOEC = 0.18 mg a.s./L $_{nom}$**	Assumption: 10x more toxic than parent
		Algae		
Pseudokirchneriella subcapitata	Mesosulfuron-methyl	72 h, s	**E_rC_{50} > 0.29 mg a.s./L $_{mm}$** E_bC_{50} = 0.18 mg a.s./L $_{mm}$ NOE_rC = 0.018 mg a.s./L $_{mm}$	EFSA Conclusion Mesosulfuron-methyl, 2016
Pseudokirchneriella subcapitata	Mesosulfuron-methyl	72 h, s	E_rC_{50} = 3.99 mg a.s./L $_{mm}$ NOE_rC = 0.143 mg a.s./L $_{mm}$	EFSA Conclusion Mesosulfuron-methyl, 2016
Navicula pelliculosa	Mesosulfuron-methyl	72 h, s	E_rC_{50} > 74.9 mg a.s./L $_{mm}$ E_bC_{50} > 74.9 mg a.s./L $_{mm}$ NOE_rC = 74.9 mg a.s./L $_{mm}$	EFSA Conclusion Mesosulfuron-methyl, 2016
Anabaena flos-aquae	Mesosulfuron-methyl	96 h, s	E_rC_{50} = 4.1 mg a.s./L $_{mm}$ E_bC_{50} = 2.4 mg a.s./L $_{mm}$ NOE_rC = 1 mg a.s./L $_{mm}$	EFSA Conclusion Mesosulfuron-methyl, 2016
Skeletonema costatum	Mesosulfuron-methyl	72 h, s	E_rC_{50} > 100 mg a.s./L $_{nom}$ E_bC_{50} = 82 mg a.s./L $_{nom}$ NOE_rC = 60 mg a.s./L $_{nom}$	EFSA Conclusion Mesosulfuron-methyl, 2016
Pseudokirchneriella subcapitata	BCS-CV14885	-	**E_rC_{50} > 0.029 mg a.s./L**	Assumption: 10x more toxic than parent

Species	Substance	Exposure System	Results	Reference
Pseudokirchneriella subcapitata	Mesosulfuron	72 h, s	E_rC_{50} = 38 mg a.s./L mm	EFSA Conclusion Mesosulfuron-methyl, 2016
		-	~~**E_rC_{50} > 0.029 mg a.s./L**~~	~~Assumption: 10x more toxic than parent~~
Pseudokirchneriella subcapitata	AE F160459	72 h, s	**E_rC_{50} > 100 mg a.s./L nom** E_bC_{50} = 92 mg a.s./L nom	EFSA Conclusion Mesosulfuron-methyl, 2016
Pseudokirchneriella subcapitata	AE F099095	72 h, s	E_rC_{50} > 100 mg a.s./L nom	EFSA Conclusion Mesosulfuron-methyl, 2016
Pseudokirchneriella subcapitata	AE F099095	72 h, s	E_rC_{50} = 99.1 mg a.s./L nom E_bC_{50} = 41.1 mg a.s./L nom	EFSA Conclusion Mesosulfuron-methyl, 2016
		-	**E_rC_{50} > 0.029 mg a.s./L**	Assumption: 10x more toxic than parent
Pseudokirchneriella subcapitata	AE F092944	72 h, s	E_rC_{50} > 120 mg a.s./L nom E_bC_{50} > 120 mg a.s./L nom NOE_rC = 7.5 mg a.s./L nom	EFSA Conclusion Mesosulfuron-methyl, 20166
		-	**E_rC_{50} > 0.029 mg a.s./L**	Assumption: 10x more toxic than parent
Scenedesmus subspicatus	AE F092944	72 h, s	E_rC_{50} > 100 mg a.s./L nom E_bC_{50} > 100 mg a.s./L nom NOE_rC = 100 mg a.s./L nom	EFSA Conclusion Mesosulfuron-methyl, 2016
Scenedesmus subspicatus	AE F160460	-	**E_rC_{50} > 0.029 mg a.s./L**	Assumption: 10x more toxic than parent
Pseudokirchneriella subcapitata	AE F147447	72 h, s	**E_rC_{50} > 100 mg a.s./L nom** E_bC_{50} > 100 mg a.s./L nom	EFSA Conclusion Mesosulfuron-methyl, 2016
Pseudokirchneriella subcapitata	AE F140584	-	**E_rC_{50} > 0.029 mg a.s./L**	Assumption: 10x more toxic than parent
Pseudokirchneriella subcapitata	BCS-CO60720	72 h, s	E_rC_{50} > 10 mg/L nom	EFSA Conclusion Mesosulfuron-methyl, 2016
		-	~~**E_rC_{50} > 0.029 mg a.s./L**~~	~~Assumption: 10x more toxic than parent~~
Pseudokirchneriella subcapitata	BCS-CO60721	72 h, s	E_rC_{50} > 10 mg/L nom	EFSA Conclusion Mesosulfuron-methyl, 2016
		-	~~**E_rC_{50} > 0.029 mg a.s./L**~~	~~Assumption: 10x more toxic than parent~~
		Higher plant		
Lemna gibba	Mesosulfuron-methyl	7 d, ss	E_rC_{50} = 0.001717 mg a.s./L nom E_bC_{50} = 0.001863 mg a.s./L nom NOE_rC < 0.00077 mg a.s./L nom	EFSA Conclusion Mesosulfuron-methyl, 2016
Lemna gibba	BCS-CV14885	-	**$E_rC_{50\ frond\ area}$ = 0.00129 mg a.s./L**	In the absence of a toxicity endpoint for the metabolite, the available toxicity endpoint of the parent compound was used since from the

Species	Substance	Exposure System	Results	Reference
				available information the toxophore appears to be lost
Lemna gibba	Mesosulfuron	7 d, s	**E_rC_{50} = 0.11 mg a.s./L $_{nom}$**	EFSA Conclusion Mesosulfuron-methyl, 2016
Lemna gibba	AE F160459	7 d, s	**E_rC_{50} = 2.6 mg a.s./L $_{nom}$** E_bC_{50} = 1.7 mg a.s./L $_{nom}$	EFSA Conclusion Mesosulfuron-methyl, 2016
Lemna gibba	AE F099095	7 d, s	**E_rC_{50} > 100 mg a.s./L $_{nom}$**	EFSA Conclusion Mesosulfuron-methyl, 2016
Lemna gibba	AE F092944	7 d, ss	**E_rC_{50} > 100 mg a.s./L $_{nom}$** E_bC_{50} > 100 mg a.s./L $_{nom}$	EFSA Conclusion Mesosulfuron-methyl, 2016
Lemna gibba	AE F160460	7 d, ss	**E_rC_{50} > 100 mg a.s./L $_{nom}$** E_bC_{50} > 100 mg a.s./L $_{nom}$	EFSA Conclusion Mesosulfuron-methyl, 2016
Lemna gibba	AE F140584	7 d, ss	**E_rC_{50} > 10 mg a.s./L $_{nom}$**	EFSA Conclusion Mesosulfuron-methyl, 2016
Lemna gibba	AE F147447	7 d, ss	**E_rC_{50} > 100 mg a.s./L $_{nom}$** E_bC_{50} > 100 mg a.s./L $_{nom}$	EFSA Conclusion Mesosulfuron-methyl, 2016
Lemna gibba	BCS-CO60720	7 d, s	**E_rC_{50} > 11.8 mg a.s./L $_{nom}$**	EFSA Conclusion Mesosulfuron-methyl, 2016
Lemna gibba	BCS-CO60721	7 d, s	E_rC_{50} > 10 mg a.s./L $_{nom}$	EFSA Conclusion Mesosulfuron-methyl, 2016
Higher-tier studies (micro- or mesocosm studies)				
Aquatic macrophytes (9 species) *Elodea canadensis* *Potamogeton pectinatus* *Pontederia cordata* *Nymphaea odorata* *Cabomba caroliniana* *Cerat. Demersum* *Glyceria maxima* *Mentha aquatica* *Myriophyll. heterophyllum*	Mesosulfuron-methyl	Outdoor growth inhibition, static, 8 weeks	<u>8-week endpoints</u> Lowest NOAEC = 0.00057 mg a.s./L $_{mm}$ (Shoot length/dry weight)	EFSA Conclusion Mesosulfuron-methyl, 2016
Lemna gibba	Mesosulfuron-methyl	Growth inhibition, mimicking exposure of outdoor study, 8 weeks	<u>7 d endpoint</u> $E_rC_{50\ frond\ number}$ = 0.00161 mg a.s./L $_{nom}$ **$E_rC_{50\ frond\ area}$ = 0.00129 mg a.s./L $_{nom}$** NOE_rC = 0.00039 mg a.s./L $_{nom}$ <u>8-week endpoint</u> $NOE_rC_{\ frond\ number}$ = 0.000388 mg a.s./L $_{nom}$, 0.00026 mg a.s./L $_{mm}$ $NOE_rC_{\ frond\ area}$ = 0.000388 mg a.s./L $_{nom}$, 0.00026 mg a.s./L $_{mm}$	EFSA Conclusion Mesosulfuron-methyl, 2016 First-tier endpoint
Lemna gibba	Mesosulfuron-methyl	Growth	<u>**$E_rC_{50\ frond\ number}$ = 2.72 µg a.s./L $_{nom}$**</u>	Lang C., 2018

Species	Substance	Exposure System	Results	Reference
		inhibition, static, 7 days	$E_yC_{50\ \text{frond number}}$ = 1.02 µg a.s./L $_{\text{nom}}$ $E_rC_{50\ \text{fresh weight}}$ = 3.30 µg a.s./L $_{\text{nom}}$ $E_yC_{50\ \text{fresh weight}}$ = 1.30 µg a.s./L $_{\text{nom}}$ $E_rC_{50\ \text{dry weight}}$ > 4.00 µg a.s./L $_{\text{nom}}$ $E_yC_{50\ \text{dry weight}}$ = 2.93 µg a.s./L $_{\text{nom}}$	
Nasturtium officinale	Mesosulfuron-methyl	Growth inhibition, static, 14 days	$E_rC_{50\ \text{shoot length}}$ = 1.60 µg a.s./L $_{\text{nom}}$ $E_yC_{50\ \text{shoot length}}$ = 1.21 µg a.s./L $_{\text{nom}}$ **$E_rC_{50\ \text{shoot fresh weight}}$ = 1.18 µg a.s./L $_{\text{nom}}$** $E_yC_{50\ \text{shoot fresh weight}}$ = 0.67 µg a.s./L $_{\text{nom}}$ $E_rC_{50\ \text{shoot dry weight}}$ = 3.99 µg a.s./L $_{\text{nom}}$ $E_yC_{50\ \text{shoot dry weight}}$ = 1.12 µg a.s./L $_{\text{nom}}$	Dill M., 2018a
Hottonia palustris	Mesosulfuron-methyl	Growth inhibition, static, 16 days	**$E_rC_{50\ \text{shoot length}}$ = 15.6 µg a.s./L $_{\text{nom}}$** $E_yC_{50\ \text{shoot length}}$ = 10.3 µg a.s./L $_{\text{nom}}$ $E_rC_{50\ \text{shoot fresh weight}}$ = 26.2 µg a.s./L $_{\text{nom}}$ $E_yC_{50\ \text{shoot fresh weight}}$ = 9.81 µg a.s./L $_{\text{nom}}$ $E_rC_{50\ \text{shoot dry weight}}$ = > 1000 µg a.s./L $_{\text{nom}}$ $E_yC_{50\ \text{shoot dry weight}}$ = > 1000 µg a.s./L $_{\text{nom}}$	Dill M., 2018b
Myriophillum sibirum	Mesosulfuron-methyl	Growth inhibition, static, 14 days	$E_rC_{50\ \text{shoot length}}$ = 32.1 µg a.s./L $_{\text{nom}}$ $E_yC_{50\ \text{shoot length}}$ = 13.9 µg a.s./L $_{\text{nom}}$ **$E_rC_{50\ \text{shoot fresh weight}}$ = 20.7 µg a.s./L $_{\text{nom}}$** $E_yC_{50\ \text{shoot fresh weight}}$ = 12.4 µg a.s./L $_{\text{nom}}$ $E_rC_{50\ \text{shoot dry weight}}$ = 101 µg a.s./L $_{\text{nom}}$ $E_yC_{50\ \text{shoot dry weight}}$ = 51.2 µg a.s./L $_{\text{nom}}$	Dill M., 2018c
Ceratophyllum demersun	Mesosulfuron-methyl	Growth inhibition, static, 14 days	**$E_rC_{50\ \text{shoot length}}$ = 7.77 µg a.s./L $_{\text{mm}}$** $E_yC_{50\ \text{shoot length}}$ = 7.37 µg a.s./L $_{\text{mm}}$ $E_rC_{50\ \text{shoot fresh weight}}$ = 16.0 µg a.s./L $_{\text{mm}}$ $E_yC_{50\ \text{shoot fresh weight}}$ = 10.6 µg a.s./L $_{\text{mm}}$ $E_rC_{50\ \text{shoot dry weight}}$ = 21.3 µg a.s./L $_{\text{mm}}$ $E_yC_{50\ \text{shoot dry weight}}$ = 8.63 µg a.s./L $_{\text{mm}}$	Dill M., 2018d
Vallisneria spiralis	Mesosulfuron-methyl	Growth inhibition, static, 21 days	$E_rC_{50\ \text{shoot length}}$ = 51.1 µg a.s./L $_{\text{mm}}$ $E_yC_{50\ \text{shoot length}}$ = 43.5 µg a.s./L $_{\text{mm}}$ $E_rC_{50\ \text{shoot fresh weight}}$ = 65.1 µg a.s./L $_{\text{mm}}$ $E_yC_{50\ \text{shoot fresh weight}}$ = 35.6 µg a.s./L $_{\text{mm}}$ **$E_rC_{50\ \text{shoot dry weight}}$ = 38.6 µg a.s./L $_{\text{mm}}$** $E_yC_{50\ \text{shoot dry weight}}$ = 22.0 µg a.s./L $_{\text{mm}}$	Dill M., 2018e
Glyceria maxima	Mesosulfuron-methyl	Growth inhibition, static, 14 days	$E_rC_{50\ \text{shoot length}}$ = 160 µg a.s./L $_{\text{nom}}$ $E_yC_{50\ \text{shoot length}}$ = 97.7 µg a.s./L $_{\text{nom}}$ **$E_rC_{50\ \text{shoot fresh weight}}$ = 65.8 µg a.s./L $_{\text{nom}}$** $E_yC_{50\ \text{shoot fresh weight}}$ = 52.7 µg a.s./L $_{\text{nom}}$ $E_rC_{50\ \text{shoot dry weight}}$ = 490 µg a.s./L $_{\text{nom}}$ $E_yC_{50\ \text{shoot dry weight}}$ = 255 µg a.s./L $_{\text{nom}}$	Dill M., 2018f
Elodea canadiensis	Mesosulfuron-methyl	Growth inhibition,	**$E_rC_{50\ \text{shoot length}}$ = 9.08 µg a.s./L $_{\text{mm}}$** $E_yC_{50\ \text{shoot length}}$ = 3.92 µg a.s./L $_{\text{mm}}$	Dill M., 2018g

Species	Substance	Exposure System	Results	Reference
		static, 14 days	$E_rC_{50\ shoot\ fresh\ weight}$ = 9.57 µg a.s./L $_{mm}$ $E_yC_{50\ shoot\ fresh\ weight}$ = 5.31 µg a.s./L $_{mm}$ $E_rC_{50\ shoot\ dry\ weight}$ = > 100 µg a.s./L $_{mm}$ $E_yC_{50\ shoot\ dry\ weight}$ = > 108 µg a.s./L $_{mm}$	
Wolffia arrhiza	Mesosulfuron-methyl	Growth inhibition, static, 7 days	$E_rC_{50\ frond\ number}$ = 7.45 µg a.s./L $_{mm}$ $E_yC_{50\ frond\ number}$ = 2.79 µg a.s./L $_{mm}$ **$E_rC_{50\ fresh\ weight}$ = 5.59 µg a.s./L $_{mm}$** $E_yC_{50\ fresh\ weight}$ = 2.08 µg a.s./L $_{mm}$ $E_rC_{50\ dry\ weight}$ = 23.2* µg a.s./L $_{mm}$ $E_yC_{50\ dry\ weight}$ = 5.23 µg a.s./L $_{mm}$	Dill M., 2018h
Spriodela polyrriza	Mesosulfuron-methyl	Growth inhibition, static, 7 days	$E_rC_{50\ frond\ number}$ = 3.35 µg a.s./L $_{nom}$ $E_yC_{50\ frond\ number}$ = 1.48 µg a.s./L $_{nom}$ **$E_rC_{50\ fresh\ weight}$ = 2.49 µg a.s./L $_{nom}$** $E_yC_{50\ fresh\ weight}$ = 1.39 µg a.s./L $_{nom}$ $E_rC_{50\ dry\ weight}$ = 12.7 µg a.s./L $_{nom}$ $E_yC_{50\ dry\ weight}$ = 4.06 µg a.s./L $_{nom}$	Dill M., 2018i
HC5 based on 11 studies with 10 species of macrophytes (presented above in bold)	Mesosulfuron-methyl	Growth inhibition test on 10 different species under laboratory conditions	HC5 = **0.9** µg a.s./L	-

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
* value not reliable. No inhibition > 50% was observed.

Refined aquatic risk assessment for active substance mesosulfuron-methyl

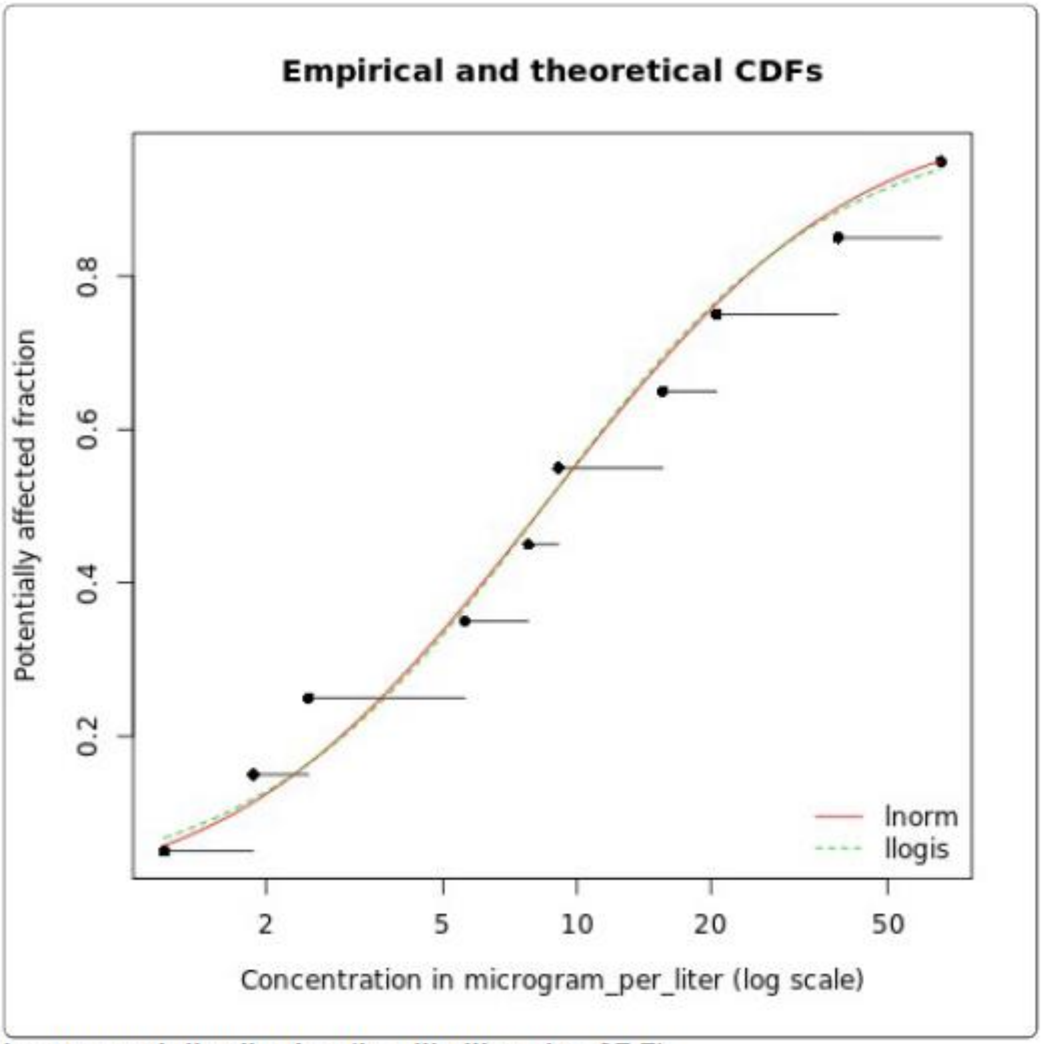
The higher tier study in the RAR resulted in the same RAC as the Tier 1 study in the RAR. Both RAC values were 0.129 µg/L. The higher tier study was therefore not useful to refine the risk on aquatic organisms. In addition, the only endpoints that could be derived from the higher tier study were NOEAEC's instead of ErC50's because no intermediate measurements were made (which was practically impossible due to the study set-up).

In order to refine the risk for aquatic organisms, further macrophyte endpoints have been generated for mesosulfuron-methyl. The new data are listed in Appendix 1 and summarised in Appendix 2.

The effect of the active substance on the growth of aquatic macrophytes was tested under laboratory conditions in the following species: *Lemna gibba*, *Elodea canadiensis*, *Spriodela polyrriza*, *Nasturtium officinale*, *Hottonia palustris*, *Myriophillum sibirum*, *Ceratophyllum demersun*, *Vallisneria spiralis*, *Glyceria maxima* and *Wolffia arrhiza*. The species tested were chosen to represent a range of aquatic macrophyte habits; rooted and unrooted, monocotyledon and dicotyledon, emergent and submerge, and based on the experience of the CRO. The studies were designed to determine ErC50 values as recommended by Section 7.2.7 of the EFSA 2013 Aquatic Guidance. *Nasturtium officinale* (worst-case species tested in the laboratory test) with a NOEC of 0.133 µg/L showed to be more sensitive than *Potamogeton pectinatus* (worst-case species tested in the mesocosm) with a NOEC of 0.51 µg/L. The direct comparison of the NOEC values derived from the mesocosm and laboratory studies shows that endpoints in the mesocosm are not lower despite inter-species competition. Mesocosm results are generally in line with those for the worst-case laboratory tests. The available laboratory data demonstrate that the use of the worst-case NOEC value from the mesocosm study is not warranted to provide a realistic risk assessment for aquatic macrophytes. This is likely to be due to various factors. Indeed, the mesocosm does not provide growth rate endpoints and thus new studies summarized under Appendix 2 are considered scientifically robust to update the risk assessment based on valid growth rate endpoints from the laboratory data.

The results of the laboratory studies allow for species sensitivity distribution (SSD) analysis. E_rC_{50} values for the 10 species tested in laboratory conditions ranged from 1.18 µg a.s./L (*Nasturium officinale*) to 65.8 µg a.s./L (*Glyceria maxima)*. The complete data set was used for the calculation of the EC_{50} based median HC_5 value, including the 7-day endpoint for *Lemna gibba* available in the EFSA review for mesosulfuron-methyl. For *Lemna gibba*, the geometric mean was taken of the two available values. The Species Sensitivity Distribution has been calculated using the **Mosaic tool.** Based on 10 species, the median HC5 was calculated to be 0.93 µg/L.

Species	**E_rC_{50} (µg/L)**	
Lemna gibba	1: 1.29 2: 2.72 Geometric mean**:** 1.87	Median HC_5 = 0.93 µg/L n = 10 = 0.93 / 3 = 0.31
Elodea canadiensis	9.08 mm shoot length	
Spriodela polyrriza	2.49 nom fresh weight	
Nasturtium officinale	1.18 nom shoot fresh weight	
Hottonia palustris	15.6 nom shoot length	
Myriophillum sibirum	20.6 nom shoot fresh weight	
Ceratophyllum demersun	7.77 mm shoot length	
Vallisneria spiralis	38.6 mm dry weight	
Glyceria maxima	65.8 nom shoot fresh weight	
Wolffia arrhiza	5.59 mm shoot fresh weight	



Log normal distribution (log-likelihood = -37.7)
meanlog: 2.1 [1.3 ; 2.9]
sdlog: 1.2 [0.64 ; 1.7]
Log logistic distribution (log-likelihood = -38.1)
shape: 8.4 [3.8 ; 19]
scale: 1.3 [0.88 ; 2.7]

Figure 9.5 1: Species Sensitivity Distribution for aquatic plants exposed to mesosulfuron-methyl, based on EC_{50} values (10 species – MOSAIC tool)

Table 9.5-3: HC5 with specified confidence interval

HC	Log-normal	Log-logistic
HC5	1.1 [0.38 ; 4.1]	**0.93** [0.24 ; 3.8]
HC10	1.7 [0.66 ; 5.5]	1.6 [0.52 ; 5.4]
HC20	2.9 [1.3 ; 7.8]	3 [1.2 ; 7.9]
HC50	8.4 [3.9 ; 18]	8.4 [3.8 ; 19]

For the derivation of a RAC, an assessment factor (AF) needs to be applied to account for uncertainties. For the risk assessment for primary producers, an AF of 3 is advised in the EFSA Journal 2013; 11(7):3290. This results in a **RAC of 0.31 µg a.s./L**.

Table 9.5-4: Endpoints and effect values relevant for the risk assessment for aquatic organisms – mefenpyr-diethyl and relevant metabolites

Species	Substance	Exposure System	Results	Reference
Fish – acute				
Cyprinus carpio	Mefenpyr-diethyl	Acute	**LC_{50} = 2.4 mg a.s./L**	DAR Mefenpyr-diethyl, 2011
Lepomis macrochirus	AE F113225	Acute	**LC_{50} = 100 mg/L**	DAR Mefenpyr-diethyl, 2011
Oncorhynchus mykiss	AE F109453	Acute	**LC_{50} > 100 mg/L**	DAR Mefenpyr-diethyl, 2011
Danio rerio	AE F094270	Acute	**LC_{50} > 72 mg/L**	DAR Mefenpyr-diethyl, 2011
Fish	*AE2211046*	Acute	**LC_{50}=0.24 mg/L**	*10 x more toxic than parent*
Fish – chronic				
Oncorhynchus mykiss	Mefenpyr-diethyl	Chronic	**NOEC = 0.1 mg a.s./L**	DAR Mefenpyr-diethyl, 2011
Oncorhynchus mykiss	AE F113225	Chronic	**NOEC = 32 mg/L**	DAR Mefenpyr-diethyl, 2011
Danio rerio	AE F094270	Chronic	**NOEC = 3.2 mg/L**	DAR Mefenpyr-diethyl, 2011
Fish	*AE2211046*	Chronic	**NOEC=0.01**	*10 x more toxic than parent*
Aquatic invertebrates – acute				
Daphnia magna	Mefenpyr-diethyl	Acute	**EC_{50} = 5.5 mg a.s./L**	DAR Mefenpyr-diethyl, 2011
Daphnia magna	AE F113225	Acute	**EC_{50} > 100 mg/L**	DAR Mefenpyr-diethyl, 2011
Daphnia magna	AE F109453	Acute	**EC_{50} > 100 mg/L**	DAR Mefenpyr-diethyl, 2011
Daphnia magna	AE F094270	Acute	**EC_{50} > 60.3 mg/L**	DAR Mefenpyr-diethyl, 2011
Daphnia magna	*AE 2211046*	Acute	**EC_{50}=0.55 mg/L**	*10 x more toxic than parent*
Aquatic invertebrates - chronic				
Daphnia magna	Mefenpyr-diethyl	Chronic	**NOEC = 0.32 mg a.s./L**	DAR Mefenpyr-diethyl, 2011
Daphnia magna	AE F113225	Chronic	**NOEC = 3.2 mg/L**	DAR Mefenpyr-diethyl, 2011
Daphnia magna	AE F094270	Chronic	**NOEC = 32 mg/L**	DAR Mefenpyr-diethyl, 2011
Daphnia magna	*AE2211046*	*Chronic*	***NOEC=0.032***	*10 x more toxic than parent*
Algae				
Navicula pelliculosa	Mefenpyr-diethyl	-	**E_bC_{50} = 1.39 mg a.s./L**	DAR Mefenpyr-diethyl, 2011
Pseudokirchneriella subcapitata	AE F113225	-	**E_bC_{50} > 100 mg a.s./L** E_rC_{50} > 100 mg a.s./L	DAR Mefenpyr-diethyl, 2011

Species	Substance	Exposure System	Results	Reference
Pseudokirchneriella subcapitata	AE F109453	-	**E_rC_{50} = 41.9 mg a.s./L** E_bC_{50} = 41.9 mg a.s./L	DAR Mefenpyr-diethyl, 2011
Pseudokirchneriella subcapitata	AE F094270	-	E_rC_{50} = 42.0 mg a.s./L **E_bC_{50} = 30.8 mg a.s./L**	DAR Mefenpyr-diethyl, 2011
Navicula pelliculosa	*AE2211046*	-	E_bC_{50}=0.139 g/L	*10 x more toxic than parent*
Sediment dwelling organisms				
Chironimus riparius	AE F094270	-	NOEC = 50 mg/L	DAR Mefenpyr-diethyl, 2011
Higher plant				
Lemna gibba	Mefenpyr-diethyl	7 days	**E_rC_{50} > 7.6 mg a.s./L** E_bC_{50} > 7.6 mg a.s./L	DAR Mefenpyr-diethyl, 2011
Lemna gibba	*AE2211046*	-	E_rC_{50} > 0.76 mg a.s./L	*10 x more toxic than parent*

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
* value not reliable. No inhibition > 50% was observed.

The endpoint ErC_{50} is selected in this Core Assessment but there are some uncertainties regarding the level of protection reached for primary producers. This is indicated for macrophytes in the aquatic Guidance Document (EFSA Journal 2013;11(7):3290) that recommends: "... a proper calibration between different tiers (higher and lower tier data) for macrophytes should be performed in the future". Such calibration should be extended to algae. Until available relevant information on the level of protection reached is considered at EU level, it is recommended to address this uncertainty at each Member State level in the National Addendum if considered necessary, although it would be highly appreciated to have a harmonised approach in the Central zone

Toxicity of the formulation

According to Aquatic Guidance Document (EFSA Journal 2013;11(7):3290) when available information on the active substance indicates that one group of aquatic organisms (fish, aquatic invertebrates and algae) is clearly more sensitive (factor of 10), then studies with the product should be carried out only on the most sensitive species. For mefenpyr-diethyl, there is no group of aquatic organisms that is clearly more sensitive than the other. However, the data presented in above Table of Agreed EU endpoints clearly demonstrate that aquatic plants are more sensitive to Iodosulfuron-methyl-sodium and Mesosulfuron-methyl technical. Based on this information, testing the formulated product on fish was not considered necessary. Apart from *lemna gibba*, the effect of the formulation on *daphnia magna* and algae was tested.

To improve efficacy of the product, an adjuvant can be added in tank mix. As non-target organisms will be exposed to the combination of the formulation and the adjuvants, the combined effect on aquatic organisms was addressed. Two different adjuvants were tested; a non-esterified rapeseed oil (Actirob) and a non-ionic surfactant (Pottok). Based on the studies with the solo formulation, *lemna gibba* could be identified as the most sensitive organism. Studies with the adjuvants were therefore only performed on *lemna gibba*. The endpoints from the studies with the adjuvants were in the same order of magnitude as the endpoint for the solo formulation. It can therefore be concluded that the adjuvants do not significantly increase the toxicity of the plant protection product to aquatic organisms.

The results of the toxicity studies on GLOB289H, GLOB289H + Actirob and GLOB289H + Pottok are summarized in Table 9.5-4.

Table 9.5-5: Endpoints and effect values relevant for the risk assessment for aquatic organisms – GLOB289H

Species	Substance	Exposure System	Results	Reference
Daphnia magna	GLOB289H	48 h, s	EC_{50} = 21.97 mg/L nom	Renner P., 2018a
Pseudokirchneriella subcapitata	GLOB289H	72 h, s	E_rC_{50} = 17.95 mg/L nom E_yC_{50} = 7.53 mg/L nom	Renner P., 2018b
Lemna gibba	GLOB289H	7d, ss	E_rC_{50} = 41.27 µg/L nom E_yC_{50} = 23.20 µg/L nom	Renner P., 2018c
Lemna gibba	GLOB289H + Actirob*	7d, ss	E_rC_{50} = 21.47 µg/L nom E_yC_{50} = 9.64 µg/L nom	Renner P., 2019a
Lemna gibba	GLOB289H + Pottok*	7d, ss	**E_rC_{50} = 18.08 µg/L nom** E_yC_{50} = 9.80 µg/L nom	Renner P., 2019b

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations
* Concentrations are related to GLOB289H only. The adjuvant was applied at the ratio advised on the label.

zRMS comments:

The applicant performed the study with adjuwants for most sensitive organism Lemna gibba.
The endpoints with the adjuvant are appromix. two times lower compared to the endpoint for the solo PPP.
However, the results are still in the same order of magnitude.The study for alge and for Daphnia was not provided with adjuwant. Taking into account higher toxicity for lemna in comparioson to the other group of organism app 1000 times the study with adjuwant might be sufficient.
The factor 10 of difference in the sensitivity as referred to in the EFSA GD on aquatic organisms justifies, that the studies on daphnia or lemna should not be carried out.

9.5.1.1 Justification for new endpoints

In general , the EU-agreed endpoints are used for the risk assessment on aquatic organisms for both active substances and their metabolites. For some metabolites, the endpoint of the parent was divided by 10 (the metabolite is assumed to be 10 times more toxic than the parent). This is considered a conservative approach and therefore acceptable.
For mesosulfuron-methyl, in addition to the 7-day endpoint for *Lemna gibba* further macrophyte endpoints have been generated. These studies were used to calculate a Species Sensitivity Distribution, from which a HC_5 value and refined RAC could be derived as described above.
For iodosulfuron-methyl-sodium, the risk was acceptable in first tier. However, as the higher tier study submitted for the renewal of the active substance results in a lower endpoint, this endpoint was included in the risk assessment as a conservative approach.
For the plant protection product, the lowest endpoint from the newly submitted studies was used for the risk assessment.

ZRMS comments:
Iodosulfuron-methyl-sodium
In opinion of the zRMS, EU agreed endpoints for the active substance should not be challenged during zonal or national evaluations, especially when they were recently agreed and selected with consideration of extent dataset.
The Applicant has not provided any new studies but challenged the EFSA conclusion. However, the first-Tier endpoint 0.74 µg iodosulfuron a.s./L derived from the study conducted with IMS+MPR OD400, which was the representative formulation in the Annex I Renewal process of iodosulfuron-methyl-sodium (containing, beside iodosulfu-

ron-methyl-sodium, the safener mefenpyr-diethyl) was considered in the first Tier evaluation by ZRMS.

For the higher Tier evaluation, in EFSA Review Report, 2016 the following information/conclusion is provided:
"A modified-exposure outdoor pond study, evaluating the effects of iodosulfuron-methyl-sodium on several macrophytes species, was available as a refinement during renewal process.
Originally, E_rC_{50} were derived from this study and then combined for building a SSD. This study was discussed during the Pesticide Peer Review meeting 139.
During the meeting it was agreed that no reliable ErC_{50} could be derived, as the biological measurements were only performed at the end of the study, when some recovery might already have taken place, given that most of the applied iodosulfuron-methyl-sodium was already degraded. Due to this concern, it was decided that the lowest NOEC (to be considered as a NOEAEC) observed in the study could be used in the risk assessment together with an assessment factor of 3. During the meeting it was also decided that the risk assessment could be performed in two different ways;
1) NOEAEC based on measured initial test concentration 0.27 µg a.s./L compared with FOCUS PECsw
or
2) geomean measured concentration 0.16 µg a.s./L compared with PECmax should be used for the risk assessment, along with an assessment factor of 3.

Therefore, in the opinion of ZRMS-PL the following endpoints can be considered in the risk assessment for a.s. - iodosulfuron-methyl-sodium

First Tier:
7 d E_rC_{50}=0.74 µg a.s./L with AF of 10 (laboratory study) → RAC = 0.074 µg a.s./L)

It is notable that the higher tier endpoints provided in the EFSA conclusion ends up to the slightly lower RAC-values than the first Tier RAC of 0.074 or 0.108 µg a.s./L:
NOEAEC = 0.27 µg a.s./L (measured initial concentration) with AF of 3 → **RAC = 0.09 µg a.s./L**
NOEAEC = 0.16 µg a.s./L (geomean measured concentration) with AF of 3 → RAC = **0.053 µg a.s./L**

However, the geomean approach with the the three valid Lemna laboratory studies in EFSA LoEP by Christ & Ruff (1997), Bruns (2013), and Dorgerloh (2004) from endpoints (7d E_rC_{50}) of 1.34, 1.08 and 0.74 µg a.s./L-
geomean 7 d E_rC_{50} = 1.02 µg a.s./L with AF of 10 (laboratory study) → **RAC = 0.102 µg a.s./L**
If only a.s. studies are included: the geomean 7 d E_rC_{50} of 1.34 and 1.08 would be 1.20 µg a.s./L and the **RAC = 0.12 µg/L**.
The geomean approach is in line with the recommendations of EFSA Aquatic Guidance Document, 2013.
The final endpoints may be decided at MSs level, if necessary.

9.5.2 Risk assessment

9.5.2.1 Iodosulfuron-methyl-sodium

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. The risk assessment on iodosulfuron-methyl-sodium is carried out for all proposed uses. For the metabolites, only the use resulting in the highest PEC_{SW} values (i.e. 1 x 3g a.s./ha in winter cereals and 1 x 1.8 g a.s./ha in spring cereals) is taken into account for the risk assessment, since it covers all other proposed uses.
In the following table, the ratios between predicted environmental concentrations in surface water bodies (PEC_{SW}, PEC_{SED}) and regulatory acceptable concentrations (RAC) for aquatic organisms are given per intended use for each FOCUS scenario and each organism group.

1. WINTER CEREALS – 3g a.s./ha

Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for iodosulfuron-methyl-sodium for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in winter cereals – 3 g a.s./gha

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. Acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**	**Higher-tier information**
Test species		***Oncorhyn-chus mykiss***	***Oncorhyn-chus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudo-kirchn. subcapitata***	***Lemna gibba***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 100000	NOEC 7790	EC_{50} > 100000	NOEC 7900	E_rC_{50}/E_yC_{50} 152	NOEC 1.08	NOEAEC 0.27
AF		100	10	100	10	10	10	3
RAC (µg/L)		> 1000	779	> 1000	790	15.2	0.108	0.09
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**							
Step 1								
	0.98	< 0.0010	0.0013	< 0.0010	0.0012	0.0645	**9.0741**	**10.8889**
Step 2								
NEU Oct-Feb	0.13	< 0.0001	0.0002	< 0.0001	0.0002	0.0086	**1.2037**	**1.4444**
NEU Mar-May	0.13	< 0.0001	0.0002	< 0.0001	0.0002	0.0086	**1.2037**	**1.4444**
SEU Oct-Feb	0.08	< 0.0001	0.0001	< 0.0001	0.0001	0.0053	0.7407	0.8889
SEU Mar-May	0.08	< 0.0001	0.0001	< 0.0001	0.0001	0.0053	0.7407	0.8889
Step 3								
D1/ Ditch	0.01965	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0013	0.1819	0.2183
D1/ Stream	0.01665	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0011	0.1542	0.1850
D2/ Ditch	0.15410	< 0.0002	0.0002	< 0.0002	0.0002	0.0101	1.4269	**1.7122**
D2/ Stream	0.09634	< 0.0001	0.0001	< 0.0001	0.0001	0.0063	0.8920	**1.0704**
D3/ Ditch	0.01898	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0012	0.1757	0.2109
D4/ Pond	0.00066	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0061	0.0073
D4/ Stream	0.01452	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0010	0.1344	0.1613
D5/ Pond	0.00066	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0061	0.0073
D5/ Stream	0.01502	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0010	0.1391	0.1669
D6/ Ditch	0.01902	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0013	0.1761	0.2113

Group		Fish acute	Fish pro-longed	Inverteb. Acute	Inverteb. prolonged	Algae	Higher plants	Higher-tier information
R1/ Pond	0.00066	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0061	0.0073
R1/ Stream	0.01387	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0009	0.1284	0.1541
R3/ Stream	0.04281	< 0.0001	0.0001	< 0.0001	0.0001	0.0028	0.3964	0.4757
R4/ Stream	0.02915	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0019	0.2699	0.3239

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 high-dose use in winter cereals, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms for the D2 scenario's. As the exceedance is caused by drainage, no further risk assessment is considered useful; However for member states where D2 is relevant, a national specific risk mitigation measure should be applied.

2. <u>WINTER CEREALS – 1.8g a.s./ha</u>

Table 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for iodosulfuron-methyl-sodium for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in winter cereals – 1.8 g a.s./ha

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. Acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**	**Higher-tier information**
Test species		***Oncorhyn-chus mykiss***	***Oncorhyn-chus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudo-kirchn. subcapitata***	***Lemna gibba***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 100000	NOEC 7790	EC_{50} > 100000	NOEC 7900	E_rC_{50}/E_yC_{50} 152	NOEC 1.08	NOEAEC 0.27
AF		100	10	100	10	10	10	3
RAC (µg/L)		> 1000	779	> 1000	790	15.2	0.108	0.09
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**							
Step 1								
	0.59	< 0.0006	0.0008	< 0.0006	0.0007	0.0388	**5.4630**	**6.5556**
Step 2								
NEU Oct-Feb	0.08	< 0.0001	0.0001	< 0.0001	0.0001	0.0053	0.7407	0.8889
NEU Mar-May	0.06	< 0.0001	0.0001	< 0.0001	0.0001	0.0039	0.5556	0.6667
SEU Oct-Feb	0.05	< 0.0001	0.0001	< 0.0001	0.0001	0.0033	0.4630	0.5556
SEU Mar-May	0.1	< 0.0001	0.0001	< 0.0001	0.0001	0.0066	0.9259	**1.1111**
Step 3								
D1/ Ditch	0.0118	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0008	0.1089	0.1307
D1 /stream	0.0100	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0007	0.0924	0.1108

Group		Fish acute	Fish pro-longed	Inverteb. Acute	Inverteb. prolonged	Algae	Higher plants	Higher-tier information
D2/ Ditch	0.0874	< 0.0001	0.0001	< 0.0001	0.0001	0.0058	0.8094	0.9713
D2/ Stream	0.0552	< 0.0001	0.0001	< 0.0001	0.0001	0.0036	0.5113	0.6136
D3/ Ditch	0.0114	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0007	0.1055	0.1266
D4/ Pond	0.0004	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0036	0.0044
D4/ Stream	0.0087	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0006	0.0806	0.0968
D5 /pond	0.0004	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0036	0.0044
D5/ Stream	0.0090	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0006	0.0834	0.1001
D6/ Ditch	0.0114	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0008	0.1056	0.1267
R1/ Pond	0.0004	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0036	0.0044
R1/ Stream	0.0081	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0005	0.0751	0.0901
R3/ Stream	0.0256	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0017	0.2370	0.2844
R4/ Stream	0.0176	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0012	0.1631	0.1957

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 low-dose use in winter cereals, calculated PEC/RAC ratios did not exceed the trigger of 1 for any of the scenario's. The risk to aquatic organisms is considered acceptable without the use of risk mitigation measures.

3. SPRING CEREALS – 1.8g a.s./ha

Table 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for iodosulfuron-methyl-sodium for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in spring cereals – 1.8 g a.s./ha

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**	**Higher-tier information**
Test species		***Oncorhyn-chus mykiss***	***Oncorhyn-chus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudo-kirchn. subcapitata***	***Lemna gibba***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 100000	NOEC 7790	EC_{50} > 100000	NOEC 7900	E_rC_{50}/E_yC_{50} 152	NOEC 1.08	NOEAEC 0.27
AF		100	10	100	10	10	10	3
RAC (µg/L)		> 1000	779	> 1000	790	15.2	0.108	0.09
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**							
Step 1								
	0.59	< 0.0006	0.0008	< 0.0006	0.0007	0.0388	**5.4630**	**6.5556**

Group		Fish acute	Fish pro-longed	Inverteb. acute	Inverteb. prolonged	Algae	Higher plants	Higher-tier information
Step 2								
NEU mar-may	0.08	< 0.0001	0.0001	< 0.0001	0.0001	0.0053	0.7407	0.8889
NEU jun-sep	0.08	< 0.0001	0.0001	< 0.0001	0.0001	0.0053	0.7407	0.8889
SEU mar-may	0.10	< 0.0001	0.0001	< 0.0001	0.0001	0.0066	0.9259	**1.1111**
SEU jun-sep	0.05	< 0.0001	0.0001	< 0.0001	0.0001	0.0033	0.4630	0.5556
Step 3								
D1/ Ditch	0.0115	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0008	0.1068	0.1281
D1/ Stream	0.0092	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0006	0.0854	0.1025
D3/ Ditch	0.0114	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0008	0.1056	0.1267
D4/ Pond	0.0004	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0036	0.0044
D4/ Stream	0.0090	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0006	0.0830	0.0996
D5/ Pond	0.0004	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0036	0.0044
D5/ Stream	0.0091	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0006	0.0839	0.1007
R4/ Stream	0.0075	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0005	0.0695	0.0834

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

PEC/RAC ratios for the use in spring cereals indicated an acceptable risk for all scenarios. No risk mitigation is considered necessary.

zRMS comments:

Iodosulfuron-methyl-sodium

In the risk assessment for aquatic macrophytes ZRMS-PL considered following endpoints for Lemna sp.: E_rC_{50} of 0.74 µg/L, of 0.16 µg/L (geomean.), E_rC_{50} of 1.2 µg/L, or 1.02 µg/L values (two last values - geometric mean values).
Justification of using endpoints by ZRMS is presented in the commentig box under Point (please see).
The risk assessment for a.s.- iodosulfuron-methyl-sodium is presented in the Table below.

Table $_{corr}$: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for iodosulfuron-methyl-sodium for aquatic macrophytes based on FOCUS Steps 1, 2, 3 calculations for the use of GLOB289H/SAP63H in cereals.

Group		Aquatic plants
Test species		***Lemna gibba***

Endpoint (**µg/L**)		**E_rC_{50}** **0.74***	**NOEAEC (Higher tier)** **0.16****	**Geomean E_rC50** **1.02*****	**Geomean E_rC_{50}** **1.20*****
AF		**10**	**3**	**10**	**10**
RAC (µg/L)		**0.074**	**0.053**	**0.10**	**0.12**
FOCUS Scenario	**$PEC_{gl\text{-}max}$ (µg/L)**				
Winter cereals – 3 g a.s./g ha					
NEU Oct-Feb	0.13	**1.76**	**2.45**	**1.3**	**1.08**
NEU Mar-May	0.13	**1.76**	**2.45**	**1.3**	**1.08**
SEU Oct-Feb	0.08	**1.08**	**1.51**	0.8	0.67
SEU Mar-May	0.08	1.08	**1.51**	0.8	0.67
STEP 3					
D1/ Ditch	0.01965	0.27	0.37	0.1965	0.16
D1/ Stream	0.01665	0.23	0.31	0.1665	0.14
D2/ Ditch	0.15410	**2.08**	**2.91**	**1.541**	**1.28**
D2/ Stream	0.09634	**1.30**	**1.82**	0.9634	0.80
D3/ Ditch	0.01898	0.26	0.36	0.1898	0.16
D4/ Pond	0.00066	0.01	0.01	0.0066	0.01
D4/ Stream	0.01452	0.20	0.27	0.1452	0.12
D5/ Pond	0.00066	0.01	0.01	0.0066	0.01
D5/ Stream	0.01502	0.20	0.28	0.1502	0.13
D6/ Ditch	0.01902	0.26	0.36	0.1902	0.16
R1/ Pond	0.00066	0.01	0.01	0.0066	0.01
R1/ Stream	0.01387	0.19	0.26	0.1387	0.12
R3/ Stream	0.04281	0.58	0.81	0.4281	0.36

R4/ Stream	0.02915	0.39	0.55	0.2915	0.24
Winter cereals – 1.8g a.s./ha					
NEU Oct-Feb	0.08	**1.08**	**1.51**	0.8	0.67
NEU Mar-May	0.06	0.81	**1.13**	0.6	0.50
SEU Oct-Feb	0.05	0.68	0.94	0.5	0.42
SEU Mar-May	0.1	**1.35**	**1.89**	**1**	0.83
STEP3					
D1/ Ditch	0.0118	0.16	0.22	0.118	0.10
D1 /stream	0.0100	0.14	0.19	0.1	0.08
D2/ Ditch	0.0874	1.18	**1.65**	0.874	0.73
D2/ Stream	0.0552	0.75	**1.04**	0.552	0.46
D3/ Ditch	0.0114	0.15	0.22	0.114	0.10
D4/ Pond	0.0004	0.01	0.01	0.004	0.00
D4/ Stream	0.0087	0.12	0.16	0.087	0.07
D5 /pond	0.0004	0.01	0.01	0.004	0.00
D5/ Stream	0.0090	0.12	0.17	0.09	0.08
D6/ Ditch	0.0114	0.15	0.22	0.114	0.10
R1/ Pond	0.0004	0.01	0.01	0.004	0.00
R1/ Stream	0.0081	0.11	0.15	0.081	0.07
R3/ Stream	0.0256	0.35	0.48	0.256	0.21
R4/ Stream	0.0176	0.24	0.33	0.176	0.15
Spring cereals – 1.8g a.s./ha					
NEU mar-may	0.08	**1.08**	**1.51**	0.8	0.67
NEU jun-sep	0.08	**1.08**	**1.51**	0.8	0.67

SEU mar-may	0.10	**1.35**	**1.89**	**1**	0.83
SEU jun-sep	0.05	0.68	0.94	0.5	0.42
STEP 3					
D1/ Ditch	0.0115	0.16	0.22	0.115	0.10
D1/ Stream	0.0092	0.12	0.17	0.092	0.08
D3/ Ditch	0.0114	0.15	0.22	0.114	0.10
D4/ Pond	0.0004	0.01	0.01	0.004	0.00
D4/ Stream	0.0090	0.12	0.17	0.09	0.08
D5/ Pond	0.0004	0.01	0.01	0.004	0.00
D5/ Stream	0.0091	0.12	0.17	0.091	0.08
R4/ Stream	0.0075	0.10	0.14	0.075	0.06

* the endpoint used in EU review report (2016). Study was done with IMS+MPR OD, which was the representative formulation in the Annex I Renewal process of iodosulfuron-methyl-sodium
** A modified outdoor exposure study, this RAC is used in the final risk assessment (EFSA Conclusion 2016)
*** Geomean approach

For aquatic plants PEC/RAC values were >1 at STEP 1-2 calculations and considering all toxicity end-points, indicating an unacceptable risk for Lemna sp.

However, taking into account the PECsw STEP 3 calculations the PEC/RAc ratio was below trigger value of 1 except the high-dose use (3 g a.s./ha) in winter cereals.
In this case the calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms for the D2 scenario's. As the exceedance is caused by drainage, no further risk assessment is considered useful.
However, for Member States where D2 is relevant, a national specific risk mitigation measure should be applied.

Metabolites of iodosulfuron-methyl-sodium

1. **WINTER CEREALS – 3g a.s./ha**

Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F075736 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in winter cereals – 3 g a.s./gha

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint		LC_{50}	NOEC	EC_{50}	ER_{10}	E_rC_{50}	NOEC

Group		Fish acute	Fish pro-longed	Inverteb. acute	Inverteb. prolonged	Algae	Higher plants
(µg/L)		> 10000*	779*	> 10000*	790*	15.2*	0.57
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	77.9	> 100	79	1.52	0.057
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	1.12	< 0.0112	0.0144	< 0.0112	0.0142	0.7368	**19.6491**
Step 2							
NEU Oct-Feb	0.31	< 0.0031	0.0040	< 0.0031	0.0039	0.2039	**5.4386**
NEU Mar-May	0.13	< 0.0013	0.0017	< 0.0013	0.0016	0.0855	**2.2807**
SEU Oct-Feb	0.25	< 0.0025	0.0032	< 0.0025	0.0032	0.1645	**4.3860**
SEU Mar-May	0.25	< 0.0025	0.0032	< 0.0025	0.0032	0.1645	**4.3860**
Step 3							
D1/ ditch	0.0282	< 0.0003	0.0004	< 0.0003	0.0004	0.0186	0.4951
D1/ stream	0.0229	< 0.0002	0.0003	< 0.0002	0.0003	0.0151	0.4021
D2/ ditch	0.1289	< 0.0013	0.0017	< 0.0013	0.0016	0.0848	**2.2614**
D2/ stream	0.1195	< 0.0012	0.0015	< 0.0012	0.0015	0.0786	**2.0965**
D3/ ditch	0.0043	< 0.0001	0.0001	< 0.0001	0.0001	0.0029	0.0762
D4/ pond	0.0101	< 0.0001	0.0001	< 0.0001	0.0001	0.0066	0.1763
D4/ stream	0.0053	< 0.0001	0.0001	< 0.0001	0.0001	0.0035	0.0928
D5/ pond	0.0020	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0013	0.0344
D5/ stream	0.0010	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0007	0.0182
D6/ ditch	0.0016	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0011	0.0287
R1/ pond	0.0003	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0002	0.0058
R1/ stream	0.0060	< 0.0001	0.0001	< 0.0001	0.0001	0.0040	0.1057
R3/ stream	0.0130	< 0.0001	0.0002	< 0.0001	0.0002	0.0086	0.2284
R4/ stream	0.0098	< 0.0001	0.0001	< 0.0001	0.0001	0.0064	0.1714

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios below the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than the parent

Table 9.5-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F145740 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in winter cereals – 3 g a.s./gha

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 779*	EC_{50} > 10000*	ER_{10} 790*	E_rC_{50} 15.2*	NOEC 10000
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	77.9	> 100	79	1.52	1000
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	0.20	< 0.0020	0.0026	< 0.0020	0.0025	0.1316	0.0002

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than the parent

Table 9.5-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F145741 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in winter cereals – 3 g a.s./gha

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. Acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 779*	EC_{50} > 10000*	ER_{10} 790*	E_rC_{50} 15.2*	NOEC 3840
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	77.9	> 100	79	1.52	384
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	0.15	< 0.0015	0.0019	< 0.0015	0.0019	0.0987	0.0004

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than the parent

Table 9.5-12: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE 0000119 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in winter cereals – 3 g a.s./gha

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 779*	EC_{50} > 10000*	ER_{10} 790*	E_rC_{50} 15.2*	NOEC 100000
AF		100	10	100	10	10	10

Group		Fish acute	Fish pro-longed	Inverteb. acute	Inverteb. prolonged	Algae	Higher plants
RAC (µg/L)		> 100	77.9	> 100	79	1.52	10000
FOCUS Scenario	PEC $_{gl-max}$ (µg/L)						
Step 1							
	0.14	< 0.0014	0.0018	< 0.0014	0.0018	0.0921	< 0.0001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than the parent

Table 9.5-13: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F161778 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in winter cereals – 3 g a.s./gha

Group		Fish acute	Fish pro-longed	Inverteb. acute	Inverteb. prolonged	Algae	Higher plants
Test species		*Oncorhynchus mykiss*	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Pseudokirchn. subcapitata*	*Lemna gibba*
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 779*	EC_{50} > 10000*	ER_{10} 790*	E_rC_{50} 15.2*	NOEC 28.1
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	77.9	> 100	79	1.52	2.81
FOCUS Scenario	PEC $_{gl-max}$ (µg/L)						
Step 1							
	0.11	< 0.0011	0.0014	< 0.0011	0.0014	0.0724	0.0391

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than the parent

Table 9.5-14: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for BCS-CW811253 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in winter cereals – 3 g a.s./gha

Group		Fish acute	Fish pro-longed	Inverteb. acute	Inverteb. prolonged	Algae	Higher plants
Test species		*Oncorhynchus mykiss*	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Pseudokirchn. subcapitata*	*Lemna gibba*
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 779*	EC_{50} > 10000*	ER_{10} 790*	E_rC_{50} 15.2*	NOEC 10000
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	77.9	> 100	79	1.52	1000
FOCUS Scenario	PEC $_{gl-max}$ (µg/L)						
Step 1							
	0.22	< 0.0022	0.0028	< < 0.0022	0.0028	0.1447	0.0002

AF: Assessment factor; PEC: Predicted environmental concentration
* Metabolite is considered 10 times more toxic than the parent

Table 9.5-15: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F059411 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in winter cereals – 3 g a.s./gha

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 779*	EC_{50} > 10000*	ER_{10} 790*	E_rC_{50} 15.2*	NOEC 100000
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	77.9	> 100	79	1.52	> 10000
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	0.17	< 0.0017	0.0022	< 0.0017	0.0022	0.1118	< 0.0001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than the parent

Table 9.5-16: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE 0014966 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in winter cereals – 3 g a.s./gha

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 779*	EC_{50} > 10000*	ER_{10} 790*	E_rC_{50} 15.2*	NOEC 575
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	77.9	> 100	79	1.52	57.5
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	0.17	< 0.0017	0.0022	< 0.0017	0.0022	0.1118	< 0.0001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than the parent

Table 9.5-17: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE 0043855 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in winter cereals – 3 g a.s./gha

Group		**Fish acute**	**Fish prolonged**	**Inverteb. Acute**	**Inverteb. pro-longed**	**Algae**	**Higher plants**
Test spe-cies		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 779*	EC_{50} > 10000*	ER_{10} 790*	E_rC_{50} 15.2*	NOEC 100000
AF		100	10	100	10	10	10

Group		Fish acute	Fish prolonged	Inverteb. Acute	Inverteb. prolonged	Algae	Higher plants
RAC (µg/L)		> 100	77.9	> 100	79	1.52	10000
FOCUS Scenario	PEC $_{gl\text{-}max}$ (µg/L)						
Step 1							
	0.08	< 0.0008	0.0010	< 0.0008	0.0010	0.0526	< 0.0001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than the parent

Table 9.5-18: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F159737 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in winter cereals – 3 g a.s./gha

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Higher plants
Test species		*Oncorhynchus mykiss*	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Pseudokirchn. subcapitata*	*Lemna gibba*
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 779*	EC_{50} > 10000*	ER_{10} 790*	E_rC_{50} 15.2*	NOEC 100000
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	77.9	> 100	79	1.52	10000
FOCUS Scenario	PEC $_{gl\text{-}max}$ (µg/L)						
Step 1							
	0.03	< 0.0003	0.0004	< 0.0003	0.0004	0.0197	< 0.0001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than the parent

Table 9.5-19: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F1234964 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in winter cereals – 3 g a.s./gha

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Higher plants
Test species		*Oncorhynchus mykiss*	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Pseudokirchn. subcapitata*	*Lemna gibba*
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 779*	EC_{50} > 10000*	ER_{10} 790*	E_rC_{50} 15.2*	NOEC 100000
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	77.9	> 100	79	1.52	10000
FOCUS Scenario	PEC $_{gl\text{-}max}$ (µg/L)						
Step 1							
	0.03	< 0.0003	0.0004	< 0.0003	0.0004	0.0197	< 0.0001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than the parent

Table 9.5-20: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F154781 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in winter cereals – 3 g a.s./gha

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. pro-longed**	**Algae**	**Higher plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 779*	EC_{50} > 10000*	ER_{10} 790*	E_rC_{50} 15.2	NOEC 10000
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	77.9	> 100	79	1.52	1000
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	0.02	< 0.0002	0.0003	< 0.0002	0.0003	0.0132	< 0.0001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than the parent

Table 9.5-21: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE 0002166 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in winter cereals – 3 g a.s./gha

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 779*	EC_{50} > 10000*	ER_{10} 790*	E_rC_{50} 15.2*	NOEC 575
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	77.9	> 100	79	1.52	57.5
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	0.34	< 0.0034	0.0044	< 0.0034	0.0043	0.2237	0.0059

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold* Metabolite is considered 10 times more toxic than the parent

For the high-dose use in winter cereals, calculated PEC/RAC ratios for metabolite AE F075736 did not indicate an acceptable risk for the most sensitive group of aquatic organisms for the D2 scenario's. As the exceedance is caused by drainage, no further risk assessment is considered useful; However for member states where D2 is relevant, a national specific risk mitigation measure should be applied. For all other metabolites, the PEC/RAC ratios did not exceed the trigger of 1, indicating an acceptable risk.

2. **WINTER CEREALS – 1.8 g a.s./ha**

The risk assessment for the use of GLOB289H in winter cereals at 0.3 kg/ha or 1.8 g iodosulfuron-methyl-sodium is covered by the risk assessment above, of 0.5 kg/ha or 3 g iodosulfuron-methyl-sodium.

3. **SPRING CEREALS – 1.8g a.s./ha**

Table 9.5-22: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F075736 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in spring cereals – 1.8 g a.s./gha

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 779*	EC_{50} > 10000*	ER_{10} 790*	E_rC_{50} 15.2*	NOEC 0.57
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	77.9	> 100	79	1.52	0.057
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	0.67	< 0.0067	0.0086	< 0.0067	0.0085	0.4408	**11.7544**
Step 2							
NEU mar-may	0.11	< 0.0011	0.0014	< 0.0011	0.0014	0.0727	**1.9298**
NEU jun-sep	0.15	< 0.0015	0.0019	< 0.0015	0.0019	0.0987	**2.6316**
SEU mar-may	0.15	< 0.0015	0.0019	< 0.0015	0.0019	0.0987	**2.6318**
SEU jun-sep	0.11	< 0.0011	0.0014	< 0.0011	0.0014	0.0724	**1.9298**
Step 3							
D1/ ditch	0.02707	< 0.0003	0.0003	< 0.0003	0.0003	0.0178	0.4749
D1/ stream	0.01741	< 0.0002	0.0002	< 0.0002	0.0002	0.0115	0.3054
D3/ ditch	0.003271	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0022	0.0574
D4/ pond	0.006464	< 0.0001	0.0001	< 0.0001	0.0001	0.0043	0.1134
D4/ stream	0.003266	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0021	0.0573
D5 /pond	0.001225	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0008	0.0215
D5 /stream	0.000627	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0004	0.0110
R4/ stream	0.000436	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0003	0.0076

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than the parent

Table 9.5-23: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F145740 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in spring cereals – 1.8 g a.s./gha

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 779*	EC_{50} > 10000*	ER_{10} 790*	E_rC_{50} 15.2*	NOEC 10000
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	77.9	> 100	79	1.52	1000
FOCUS Scenario	**PEC** $_{\text{gl-max}}$ **(µg/L)**						
Step 1							
	0.12	< 0.0012	0.0015	< 0.0012	0.0015	0.0789	0.0001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than the parent

Table 9.5-24: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F145741 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in spring cereals – 1.8 g a.s./gha

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 779*	EC_{50} > 10000*	ER_{10} 790*	E_rC_{50} 15.2*	NOEC 3840
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	77.9	> 100	79	1.52	384
FOCUS Scenario	**PEC** $_{\text{gl-max}}$ **(µg/L)**						
Step 1							
	0.09	< 0.0009	0.0012	< 0.0009	0.0011	0.0592	0.0002

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than the parent

Table 9.5-25: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE 0000119 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in spring cereals – 1.8 g a.s./gha

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**
Test species		***Oncorhyn-chus mykiss***	***Oncorhyn-chus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudo-kirchn. sub-capitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 779*	EC_{50} > 10000*	ER_{10} 790*	E_rC_{50} 15.2*	NOEC 100000
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	77.9	> 100	79	1.52	10000

Group		Fish acute	Fish pro-longed	Inverteb. acute	Inverteb. prolonged	Algae	Higher plants
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	0.08	< 0.0008	0.0010	< 0.0008	0.0010	0.0526	< 0.0001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than the parent

Table 9.5-26: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F161778 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in spring cereals – 1.8 g a.s./gha

Group		Fish acute	Fish pro-longed	Inverteb. acute	Inverteb. prolonged	Algae	Higher plants
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 779*	EC_{50} > 10000*	ER_{10} 790*	E_rC_{50} 15.2*	NOEC 28.1
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	77.9	> 100	79	1.52	2.81
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	0.07	< 0.0007	0.0009	< 0.0007	0.0009	0.0461	0.0249

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios below the relevant trigger of 1 are shown in bold; RAC: Regulatory acceptable concentration; PEC/RAC ratios below the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than the parent

Table 9.5-27: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for BCS-CW811253 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in spring cereals – 1.8 g a.s./gha

Group		Fish acute	Fish pro-longed	Inverteb. acute	Inverteb. prolonged	Algae	Higher plants
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 779*	EC_{50} > 10000*	ER_{10} 790*	E_rC_{50} 15.2*	NOEC > 10000
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	77.9	> 100	79	1.52	> 1000
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	0.13	< 0.0013	0.0017	< 0.0013	0.0016	0.0855	< 0.0001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than the parent

Table 9.5-28: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F059411 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in spring cereals – 1.8 g a.s./gha

Group		Fish acute	Fish pro-longed	Inverteb. acute	Inverteb. prolonged	Algae	Higher plants
Test species		*Oncorhynchus mykiss*	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Pseudokirchn. subcapitata*	*Lemna gibba*
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 779*	EC_{50} > 10000*	ER_{10} 790*	E_rC_{50} 15.2*	NOEC 100000
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	77.9	> 100	79	1.52	10000
FOCUS Scenario	PEC $_{gl-max}$ (µg/L)						
Step 1							
	0.10	< 0.0010	0.0013	< 0.0010	0.0013	0.0658	< 0.0001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than the parent

Table 9.5-29: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE 0014966 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in spring cereals – 1.8 g a.s./gha

Group		Fish acute	Fish pro-longed	Inverteb. acute	Inverteb. prolonged	Algae	Higher plants
Test species		*Oncorhynchus mykiss*	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Pseudokirchn. subcapitata*	*Lemna gibba*
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 779*	EC_{50} > 10000*	ER_{10} 790*	E_rC_{50} 15.2*	NOEC 575
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	77.9	> 100	79	1.52	57.5
FOCUS Scenario	PEC $_{gl-max}$ (µg/L)						
Step 1							
	0.07	< 0.0007	0.0009	< 0.0007	0.0009	0.0461	0.0012

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than the parent

Table 9.5-30: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE 0043855 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in spring cereals – 1.8 g a.s./gha

Group		Fish acute	Fish pro-longed	Inverteb. acute	Inverteb. prolonged	Algae	Higher plants
Test species		*Oncorhynchus mykiss*	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Pseudokirchn. subcapitata*	*Lemna gibba*
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 779*	EC_{50} > 10000*	ER_{10} 790*	E_rC_{50} 15.2*	NOEC 100
AF		100	10	100	10	10	10

Group		Fish acute	Fish pro-longed	Inverteb. acute	Inverteb. prolonged	Algae	Higher plants
RAC (µg/L)		> 100	77.9	> 100	79	1.52	10
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1							
	0.05	< 0.0005	0.0006	< 0.0005	0.0006	0.0329	< 0.0001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than the parent

Table 9.5-31: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F159737 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in spring cereals – 1.8 g a.s./gha

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 779*	EC_{50} > 10000*	ER_{10} 790*	E_rC_{50} 15.2*	NOEC 100000
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	77.9	> 100	79	1.52	10000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1							
	0.02	< 0.0002	0.0003	< 0.0002	0.0003	0.0132	< 0.0001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than the parent

Table 9.5-32: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F1234964 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in spring cereals – 1.8 g a.s./gha

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 779*	EC_{50} > 10000*	ER_{10} 790*	E_rC_{50} 15.2*	NOEC 100000
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	77.9	> 100	79	1.52	10000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1							
	0.02	< 0.0002	0.0003	< 0.0002	0.0003	0.0132	< 0.0001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than the parent

Table 9.5-33: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F154781 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in spring cereals – 1.8 g a.s./gha

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 779*	EC_{50} > 10000*	ER_{10} 790*	E_rC_{50} 15.2	NOEC > 10000
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	77.9	> 100	79	1.52	> 1000
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	0.01	< 0.0001	0.0001	< 0.0001	0.0001	0.0066	< 0.0001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold* Metabolite is considered 10 times more toxic than the parent

Table 9.5-34: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE 0002166 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in spring cereals – 1.8 g a.s./gha

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 779*	EC_{50} > 10000*	ER_{10} 790*	E_rC_{50} 15.2*	NOEC 575
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	77.9	> 100	79	1.52	57.5
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	0.21	< 0.0021	0.0027	< 0.0021	0.0027	0.1382	0.0037

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

PEC/RAC ratios for the use in spring cereals indicated an acceptable risk for all scenarios. No risk mitigation is considered necessary.

zRMS comments:

zRMS agrees with the calculations of the risk assessment for all metabolites provided in the Tables above.
PEC/RAC ratios for all proposed uses indicated an acceptable risk. No risk mitigation is considered necessary.

9.5.2.2 Mesosulfuron-methyl

The evaluation of the risk for aquatic and sediment-dwelling organisms was performed in accordance

with the recommendations of the "Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters in the context of Regulation (EC) No 1107/2009", as provided by the Commission Services (SANTE-2015-00080, 15 January 2015).
The relevant global maximum FOCUS Step 1, 2, 3 and 4 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the table below. The risk assessment mesosulfuron-methyl is carried out for 15 g a.s./ha in winter cereals and 9 g a.s./ha in both winter and spring cereals, covering for all uses requested. For the metabolites, only the use resulting in the highest PEC_{SW} values (i.e. 1 x 15 g a.s./ha in winter cereals and 1 x 9 g a.s./ha in spring cereals) is taken into account for the risk assessment, since it covers all other proposed uses.
In the following table, the ratios between predicted environmental concentrations in surface water bodies (PEC_{SW}, PEC_{SED}) and regulatory acceptable concentrations (RAC) for aquatic organisms are given per intended use for each FOCUS scenario and each organism group.

1. **WINTER CEREALS – 15g a.s./ha**

Table 9.5-35: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mesosulfuron-methyl for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in winter cereals – 15 g a.s./ha

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**	**Refinement**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudo-kirchn. subcapitata***	***Lemna gibba***	***Macrophyt SSD***
End-point		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}/E_yC_{50}	ER50 1.29 (1st tier)	HC_5
(µg/L)		> 100000	32000	> 100000	1800	> 290	NOEAEC 0.388 (higher tier)	0.93
AF		100	10	100	10	10	10 / 3	3
RAC (µg/L)		> 1000	3200	> 1000	180	> 29	0.129	0.31
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**							
Step 1								
	4.74	< 0.0047	0.0015	< 0.0047	0.0263	< 0.1634	**36.7442**	**15.2903**
Step 2								
NEU Oct-Feb	1.17	< 0.0012	0.0004	< 0.0012	0.0065	< 0.0403	**9.0698**	**3.7742**
NEU Mar-May	1.17	< 0.0012	0.0004	< 0.0012	0.0065	< 0.0403	**9.0698**	**3.7742**
SEU Oct-Feb	1.17	< 0.0012	0.0004	< 0.0012	0.0065	< 0.0403	**9.0698**	**3.7742**
SEU Mar-May	1.52	< 0.0015	0.0005	< 0.0015	0.0084	< 0.0524	**11.7829**	**4.9032**
Step 3								
D1/ ditch	0.2188	< 0.0002	0.0001	< 0.0002	0.0012	< 0.0075	**1.6961**	0.7058
D1/ stream	0.1411	< 0.0001	< 0.0001	< 0.0001	0.0008	< 0.0049	**1.0938**	0.4552

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**	**Refinement**
D2/ ditch	1.6020	< 0.0016	0.0005	< 0.0016	0.0089	< 0.0552	**12.4186**	**5.1677**
D2/ stream	1.0220	< 0.0010	0.0003	< 0.0010	0.0057	< 0.0352	**7.9225**	**3.2968**
D3/ ditch	0.0982	< 0.0001	< 0.0001	< 0.0001	0.0005	< 0.0034	0.7610	0.3167
D4/ pond	0.0412	< 0.0001	< 0.0001	< 0.0001	0.0002	< 0.0014	0.3190	0.1327
D4 /stream	0.0771	< 0.0001	< 0.0001	< 0.0001	0.0004	< 0.0027	0.5979	0.2488
D5 /pond	0.0198	< 0.0001	< 0.0001	< 0.0001	0.0001	< 0.0007	0.1531	0.0637
D5/ stream	0.0828	< 0.0001	< 0.0001	< 0.0001	0.0005	< 0.0029	0.6420	0.2672
D6/ ditch	0.1058	< 0.0001	< 0.0001	< 0.0001	0.0006	< 0.0036	0.8202	0.3413
R1/ pond	0.0063	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0002	0.0486	0.0202
R1/ stream	0.1008	< 0.0001	< 0.0001	< 0.0001	0.0006	< 0.0035	0.7814	0.3252
R3/ stream	0.3099	< 0.0003	0.0001	< 0.0003	0.0017	< 0.0107	**2.4023**	0.9997
R4/ stream	0.2646	< 0.0003	0.0001	< 0.0003	0.0015	< 0.0091	**2.0512**	0.8535

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

* As only a NOAEC and no ER50 could be derived from the mesocosm, a suggestion was made in the EFSA Conclusion to lower the AF.

Table 9.5- 37.~~36:~~ Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for mesosulfuron-methyl based on FOCUS Step 4 calculations and toxicity data for aquatic macrophyes with mitigation of spray drift and run-off for the use of GLOB289H in winter cereals – 15 g a.s./ha

~~Intended use~~		~~Winter cereals~~	
~~Active substance~~		~~Mesosulfuron-methyl~~	
~~Application rate (g/ha)~~		~~1 × 15~~	
~~Nozzle reduction~~	**~~No-spray buffer (m)~~**	~~10~~	~~20~~
	~~Vegetated filter strip (m)~~	~~10~~	~~20~~
~~None~~	~~R3/stream~~	~~0.1370~~	~~0.071~~
~~None~~	~~R4/stream~~	~~0.1203~~	-
~~RAC (µg/L)~~ ~~0.129~~		**~~PEC/RAC ratio~~**	
~~None~~	~~R3/stream~~	**~~1.0620~~**	~~0.5502~~
~~None~~	~~R4/stream~~	~~0.9326~~	-

~~RAC (µg/L)~~ ~~0.194~~		~~PEC/RAC ratio~~	
~~None~~	~~R3/stream~~	~~0.7062~~	~~-~~
~~None~~	~~R4/stream~~	~~0.6201~~	~~-~~

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

Intended use		Winter cereals	
Active substance		Mesosulfuron-methyl	
Application rate (g/ha)		1 × 15	
Nozzle reduction	**No-spray buffer (m)**	10	20
	Vegetated filter strip (m)	10	20
None	R3/stream	0.1370	0.071
None	R4/stream	0.1203	-
		STEP 3	
	D1	0.2188	
	D1	0.1411	
RAC (µg/L) 0.129		**PEC/RAC ratio**	
None	R3/stream	**1.0620**	0.5502
None	R4/stream	0.9326	-
RAC (µg/L) ~~0.194~~ 0.196*		**PEC/RAC ratio**	
None	R3/stream	0.70 ~~62~~	-
None	R4/stream	~~0.6201~~ 0.61	-
		PEC $_{STEP3}$/RAC ratio	
None	D1 ditch	**1.11**	
None	D1 stream	0.721	

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

For the high-dose use in winter cereals, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms in the D1, D2, R3 and R4 FOCUS Step 3 scenarios under Tier 1 assumptions.
As the exceedance in D2 is caused by drainage, no further risk assessment is considered useful. For member states where D2 is relevant, a national specific risk mitigation measure should be applied.
For the R3 and R4 scenario, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{sw} considering reduced exposure of surface water bodies and tier 1 data (ErC50 = 1.29 µg/L). Based on the refined risk assessment considering Tier 1 data, the risk is considered acceptable when a vegetative filter

strip of 20/10m is used, or other country-specific risk mitigation measures.

If new higher data is considered (RAC = 0.3 µg/L) just drainage mitigation measures will be proposed for member states where D2 is relevant.

2. **WINTER CEREALS – 9g a.s./ha**

Table 9.5-37: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mesosulfuron-methyl for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in winter cereals – 9 g a.s./ha

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. Prolonged**	**Algae**	**Higher plants**	**Higher-tier information**	**Higher-tier information**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***	***Lemna gibba***	***macrophyt SSD***
Endpoint (µg/L)		LC_{50} > 100000	NOEC 32000	EC_{50} > 100000	NOEC 1800	E_rC_{50}/E_yC_{50} > 290	NOEC 1.29	NOEAEC 0.388	HC_5 0.9
AF		100	10	100	10	10	10	3	3
RAC (µg/L)		> 1000	3200	> 1000	180	> 29	0.129	0.129	0.3
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**								
Step 1									
	2.85	< 0.0029	0.0009	< 0.0029	0.0158	< 0.0983	22.0930	14.6907	9.1935
Step 2									
NEU Oct-Feb	0.49	< 0.0005	0.0002	< 0.0005	0.0027	< 0.0169	3.7985	2.5258	1.5806
NEU Mar-May	0.49	< 0.0005	0.0002	< 0.0005	0.0027	< 0.0169	**3.7984**	**2.5258**	**1.5806**
SEU Oct-Feb	0.91	< 0.0009	0.0003	< 0.0009	0.0051	< 0.0314	**7.0543**	**4.6907**	**2.9355**
SEU Mar-May	0.91	< 0.0009	0.0003	< 0.0009	0.0051	< 0.0314	**7.0543**	**4.6907**	**2.9355**
Step 3									
D1/ ditch	0.1343	< 0.0001	< 0.0001	< 0.0001	0.0007	< 0.0046	**1.0411**	0.6923	0.4332
D1/ stream	0.0235	< 0.0001	< 0.0001	< 0.0001	0.0001	< 0.0008	0.1819	0.1210	0.0757
D2/ ditch	0.8823	< 0.0009	0.0003	< 0.0009	0.0049	< 0.0304	**6.8395**	**4.5479**	**2.8461**
D2/ stream	0.5709	< 0.0006	0.0002	< 0.0006	0.0032	< 0.0197	**4.4256**	**2.9428**	**1.8416**
D3/ ditch	0.0585	< 0.0001	< 0.0001	< 0.0001	0.0003	< 0.0020	0.4533	0.3014	0.1886
D4/ pond	0.0234	< 0.0001	< 0.0001	< 0.0001	0.0001	< 0.0008	0.1810	0.1204	0.0753
D4/ stream	0.0459	< 0.0001	< 0.0001	< 0.0001	0.0003	< 0.0016	0.3558	0.2366	0.1481

Group		Fish acute	Fish pro-longed	Inverteb. acute	Inverteb. Prolonged	Algae	Higher plants	Higher-tier infor-mation	Higher-tier infor-mation
D5/ pond	0.0115	< 0.0001	< 0.0001	< 0.0001	0.0001	< 0.0004	0.0895	0.0595	0.0372
D5/ stream	0.0496	< 0.0001	< 0.0001	< 0.0001	0.0003	< 0.0017	0.3842	0.2555	0.1599
D6/ ditch	0.0632	< 0.0001	< 0.0001	< 0.0001	0.0004	< 0.0022	0.4901	0.3259	0.2039
R1/ pond	0.0038	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0298	0.0198	0.0124
R1/ stream	0.0594	< 0.0001	< 0.0001	< 0.0001	0.0003	< 0.0020	0.4602	0.3060	0.1915
R3/ stream	0.1853	< 0.0002	0.0001	< 0.0002	0.0010	< 0.0064	**1.4364**	0.9552	0.5977
R4/ stream	0.1601	< 0.0002	0.0001	< 0.0002	0.0009	< 0.0055	**1.2411**	0.8253	0.5165

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

For the 0.48kg/ha use in winter cereals, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms in the D2 FOCUS Step 3 scenarios. As the exceedance is caused by drainage, further refinements are not considered useful.
For member states where D2 is relevant, national specific risk mitigation should be considered.

3. SPRING CEREALS – 9g a.s./ha

Table 9.5-38: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mesosulfuron-methyl for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in spring cereals – 9 g a.s./ha

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. Prolonged**	**Algae**	**Higher plants**	**Higher-tier in-formation**	**Higher-tier in-formation**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***	***Lemna gibba***	***Macrophyt SSD***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}/E_yC_{50}	NOEC	NOEAEC	HC_5
(µg/L)		> 100000	32000	> 100000	1800	> 290	1.29	0.388	0.9
AF		100	10	100	10	10	10	3	3
RAC (µg/L)		> 1000	3200	> 1000	180	> 29	0.129	0.129	0.3
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**								
Step 1									
	2.85	0.0029	0.0009	0.0029	0.0158	0.0983	**22.0930**	**14.6907**	**9.1935**
Step 2									
N-Europe mar-may	0.91	0.0009	0.0003	0.0009	0.0051	0.0314	**7.0543**	**4.6907**	**2.9355**
N-Europe jun-sep	0.70	0.0007	0.0002	0.0007	0.0039	0.0241	**5.4264**	**3.6082**	**2.2581**

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. Prolonged	Algae	Higher plants	Higher-tier information	Higher-tier information
S-Europe mar-may	0.49	0.0005	0.0002	0.0005	0.0027	0.0169	**3.7984**	**2.5258**	**1.5806**
S-Europe jun-sep	0.49	0.0005	0.0002	0.0005	0.0027	0.0169	**3.7984**	**2.5258**	**1.5806**
Step 3									
D1/ditch	0.1884	0.0002	0.0001	0.0002	0.0010	0.0065	**1.4605**	0.9711	0.6077
D1/stream	0.1564	0.0002	< 0.0001	0.0002	0.0009	0.0054	**1.2124**	0.8062	0.5045
D3/ditch	0.0590	0.0001	< 0.0001	0.0001	0.0003	0.0020	0.4572	0.3040	0.1903
D4/pond	0.0270	< 0.0001	< 0.0001	< 0.0001	0.0001	0.0009	0.2092	0.1391	0.0871
D4/stream	0.0480	< 0.0001	< 0.0001	< 0.0001	0.0003	0.0017	0.3717	0.2472	0.1547
D5/pond	0.0107	< 0.0001	< 0.0001	< 0.0001	0.0001	0.0004	0.0833	0.0554	0.0346
D5/stream	0.0489	< 0.0001	< 0.0001	< 0.0001	0.0003	0.0017	0.3790	0.2520	0.1577
R4/stream	0.0375	< 0.0001	< 0.0001	< 0.0001	0.0002	0.0013	0.2909	0.1935	0.1211

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

PEC/RAC ratios for the use in spring cereals indicated an acceptable risk for all scenarios. No risk mitigation is considered necessary.

ZRMS comments:

zRMS agrees with the risk assessment based on the Tier 1 study with RAC=0.129 µg a.s./L (for the most sensitive species) and FOCUS STEP 1-4 calculations for application winter cereals at rate 15 g a.s./L (covering the risk for application rate of 9 g a.s./ha) and for spring cereals.

The conclusion of the risk assessment was summarised below:

Tier 1 data (RAC =0.129 µg a.s./L)

The risk for winter cereals at rate 15 g a.s./ha is considered acceptable when:

- A vegetative filter strip of 10 m for **R3 scenario** and a vegetative buffer strip of 20 m for **R4 scenario** will be applied to surface water bodies
- For D1 scenarios – further refinement is required.
- For member states where D2 is relevant, a national specific risk mitigation measures should be applied.

The risk for winter cereals at rate 9 g a.s./ha is considered acceptable when:

- A vegetative filter strip of 10 m for **R3 scenario** and a vegetative buffer strip of 20 m for **R4 scenario** will be applied to surface water bodies
- For member states where D2 is relevant, a national specific risk mitigation measure should beapplied.

The risk for spring cereals at rate 9 g a.s./ha is considered acceptable except For D1 scenario – further refinement is required.

Refinment based on geomean approach Tier 2A (RAC=0.196 µg a.s./L))

Refinement of the Lemna Tier 1 based on the geomean of the growth rate endpoints (Tier 2A) based on

frond number from the Lemna EU-reviewed studies (1.71, 1.61 µg a.s./L) and the applicant's study (2.72 µg a.s./L). This is a justified approach as frond number is the primary measurement variable according to the OECD 221-guideline and the same measured variable should be used for a geomean calculation.
This results in a geomean ErC50 of 1.96 µg a.s./L. Using an AF of 10, this results in a RAC for risk assessment of 0.196 µg/L (Table 9.5-37).

Based on this approach the following risk mitigation measures can be concluded:

Winter cereals 15 g a.s./ha:

- A risk mitigation of 10m + DVP 10m for R3 and R4 scenarios.
- For D1 (stream) scenario the risk is acceptable with STEP 3. For D1 (ditch) further refinement is required)
- For member states where D2 is relevant, a national specific risk mitigation measure should beapplied

Winter cereals at rate 9 g a.s./ha

- A risk mitigation of 10m + DVP 10m for R3 and R4 scenarios
- For D1 scenarios the risk is considered acceptable with FOCUS Step 3.
- For member states where D2 is relevant, a national specific risk mitigation measure should beapplied

Spring cereals:

- Acceptable risk for all scenarios

Refinment based on geomean approach Tier 2A based on the value agreed at EU level (RAC=0.149 µg a.s./L)

During commenting period process furter refinement based on **Tier 2A based on the value agreed at EU level** was considered by the applicant

EU agreed ErC_{50} endpoints for Lemna were considered as a weight of evidence (i.e, 1.717-frond number, 1.29-frond area) the resulting **RAC of 0.149 µg a.s. /L** from the geomean approach (Tier 2A).
The following risk mitigation measures are proposed:

Winter cereals at rate 15 g a.s./ha:

- A risk mitigation of 10m + DVP 10m for R3 and R4 scenarios.
- For D1 (stream) scenario the risk is acceptable with STEP 3. For D1 (ditch further refinement is required)
- For member states where D2 is relevant, a national specific risk mitigation measures should be applied

Winter cereals at rate 9 g a.s./ha

- A risk mitigation of 10m + DVP 10m for R3 and R4 scenarios
- For D1 scenarios the risk is considered acceptable with FOCUS Step 3.
- For member states where D2 is relevant, a national specific risk mitigation measure should be applied

Spring cereals:

- For D1 scenarios the risk further needs further refinement

Refinment based on SSD approach :

The one of the refinement options was SSD approach with HC_5 of 0.90 µg a.s./L value for mesosulforon methyl obtained from the new data for the active substance .
The HC_5 of 0.90 µg a.s./L value was not considered by some MSs to use in the risk assessment.
Therefore, the applicant provided the justification of using the additional data for the a.s. and provided

the alternative approach which may be considered at MSs level, if relevant

Applicant's approach during commenting period process for using by MSs and their national level, if relevant:

Taking into account that the new active substance data submitted has been already assessed under Data Matching process resulting in a non-change of agreed EU endpoints of toxicological reference values, residue definitions or relevance of metabolites, the applicant proposes to use an SSD-approach (Tier 2B) using the Lemna Tier 1 endpoint from the EU-review together with the endpoints of the 9 additional aquatic species tested by the applicant according to "Guidance on tiered risk assessment for edge-of-field surface waters, (EFSA Journal 2013;11(7):3290).

The HC5 calculated can then be used to refine the Tier 1 Lemna endpoint from the EU-review. This SSD-approach leads to an HC5 of 0.83 µg/L. Using an AF of 3, as advised in the EFSA Journal 2013; 11(7):3290 for the risk assessment for primary producers, the RAC will be 0.28 µg a.s./L. This would result in an acceptable risk for aquatic organisms when respecting a risk mitigation measure of 10m + DVP 10m.

In conclusion:

The final decision of the refinement for scenarios D1, R3 and R4, D2 scenarios should be decided at MSs level.

Formulation GLOB389H/SAP63H:

For acute risk assessment of the formulation, PEC_{sw} based on drift events were calculated using the SWASH Drift Calculator tool in ditch, pond and stream surface waters. The resulting PEC_{sw} were used for comparison with the measured mixture toxicity for aquatic plants. The risk for aquatic invertebrates and algae is covered by the risk assessment for aquatic plants, as *lemna gibba* is by far the most sensitive species (more than factor 10 difference).
The PEC/RAC ratio is <1 for product if a 5m buffer zone is considered. An acceptable risk for the formulated product GLOB289H can be concluded based on the proposed use pattern, further consideration is not required.

Metabolites of mesosulfuron-methyl

WINTER CEREALS – 15g a.s./ha

Table 9.5-39: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for BCS-CV14885 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in winter cereals – 15 g a.s./ha

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**
Test species		***Oncorhyn-chus mykiss***	***Oncorhyn-chus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudo-kirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 3200*	EC_{50} > 10000*	NOEC 180*	E_rC_{50}/E_yC_{50} > 29*	NOEC 1.29**
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	320	> 100	18	> 2.9	0.129
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	1,05	< 0,0105	0,0033	0,0105	0,0583	0,3621	**8,1395**
Step 2							
NEU Oct-Feb	0.18	< 0.0018	0.0006	0.0018	0.0100	0.0621	**1.3953**
NEU Mar-May	0.18	< 0.0018	0.0006	0.0018	0.0100	0.0621	**1.3953**
SEU Oct-Feb	0.34	< 0.0034	0.0011	0.0034	0.0189	0.1172	**2.6357**
SEU Mar-May	0.26	< 0.0026	0.0008	0.0026	0.0144	0.0897	**2.0155**
Step 3							
D1/ditch	0.0533	< 0.0005	0.0002	< 0.0005	0.0030	< 0.0184	0.4129
D1/stream	0.0389	< 0.0004	0.0001	< 0.0004	0.0022	< 0.0134	0.3014
D2/ditch	0.0711	< 0.0007	0.0002	< 0.0007	0.0039	< 0.0245	0.5511
D2/stream	0.3156	< 0.0032	0.0010	< 0.0032	0.0175	< 0.1088	**2.4465**
D3/ditch	0.0632	< 0.0006	0.0002	< 0.0006	0.0035	< 0.0218	0.4899
D4/pond	0.1228	< 0.0012	0.0004	< 0.0012	0.0068	< 0.0423	0.9519
D4/stream	0.0526	< 0.0005	0.0002	< 0.0005	0.0029	< 0.0181	0.4075
D5/pond	0.0898	< 0.0009	0.0003	< 0.0009	0.0050	< 0.0310	0.6963
D5/stream	0.0346	< 0.0003	0.0001	< 0.0003	0.0019	< 0.0119	0.2684
D6/ditch	0.0191	< 0.0002	0.0001	< 0.0002	0.0011	< 0.0066	0.1478
R1/pond	0.0019	< 0.0001	< 0.0001	< 0.0001	0.0001	< 0.0006	0.0145
R1/stream	0.0028	< 0.0001	< 0.0001	< 0.0001	0.0002	< 0.0010	0.0215
R3/stream	0.0203	< 0.0002	0.0001	< 0.0002	0.0011	< 0.0070	0.1574
R4/stream	0.0092	< 0.0001	< 0.0001	< 0.0001	0.0005	< 0.0032	0.0715

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than the parent
** BCS-CV14885 has lost the toxophore and should be less toxic than the parent. Therefore, it is considered acceptable to use the parent endpoint.

Table 9.5-40: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mesosulfuron for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in winter cereals – 15 g a.s./ha

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. Acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 3200*	EC_{50} > 10000*	NOEC 180*	E_rC_{50}/E_yC_{50} > 29*	NOEC 110
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	320	> 100	18	> 2.9	11
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	0.95	< 0.0095	0.0030	< 0.0095	0.0528	< 0.3276	0.0864

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than parent

Table 9.5-41: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F160459 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in winter cereals – 15 g a.s./ha

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. Acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 3200*	EC_{50} > 10000*	NOEC 180*	E_rC_{50}/E_yC_{50} > 100000	NOEC 2600
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	320	> 100	18	> 10000	260
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	1.47	< 0.0147	0.0046	< 0.0147	0.0817	< 0.0001	0.0057

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than parent

Table 9.5-42: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F099095 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in winter cereals – 15 g a.s./ha

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. Acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 3200*	EC_{50} > 10000*	NOEC 180*	E_rC_{50}/E_yC_{50} > 29*	NOEC 100000
AF		100	10	100	10	10	10

Group		Fish acute	Fish pro-longed	Inverteb. Acute	Inverteb. prolonged	Algae	Higher plants
RAC (µg/L)		> 100	320	> 100	18	> 2.9	10000
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	0.41	< 0.0041	0.0013	< 0.0041	0.0228	< 0.1414	0.0000

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than parent

Table 9.5-43: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F092944 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in winter cereals – 15 g a.s./ha

Group		Fish acute	Fish pro-longed	Inverteb. Acute	Inverteb. prolonged	Algae	Higher plants
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 3200*	EC_{50} > 10000*	NOEC 180*	E_rC_{50}/E_yC_{50} > 29*	NOEC 100000
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	320	> 100	18	> 2.9	10000
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	0.15	< 0.0015	0.0005	< 0.0015	0.0083	< 0.0517	< 0.0001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than parent

Table 9.5-44: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F160460 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in winter cereals – 15 g a.s./ha

Group		Fish acute	Fish pro-longed	Inverteb. Acute	Inverteb. prolonged	Algae	Higher plants
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 3200*	EC_{50} > 10000*	NOEC 180*	E_rC_{50}/E_yC_{50} > 29*	NOEC 100000
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	320	> 100	18	> 2.9	10000
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	0.80	< 0.0080	0.0025	< 0.0080	0.0444	< 0.2759	0.0001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than parent

Table 9.5-45: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F147447 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in winter cereals – 15 g a.s./ha

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. Acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 3200*	EC_{50} > 10000*	NOEC 180*	E_rC_{50}/E_yC_{50} > 100000	NOEC 100000
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	320	> 100	18	> 10000	10000
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	0.51	< 0.0051	0.0016	< 0.0051	0.0283	< 0.0001	0.0001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than parent

Table 9.5-46: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F140584 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in winter cereals – 15 g a.s./ha

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. Acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 3200*	EC_{50} > 10000*	NOEC 180*	E_rC_{50}/E_yC_{50} > 29*	NOEC 100000
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	320	> 100	18	> 2.9	10000
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	0.29	< 0.0029	0.0009	< 0.0029	0.0161	< 0.1000	< 0.0001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than parent

Table 9.5-47: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for BCS-CO60720 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in winter cereals – 15 g a.s./ha

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. Acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 3200*	EC_{50} > 10000*	NOEC 180*	E_rC_{50}/E_yC_{50} > 29*	NOEC 11800
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	320	> 100	18	> 2.9	1180

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. Acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	0.54	< 0.0054	0.0017	< 0.0054	0.0300	< 0.1862	0.0005

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than parent

For the high-dose use in winter cereals, calculated PEC/RAC ratios for metabolite BCS-CV14885 did not indicate an acceptable risk for the most sensitive group of aquatic organisms for the D2 scenario's. As the exceedance is caused by drainage, no further risk assessment is considered useful; However for member states where D2 is relevant, a national specific risk mitigation measure should be applied.
For all other metabolites, the PEC/RAC ratios indicated an acceptable risk.

SPRING CEREALS – 9g a.s./ha

Table 9.5-48: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for BCS-CV14885 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in spring cereals – 9 g a.s./ha

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. pro-longed**	**Algae**	**Higher plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 3200*	EC_{50} > 10000*	NOEC 180*	E_rC_{50}/E_yC_{50} > 29*	NOEC 1.29**
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	320	> 100	18	> 2.9	0.129
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	0.63	< 0.0063	0.0020	< 0.0063	0.0350	< 0.2172	**4.8837**
Step 2							
N-Europe mar-may	0.16	< 0.0016	0.0005	< 0.0016	0.0089	< 0.0552	**1.2403**
N-Europe june-sep	0.20	< 0.0020	0.0006	< 0.0020	0.0111	< 0.0690	**1.5504**
S-Europe mar-may	0.20	< 0.0020	0.0006	< 0.0020	0.0111	< 0.0690	**1.5504**
S-Europe jun-sep	0.11	< 0.0011	0.0003	< 0.0011	0.0061	< 0.0379	0.8527
Step 3							
D1/ditch	0.0244	< 0.0002	0.0001	< 0.0002	0.0014	< 0.0084	0.1888
D1/stream	0.0270	< 0.0003	0.0001	< 0.0003	0.0015	< 0.0093	0.2096
D3/ditch	0.0413	< 0.0004	0.0001	< 0.0004	0.0023	< 0.0143	0.3204
D4/pond	0.0808	< 0.0008	0.0003	< 0.0008	0.0045	< 0.0278	0.6260
D4/stream	0.0322	< 0.0003	0.0001	< 0.0003	0.0018	< 0.0111	0.2496

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. pro-longed	Algae	Higher plants
D5/pond	0.0536	< 0.0005	0.0002	< 0.0005	0.0030	< 0.0185	0.4157
D5/stream	0.0204	< 0.0002	0.0001	< 0.0002	0.0011	< 0.0070	0.1582
R4/stream	0.0008	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0003	0.0059

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than parent
** BCS-CV14885 has lost the toxophore and should be less toxic than the parent. Therefore, it is considered acceptable to use the parent endpoint.

Table 9.5-49: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mesosulfuron for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in spring cereals – 9 g a.s./ha

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. Acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 3200*	EC_{50} > 10000*	NOEC 180*	E_rC_{50}/E_yC_{50} > 29*	ErC50 110
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	320	> 100	18	> 2.9	11
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	0.57	< 0.0057	0.0018	< 0.0057	0.0317	< 0.1966	0.0518

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than parent

Table 9.5-50: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F160459 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in spring cereals – 9 g a.s./ha

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. Acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**
Test species		***Oncorhyn-chus mykiss***	***Oncorhyn-chus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudo-kirchn. sub-capitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 3200*	EC_{50} > 10000*	NOEC 180*	E_rC_{50}/E_yC_{50} > 100000	NOEC 2600
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	320	> 100	18	> 10000	260
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	0.88	< 0.0088	0.0028	< 0.0088	0.0489	< 0.0001	0.0034

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than parent

Table 9.5-51: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F099095 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in spring cereals – 9 g a.s./ha

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. Acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. Subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 3200*	EC_{50} > 10000*	NOEC 180*	E_rC_{50}/E_yC_{50} > 29*	NOEC > 100000
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	320	> 100	18	> 2.9	> 10000
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	0.25	< 0.0025	0.0008	< 0.0025	0.0139	< 0.0862	< 0.0001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than parent

Table 9.5-52: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F092944 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in spring cereals – 9 g a.s./ha

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. Acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 3200	EC_{50} > 10000*	NOEC 24900	E_rC_{50}/E_yC_{50} > 29*	NOEC > 100000
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	320	> 100	2490	> 2.9	> 10000
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	0.09	< 0.0009	0.0003	< 0.0009	0.0050	< 0.0310	< 0.0001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than parent

Table 9.5-53: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F160460 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in spring cereals – 9 g a.s./ha

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 3200*	EC_{50} > 10000*	NOEC 180*	E_rC_{50}/E_yC_{50} > 29*	NOEC > 100000
AF		100	10	100	10	10	10

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Higher plants
RAC (µg/L)		> 100	320	> 100	18	> 33.9	> 10000
FOCUS Scenario	PEC $_{gl\text{-}max}$ (µg/L)						
Step 1							
	0.48	< 0.0048	0.0015	< 0.0048	0.0267	< 0.1655	< 0.0001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than parent

Table 9.5-54: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F147447 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in spring cereals – 9 g a.s./ha

Group		Fish acute	Fish pro-longed	Inverteb. acute	Inverteb. prolonged	Algae	Higher plants
Test species		*Oncorhynchus mykiss*	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Pseudokirchn. subcapitata*	*Lemna gibba*
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 3200*	EC_{50} > 10000*	NOEC 180*	E_rC_{50}/E_yC_{50} > 100000	NOEC 100000
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	320	> 100	18	> 10000	10000
FOCUS Scenario	PEC $_{gl\text{-}max}$ (µg/L)						
Step 1							
	0.29	< 0.0029	0.0009	< 0.0029	0.0161	< 0.0001	< 0.0001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than parent

Table 9.5-55: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F140584 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in spring cereals – 9 g a.s./ha

Group		Fish acute	Fish pro-longed	Inverteb. acute	Inverteb. prolonged	Algae	Higher plants
Test species		*Oncorhynchus mykiss*	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Pseudokirchn. subcapitata*	*Lemna gibba*
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 3200*	EC_{50} > 10000*	NOEC 180*	E_rC_{50}/E_yC_{50} > 29*	NOEC > 100000
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	320	> 100	18	> 2.9	> 10000
FOCUS Scenario	PEC $_{gl\text{-}max}$ (µg/L)						
Step 1							
	0.17	< 0.0017	0.0005	< 0.0017	0.0094	< 0.0586	< 0.0001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than parent

Table 9.5-56: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for BCS-CO60720 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in spring cereals – 9 g a.s./ha

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. Prolonged**	**Algae**	**Higher plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magn***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 10000*	NOEC 3200*	EC_{50} > 10000*	NOEC 180*	E_rC_{50}/E_yC_{50} > 29*	NOEC > 11800
AF		100	10	100	10	10	10
RAC (µg/L)		> 100	320	> 100	18	> 2.9	> 1180
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	0.33	< 0.0033	0.0010	< 0.0033	0.0183	< 0.1138	< 0.0003

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Metabolite is considered 10 times more toxic than parent

PEC/RAC ratios for the use in spring cereals indicated an acceptable risk for all scenarios for all metabolites. No risk mitigation is considered necessary.

9.5.2.3 Mefenpyr-diethyl

The evaluation of the risk for aquatic and sediment-dwelling organisms was performed in accordance with the recommendations of the "Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters in the context of Regulation (EC) No 1107/2009", as provided by the Commission Services (SANTE-2015-00080, 15 January 2015).
The relevant global maximum FOCUS Step 1, 2, 3 and 4 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the table below. The risk assessment mefenpyr-diethyl is carried out for the use resulting in the highest PEC_{SW} values (i.e. 1 x 15 g a.s./ha in winter cereals and 1 x 9 g a.s./ha in spring cereals), since it covers all other proposed uses.
In the following table, the ratios between predicted environmental concentrations in surface water bodies (PEC_{SW}, PEC_{SED}) and regulatory acceptable concentrations (RAC) for aquatic organisms are given per intended use for each FOCUS scenario and each organism group.

1. **<u>WINTER CEREALS – 45g a.s./ha</u>**

Table 9.5-57: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mefenpyr-diethyl for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in winter cereals – 45 g a.s./gha

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. Acute**	**Inverteb. prolonged**	**Algae**	**Higher plants**
Test species		***Cyprinus carpio***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Navicula pelliculosa***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} 2400	NOEC 100	EC_{50} 5500	NOEC 320	E_rC_{50}/E_yC_{50} 1390	NOEC > 7600
AF		100	10	100	10	10	10

Group		Fish acute	Fish pro-longed	Inverteb. Acute	Inverteb. prolonged	Algae	Higher plants
RAC (µg/L)		24	10	55	32	139	> 760
FOCUS Scenario	PEC $_{gl-max}$ (µg/L)						
Step 1							
	8.69	0.3621	0.8690	0.1580	0.2716	0.0625	< 0.0114

PEC/RAC ratios for the use in winter cereals indicated an acceptable risk for all scenarios for mefenpyr-diethyl. No risk mitigation is considered necessary.

2. **SPRING CEREALS – 27 g a.s./ha**

Table 9.5-58: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mefenpyr-diethy for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB289H in spring cereals – 27 g a.s./gha

Group		Fish acute	Fish pro-longed	Inverteb. Acute	Inverteb. prolonged	Algae	Higher plants
Test species		*Cyprinus carpio*	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Navicula pelliculosa*	*Lemna gibba*
Endpoint (µg/L)		LC_{50} 2400	NOEC 100	EC_{50} 5500	NOEC 320	E_rC_{50}/E_yC_{50} 1390	NOEC > 7600
AF		100	10	100	10	10	10
RAC (µg/L)		21	10	55	32	139	> 760
FOCUS Scenario	PEC $_{gl-max}$ (µg/L)						
Step 1							
	5.21	0.2171	0.5210	0.0947	0.1628	0.0375	< 0.0069

PEC/RAC ratios for the use in spring cereals indicated an acceptable risk for all scenarios for mefenpyr-diethyl. No risk mitigation is considered necessary.

Metabolites of mefenpyr-diethyl

1. **WINTER CEREALS – 45 g a.s./ha**

Table 9.5-59: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F113225 for each organism group based on FOCUS Step calculations for the use of GLOB289H in winter cereals – 45 g a.s./gha

Group		Fish acute	Fish prolonged	Inverteb. Acute	Inverteb. pro-longed	Algae
Test species		*Lepomis mcro-chirus*	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Pseudokirchneri-ella subcapitata*
Endpoint (µg/L)		LC_{50} 100000	NOEC 32000	EC_{50} > 100000	NOEC 3200	E_rC_{50}/E_yC_{50} > 100000
AF		100	10	100	10	10
RAC (µg/L)		1000	3200	> 1000	320	> 10000
FOCUS Scenario	PEC $_{gl-max}$ (µg/L)					

Step 1						
	15.68	0.0157	0.0049	< 0.0157	0.0490	< 0.0016

Table 9.5-60: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F094270 for each organism group based on FOCUS Step calculations for the use of GLOB289H in winter cereals – 45 g a.s./gha

Group		**Fish acute**	**Fish prolonged**	**Inverteb. Acute**	**Inverteb. prolonged**	**Algae**
Test species		***Danio rerio***	***Danio rerio***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchneriella subcapitata***
Endpoint (µg/L)		LC_{50} > 72000	NOEC 3200	EC_{50} > 60300	NOEC 32000	E_rC_{50}/E_yC_{50} 30800
AF		100	10	100	10	10
RAC (µg/L)		> 720	320	> 603	3200	3800
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**					
Step 1						
	12.05	< 0.0167	0.0377	< 0.0200	0.0038	0.0039

Table 9.5-61: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F109453 for each organism group based on FOCUS Step calculations for the use of GLOB289H in winter cereals – 45 g a.s./gha

Group		**Fish acute**	**Inverteb. Acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Pseudokirchneriella subcapitata***
Endpoint (µg/L)		LC_{50} > 100000	EC_{50} > 100000	NOEC 41900
AF		100	100	10
RAC (µg/L)		> 1000	> 1000	4190
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**			
Step 1				
	18.83	< 0.0188	< 0.0188	0.0045

PEC/RAC ratios for the use in winter cereals indicated an acceptable risk for all scenarios for all mefenpyr-diethyl metabolites. No risk mitigation is considered necessary.

2. <u>SPRING CEREALS – 27 g a.s./ha</u>

Table 9.5-62: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F113225 for each organism group based on FOCUS Step calculations for the use of GLOB289H in spring cereals – 27 g a.s./gha

Group		**Fish acute**	**Fish prolonged**	**Inverteb. Acute**	**Inverteb. prolonged**	**Algae**
Test species		***Lepomis mcrochirus***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchneriella subcapitata***

Endpoint (µg/L)		LC_{50} 100000	NOEC 32000	EC_{50} > 100000	NOEC 3200	E_rC_{50}/E_yC_{50} > 100000
AF		100	10	100	10	10
RAC (µg/L)		1000	3200	> 1000	320	> 10000
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**					
Step 1						
	9.41	0.0094	0.0029	< 0.0094	0.0294	< 0.0009

Table 9.5-63: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F094270 for each organism group based on FOCUS Step calculations for the use of GLOB289H in winter cereals – 45 g a.s./gha

Group		**Fish acute**	**Fish prolonged**	**Inverteb. Acute**	**Inverteb. prolonged**	**Algae**
Test species		***Danio rerio***	***Danio rerio***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchneriella subcapitata***
Endpoint (µg/L)		LC_{50} > 72000	NOEC 3200	EC_{50} > 60300	NOEC 32000	E_rC_{50}/E_yC_{50} 30800
AF		100	10	100	10	10
RAC (µg/L)		> 720	320	> 603	3200	380
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**					
Step 1						
	7.23	< 0.0100	0.0226	< 0.0120	0.0023	0.0023

Table 9.5-64: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE F109453 for each organism group based on FOCUS Step calculations for the use of GLOB289H in winter cereals – 45 g a.s./gha

Group		**Fish acute**	**Inverteb. Acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Pseudokirchneriella subcapitata***
Endpoint (µg/L)		LC_{50} > 100000	EC_{50} > 100000	NOEC 41900
AF		100	100	10
RAC (µg/L)		> 1000	> 1000	4190
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**			
Step 1				
	11.3	< 0.0113	< 0.0113	0.0027

PEC/RAC ratios for the use in spring cereals indicated an acceptable risk for all scenarios for all mefenpyr-diethyl metabolites. No risk mitigation is considered necessary.

Table 9.5-66: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AE 2211046 for each organism group based on FOCUS Step calculations for the use of GLOB289H in winter cereals – 45 g a.s./gha

Group		*Fish acute*	*Fish Long-term*	*Inverteb. Acute*	*Inverteb. Chronic*	*Algae*	*Aquatic macrophytes*
Test species		*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Daphnia magna*	*Pseudokirchneriella subcapitata*	*Lemna sp.*
Endpoint (µg/L)		LC_{50} >240	NOEC 10	EC_{50} 55000	NOEC 32	NOEC 139	ErC_{50} 760
AF		100	10	100	10	10	10
RAC (µg/L)		2.4	1	55	3.2	13.9	76
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	0.46	0.19	0.46	0.0083	0.143	0.033	0.0060

PEC/RAC ratios for the use in spring cereals indicated an acceptable risk for all scenarios for all mefenpyr-diethyl metabolites. No risk mitigation is considered necessary.

9.5.2.4 Mixture toxicity

In the studies on the formulated product, aquatic plants by far proved to be the most sensitive group of organisms (see Table 9.5-5). For this reason, calculation of the theoretical mixture toxicity was confined to the most sensitive species *Lemna gibba*, using following formula of the concentration addition model (CA model):

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

where:

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

Measured toxicity data are available for both the active substances and the formulation. The plausibility of the measured formulation toxicity is checked against the calculated mixture toxicity by means of the model deviation ratio (MDR = ECx_{mix-ca} / ECx_{ppp}).
If MDR = 0.2 – 5, CA approximately holds for the mixture
If MDR > 5, the mixture is more toxic than CA
If MDR < 0.2, the mixture is less toxic than CA

The calculation is presented in the table below:

Table 9.5-65: Aquatic organisms – comparison of the calculated mixture toxicity and measured formulation toxicity.

Test substance	[Xa.s.] in formulation (g/kg)	p_i	ECx (µg a.s./L)	p_i / ECx	ECx_{MIX-CA} (µg/L)	ECxppp (µg/L)	MDR
Iodosulfuron-methyl-sodium	6	0.048	1.08	0,0444	4,3662	18.08	0.24
Mesosulfuron-methyl	30	0.238	1.29	0,1845			
Mefenpyr-diethyl	90	0.714	> 7600	0,0001			
Total	126	1	-	0,2290			

The lowest measured formulation toxicity for *Lemna gibba* was 18.08 µg/L, observed in the study where *Lemna gibba* was exposed to GLOB289H + Pottok (see Table 9.5-5). Dividing the calculated mixture toxicity of 4.3662 µg/L by the measured formulation toxicity of 18.08 µg/L results in an MDR between 0.2 and 0.5, which means the CA approximately holds for the mixture and hence toxicity can be predicted from the toxicities of the constituents. In conclusion, the risk assessment conducted with the a.s. end-points is considered adequate and conservative.

For completeness,theoretical mixture toxicity and MDR were also calculated for aquatic invertebrates and algae, presented in the tables below. .

Table 9.5-66: Aquatic invertebrates – comparison of the calculated mixture toxicity and measured formulation toxicity.

Test substance	[Xa.s.] in formulation (g/kg)	p_i	ECx (µg a.s./L)	p_i / ECx	ECx_{MIX-CA} (µg/L)	ECxppp (µg/L)	MDR
Iodosulfuron-methyl-sodium	6	0.048	> 100000	0,0000	100000	**21970**	4.55
Mesosulfuron-methyl	30	0.238	> 100000	0,0000			
Mefenpyr-diethyl	90	0.714	> 100000	0,0000			
Total	126	1	-	0,0000			

Table 9.5-67: Algae – comparison of the calculated mixture toxicity and measured formulation toxicity.

Test substance	[Xa.s.] in formulation (g/kg)	p_i	ECx (µg a.s./L)	p_i / ECx	ECx_{MIX-CA} (µg/L)	ECxppp (µg/L)	MDR
Iodosulfuron-methyl-sodium	6	0.048	152	0.0003	**876.08**	17950	0.05
Mesosulfuron-methyl	30	0.238	290	0.0008			
Mefenpyr-diethyl	90	0.714	> 100000	0.0000			
Total	126	1	-	0.0011			

For aquatic invertebrates, the MDR is between 0.2 and 5. In this case, the observed and calculated mixture toxicity is considered in agreement and it is advised to use the measured mixture toxicity (ECx_{ppp}) in

the RA.
For algae, the MDR is < 0.2, wich indicates a less-than additive effect. No plausible toxicological explanation for this apparent antagonism can be provided, therefore it is advised to perform the RA using the calculated mixture toxicity (ECx_{mix-CA}).

For acute risk assessment of the formulation, PEC_{sw} based on drift events were calculated using the SWASH Drift Calculator tool in ditch, pond and stream surface waters. The resulting PEC_{sw} were used for comparison with the measured mixture toxicity for aquatic plants. The risk for aquatic invertebrates and algae is covered by the risk assessment for aquatic plants, as *lemna gibba* is by far the most sensitive species (more than factor 10 difference)

Table 9.5-68: Aquatic organisms: acceptability of risk (PEC/RAC ≤1) for GLOB289H for each organism group based on FOCUS drift Swash tool calculations (no mitigation measures)

GLOB289H		
Group		**Aquatic plants**
Test species		*L. gibba*
Endpoint (µg/L)		EC_{50}
		18.08
AF		10
RAC (µg/L)		1.8
FOCUS Scenario Drift SWASH calculator	**PEC_{max} (µg/L)**	
	Winter cereals 0.5 L/ha – no buffer zone	
Ditch	3.2123	1.78
Pond	0.1095	0.06
Stream*	2.8607	1.32
	Winter / spring cereals (0.3 L/ha) – no buffer zone	
Ditch	1.9274	1.07
Pond	0.0657	0.04
Stream*	1.7165	0.95

The PEC/RAC ratio is >1 for the Ditch and Stream scenario. No acceptable risk for the formulated product GLOB289H can be concluded based on the proposed use pattern, further consideration is required.

To refine the risk, a 5m buffer zone was considered. The results of the corresponding risk assessment are presented below.

Table 9.5-69: Aquatic organisms: acceptability of risk (PEC/RAC ≤1) for GLOB289H for each organism group based on FOCUS drift Swash tool calculations (with mitigation measures)

GLOB289H		
Group		**Aquatic plants**
Test species		*L. gibba*
Endpoint (µg/L)		EC_{50}
		18.08
AF		10
RAC (µg/L)		1.8
FOCUS Scenario Drift SWASH calculator	**PEC_{max} (µg/L)**	
	Winter cereals 0.5 L/ha – 5m buffer zone	
Ditch	0.8707	0.48
Pond	0.0948	0.05
Stream*	1.04484	0.58

	Winter / spring cereals (0.3 L/ha) – 5m buffer zone	
Ditch	0.5224	0.29
Pond	0.0569	0.03
Stream*	0.62688	0.35

The PEC/RAC ratio is <1 for all scenarios if a 5m buffer zone is considered. An acceptable risk for the formulated product GLOB289H can be concluded based on the proposed use pattern, further consideration is not required.

Although a buffer zone of 5m is considered necessary based on the risk assessment for the formulation, several studies are available on the active substances to refine the risk for aquatic plants, showing an acceptable risk without risk mitigation.

zRMS comment:

The mixture toxicity assessment provided by the applicant is considered as acceptable.

In addition, the PEC/RAC ratio is <1 for product if a 5m buffer zone is considered. An acceptable risk for the formulated product GLOB289H can be concluded based on the proposed use pattern, further consideration is not required.

9.5.2.5 Mixture toxicity provided during Commenting period process:

In the studies on the formulated product, aquatic plants by far proved to be the most sensitive group of organisms (see Table 9.5-5). For this reason, calculation of the theoretical mixture toxicity was confined to the most sensitive species *Lemna gibba*, using following formula of the concentration addition model (CA model):

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

where:

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

Measured toxicity data are available for both the active substances and the formulation. The plausibility of the measured formulation toxicity is checked against the calculated mixture toxicity by means of the model deviation ratio (MDR = ECx_{mix-ca} / ECx_{ppp}).

If MDR = 0.2 – 5, CA approximately holds for the mixture
If MDR > 5, the mixture is more toxic than CA
If MDR < 0.2, the mixture is less toxic than CA

The calculations are presented in the Tables below with consideration diffrent results for product for Lemna sp. from formulation studies such as E_rC_{50}=18.08 µg product/L (PPP +Pottock) and E_rC_{50} = 41.27 µg product./L (PPP+ Actirob).

1) Mixture toxicity assessment with E_rC_{50}=18.08 µg product /L for Lemna sp.

Product data	
Product name	
Density of product [g/cm^3]	1
LC_{50}fish [mg prod./L]	0
LC_{50}fish a.s. based [mg sum of a.s/L]	0,0000
EC_{50}invertrebrates [mg prod./L]	21,97
LC_{50}invertrebrates a.s. based [mg sum of a.s/L]	2,7682
EC_{50}algae [mg prod./L]	17,95
EC_{50}algae a.s. based [mg sum of a.s/L]	2,2617
EC_{50}macrophytes [mg prod./L]	18,08
EC_{50}macrophytes a.s. based [mg sum of a.s/L]	2,2781

Calculated mixture toxicity (Eq. 13) based on Tier 1 data only	[mg/L]
$ECx_{mix\text{-}Ca}$fish	3,328050713
$ECx_{mix\text{-}Ca}$invertrebrates	7,534246575
$ECx_{mix\text{-}Ca}$algae	0,606731362
$ECx_{mix\text{-}Ca}$macrophytes	0,004371477

Calculated mixture toxicity (Eq. 13) based also on additional data	[mg/L]
$ECx_{mix\text{-}Ca}$fish	3,328050713
$ECx_{mix\text{-}Ca}$invertrebrates	7,534246575
$ECx_{mix\text{-}Ca}$algae	0,606731362
$ECx_{mix\text{-}Ca}$macrophytes	0,022497063

Options

Show Species

Unlock sum of a.s. for product data

Active Substance (a.s.) standard data (Tier 1 EP)				
Active substance names	Iodo	Meso	Mefenpyr	
Concentration in Product [g a.s./L or g a.s./kg]	6	30	90	
p(X) (fraction in product)	0,05	0,24	0,71	
LC_{50}fish [mg a.s./L]	100	100	2,4	
LC_{50}invertrebrates [mg a.s./L]	100	100	5,5	
EC_{50}algae [mg a.s./L]	0,152	0,29	1,39	
EC_{50}macrophytes [mg a.s./L]	0,00108	0,00129	7,6	
Additional a.s. data (i.e. most sensitive species tested as Tier 1 data or refinements Tier 2A/B EP)				
LC_{50}fish [mg a.s./L]				
LC_{50}invertrebrates [mg a.s./L]				
EC_{50}algae [mg a.s./L]				
EC_{50}macrophytes [mg a.s./L]		0,9		
AF for RAC				
Fish	100	100	100	100
Invertrebrates	100	100	100	100
Algae	10	10	10	10
Macrophytes	10	3	10	10
RAC				
Fish	1	1	0,024	
Invertrebrates	1	1	0,055	
Algae	0,0152	0,029	0,139	
Macrophytes	0,000108	0,3	0,76	

Data used for calculation (after Step 3)				
Active substances	Iodo	Meso	Mefenpyr	
Concentration in Product [g a.s./L]	6	30	90	
p(X) (fraction in product)	0,05	0,24	0,71	
LC_{50}fish [mg a.s./L]	100	100	2,4	
LC_{50}invertrebrates [mg a.s./L]	100	100	5,5	
EC_{50}algae [mg a.s./L]	0,152	0,29	1,39	
EC_{50}macrophytes [mg a.s./L]	0,00108	0,9	7,6	

Species	Substance	Concentration (C_i) in formulation (g a.s./L)	P_i	ECxi (mg a.s./L)	$ECx_{mix\text{-}CA}$ (mg sum a.s. /L)	ECx_{PPP} (mg sum a.s. /L)	MDR
Fish, acute toxicity							
species sp.	Iodo	6	0,05	100	3,33	0	
	Meso	30	0,24	100			
	Mefenpyr	90	0,71	2,4			
Invertebrates, acute toxicity							
species sp.	Iodo	6	0,05	100	7,53	2,76822	2,72
	Meso	30	0,24	100			
	Mefenpyr	90	0,71	5,5			
species sp.	Iodo	6	0,05	0,152	0,61	2,2617	0,27
	Meso	30	0,24	0,29			
	Mefenpyr	90	0,71	1,39			
Macrophytes							
species sp.	Iodo	6	0,05	0,00108	0,00	2,27808	0,00
	Meso	30	0,24	0,00129			
	Mefenpyr	90	0,71	7,6			

Final conclusions

Steps	Conclusion on the steps			
	Fish	Invetrates	Algae	Macrophytes
Step 1: data available?	Endpoints only available for the a.s., go to 7.	Endpoints available for a.s. and the ppp, go to 2.	Endpoints available for a.s. and the ppp, go to 2.	Endpoints available for a.s. and the ppp, go to 2.
Step 2: apparent synergism or antagonism?	#N/D!	The MDR is between 0.2-5. No antagonism or synergism is indicated. Thus, the "concentration addition" concept holds, go	The MDR is between 0.2-5. No antagonism or synergism is indicated. Thus, the "concentration addition" concept holds, go	The MDR is <0.2. Thus, antagonism is indicated, go to 9.
Step 3: mixture similar or not?		Mixture sometimes similar. Some scenario can be assessed via product test, go either to 4 or 5.	Mixture not similar every scenario. No scenario can be assessed via product test, go to 5.	
Step 4: ETRmix assessment (ECxPPP)		Acceptable risk have been found in all scenarios in FOCUS step 1-3.		
Step 5: driver available?		There is no driver for invertebrates in all scenarios. Go to 8.	There is no driver for algae in all scenarios. Go to 8.	
Step 6: driver assessment				
Step 7: synergism assessment (few data)		Mixture toxicity calculation feasible: Go to 8	Mixture toxicity calculation feasible: Go to 8	Mixture toxicity calculation feasible: Go to 8
Step 8a: ETRmix assessment				
Step 8b: RQmix assessment	Risk acceptable for all scenarios in FOCUS step 1-3.	Risk acceptable for all scenarios in FOCUS step 1-3.	Risk acceptable for all scenarios in FOCUS step 1-3.	Risk acceptable for some FOCUS scenarios, if risk mitigation is applied (FOCUS Step 4).
Step 9: anatagonism assessment	Measured mixture toxicity not plausible: Go to 8	Measured mixture toxicity plausible: Go to 3	Measured mixture toxicity plausible: Go to 3	Measured mixture toxicity plausible: Go to 3
Step 10: synergism assessment				

Please details orf the calculation are provided in the following exe.file:

Kopia
AGD_AquaMix_v1.15

Overall conclusion : (PPP+Pottock)

Fish (Trigger: ETR ≤ 0.01 or RQ ≤ 1)					
FOCUS Scenario	Synergism or Antagonism?	Driver? (Step 5)	ETRmix-PPP (Step 4)	ETRmix-CA (Step 8a)	RQmix (Step 8b)
Step 1	#DZIEL/0!	Mefenpyr	#DZIEL/0!	Go to 4	
Step 2					
N-Europe		Meso	Go to 5/8	0,00	
S-Europe		Meso	Go to 5/8	0,00	
Step 3					
D1 Ditch		Meso	Go to 5/8	0,00	
D1 Stream		no driver	Go to 5/8	0,00	
D2 Ditch		Meso	Go to 5/8	0,00	
D2 Stream		Meso	Go to 5/8	0,00	
D3 Ditch		no driver	Go to 5/8	0,00	
D4 Pond		Meso	Go to 5/8	0,00	
D4 Stream		no driver	Go to 5/8	0,00	
D5 Pond		Meso	Go to 5/8	0,00	
D5 Stream		no driver	Go to 5/8	0,00	
D6 Ditch		no driver	Go to 5/8	0,00	
R1 Pond		Meso	Go to 5/8	0,00	
R1 Stream		no driver	Go to 5/8	0,00	
R2 Stream					
R3 Stream		no driver	Go to 5/8	0,00	
R4 Stream		Meso	Go to 5/8	0,00	

Invertebrates (Trigger: ETR ≤ 0.01 or RQ ≤ 1)					
FOCUS Scenario	Synergism or Antagonism?	Driver? (Step 5)	ETRmix-PPP (Step 4)	ETRmix-CA (Step 8a)	RQmix (Step 8b)
Step 1	concentration addition can be assumed	Mefenpyr	0,01	Go to 4	
Step 2					
N-Europe		Meso	Go to 5/8	0,00	
S-Europe		Meso	Go to 5/8	0,00	
Step 3					
D1 Ditch		Meso	Go to 5/8	0,00	
D1 Stream		no driver	Go to 5/8	0,00	
D2 Ditch		Meso	Go to 5/8	0,00	
D2 Stream		Meso	Go to 5/8	0,00	
D3 Ditch		no driver	Go to 5/8	0,00	
D4 Pond		Meso	Go to 5/8	0,00	
D4 Stream		no driver	Go to 5/8	0,00	
D5 Pond		Meso	Go to 5/8	0,00	
D5 Stream		no driver	Go to 5/8	0,00	
D6 Ditch		no driver	Go to 5/8	0,00	
R1 Pond		Meso	Go to 5/8	0,00	
R1 Stream		no driver	Go to 5/8	0,00	
R2 Stream					
R3 Stream		no driver	Go to 5/8	0,00	
R4 Stream		Meso	Go to 5/8	0,00	

Algae (Trigger: ETR ≤ 0.1 or RQ ≤ 1)					
FOCUS Scenario	Synergism or Antagonism?	Driver? (Step 5)	ETRmix-PPP (Step 4)	ETRmix-CA (Step 8a)	RQmix (Step 8b)
Step 1	concentration addition can be assumed	no driver	Go to 5/8	0,03	
Step 2					
N-Europe		no driver	Go to 5/8	0,00	
S-Europe		Meso	Go to 5/8	0,01	
Step 3					
D1 Ditch		no driver	Go to 5/8	0,00	
D1 Stream		no driver	Go to 5/8	0,00	
D2 Ditch		no driver	Go to 5/8	0,01	
D2 Stream		no driver	Go to 5/8	0,00	
D3 Ditch		no driver	Go to 5/8	0,00	
D4 Pond		Meso	Go to 5/8	0,00	
D4 Stream		no driver	Go to 5/8	0,00	
D5 Pond		Meso	Go to 5/8	0,00	
D5 Stream		no driver	Go to 5/8	0,00	
D6 Ditch		no driver	Go to 5/8	0,00	
R1 Pond		no driver	Go to 5/8	0,00	
R1 Stream		no driver	Go to 5/8	0,00	
R2 Stream					
R3 Stream		no driver	Go to 5/8	0,00	
R4 Stream		no driver	Go to 5/8	0,00	

Macrophytes (Trigger: ETR ≤ 0.1 or RQ ≤ 1)					
FOCUS Scenario	Synergism or Antagonism?	Driver? (Step 5)	ETRmix-PPP (Step 4)	ETRmix-CA (Step 8a)	RQmix (Step 8b)
Step 1	not applicable	Iodo			9,10
Step 2					
N-Europe		Iodo			1,21
S-Europe		Iodo			0,75
Step 3					
D1 Ditch		Iodo			0,18
D1 Stream		Iodo			0,15
D2 Ditch		Iodo			1,43
D2 Stream		Iodo			0,90
D3 Ditch		Iodo			0,18
D4 Pond		Iodo			0,01
D4 Stream		Iodo			0,13
D5 Pond		Iodo			0,01
D5 Stream		Iodo			0,14
D6 Ditch		Iodo			0,18
R1 Pond		Iodo			0,01
R1 Stream		Iodo			0,13
R2 Stream					
R3 Stream		Iodo			0,40
R4 Stream		Iodo			0,27

2) Mixture toxicity assessment with $\mathbf{E_rC_{50}}$= **41.27** µg product/L for Lemna sp.

Product data	
Product name	
Density of product [g/cm^3]	1
LC_{50}fish [mg prod./L]	0
LC_{50}fish a.s. based [mg sum of a.s/L]	0,0000
EC_{50}invertrebrates [mg prod./L]	21,97
LC_{50}invertrebrates a.s. based [mg sum of a.s/L]	2,7682
EC_{50}algae [mg prod./L]	17,95
EC_{50}algae a.s. based [mg sum of a.s/L]	2,2617
EC_{50}macrophytes [mg prod./L]	41,27
EC_{50}macrophytes a.s. based [mg sum of a.s/L]	5,2000

Calculated mixture toxicity (Eq. 13) based on Tier 1 data only	[mg/L]
ECx_{mix-Ca}fish	3,328050713
ECx_{mix-Ca}invertrebrates	7,534246575
ECx_{mix-Ca}algae	0,606731362
ECx_{mix-Ca}macrophytes	0,004371477

Calculated mixture toxicity (Eq. 13) based also on additional data	[mg/L]
ECx_{mix-Ca}fish	3,328050713
ECx_{mix-Ca}invertrebrates	7,534246575
ECx_{mix-Ca}algae	0,606731362
ECx_{mix-Ca}macrophytes	0,022497063

Options

Show Species

Unlock sum of a.s. for product data

Active Substance (a.s.) standard data (Tier 1 EP)				
Active substance names	Iodo	Meso	Mefenpyr	
Concentration in Product [g a.s./L or g a.s./kg]	6	30	90	
p(X) (fraction in product)	0,05	0,24	0,71	
LC_{50}fish [mg a.s./L]	100	100	2,4	
LC_{50}invertrebrates [mg a.s./L]	100	100	5,5	
EC_{50}algae [mg a.s./L]	0,152	0,29	1,39	
EC_{50}macrophytes [mg a.s./L]	0,00108	0,00129	7,6	
Additional a.s. data (i.e. most sensitive species tested as Tier 1 data or refinements Tier 2A/B EP)				
LC_{50}fish [mg a.s./L]				
LC_{50}invertrebrates [mg a.s./L]				
EC_{50}algae [mg a.s./L]				
EC_{50}macrophytes [mg a.s./L]		0,9		
AF for RAC				
Fish	100	100	100	100
Invertrebrates	100	100	100	100
Algae	10	10	10	10
Macrophytes	10	3	10	10
RAC				
Fish	1	1	0,024	
Invertrebrates	1	1	0,055	
Algae	0,0152	0,029	0,139	
Macrophytes	0,000108	0,3	0,76	

Data used for calculation (after Step 3)				
Active substances	Iodo	Meso	Mefenpyr	
Concentration in Product [g a.s./L]	6	30	90	
p(X) (fraction in product)	0,05	0,24	0,71	
LC_{50}fish [mg a.s./L]	100	100	2,4	
LC_{50}invertrebrates [mg a.s./L]	100	100	5,5	
EC_{50}algae [mg a.s./L]	0,152	0,29	1,39	
EC_{50}macrophytes [mg a.s./L]	0,00108	0,9	7,6	

Species	Substance	Concentration (C_i) in formulation (g a.s./L)	P_i	ECxi (mg a.s./L)	ECx_{mix-CA} (mg sum a.s. /L)	ECx_{PPP} (mg sum a.s. /L)	MDR
Fish, acute toxicity							
species sp.	Iodo	6	0,05	100	3,33	0	
	Meso	30	0,24	100			
	Mefenpyr	90	0,71	2,4			
Invertebrates, acute toxicity							
species sp.	Iodo	6	0,05	100	7,53	2,76822	2,72
	Meso	30	0,24	100			
	Mefenpyr	90	0,71	5,5			
species sp.	Iodo	6	0,05	0,152	0,61	2,2617	0,27
	Meso	30	0,24	0,29			
	Mefenpyr	90	0,71	1,39			
Macrophytes							
species sp.	Iodo	6	0,05	0,00108	0,00	5,20002	0,00
	Meso	30	0,24	0,00129			
	Mefenpyr	90	0,71	7,6			

Steps	Conclusion on the steps			
	Fish	Invetrates	Algae	Macrophytes
Step 1: data available?	Endpoints only available for the a.s., go to 7.	Endpoints available for a.s. and the ppp, go to 2.	Endpoints available for a.s. and the ppp, go to 2.	Endpoints available for a.s. and the ppp, go to 2.
Step 2: apparent synergism or antagonism?	#N/D!	The MDR is between 0.2-5. No antagonism or synergism is indicated. Thus, the "concentration addition" concept holds, go	The MDR is between 0.2-5. No antagonism or synergism is indicated. Thus, the "concentration addition" concept holds, go	The MDR is <0.2. Thus, antagonism is indicated, go to 9.
Step 3: mixture similar or not?		Mixture sometimes similar. Some scenario can be assessed via product test, go either to 4 or 5.	Mixture not similar every scenario. No scenario can be assessed via product test, go to 5.	
Step 4: ETRmix assessment (ECxPPP)		Acceptable risk have been found in all scenarios in FOCUS step 1-3.		
Step 5: driver available?		There is no driver for invertebrates in all scenarios. Go to 8.	There is no driver for algae in all scenarios. Go to 8.	
Step 6: driver assessment				
Step 7: synergism assessment (few data)		Mixture toxicity calculation feasible: Go to 8	Mixture toxicity calculation feasible: Go to 8	Mixture toxicity calculation feasible: Go to 8
Step 8a: ETRmix assessment				
Step 8b: RQmix assessment	Risk acceptable for all scenarios in FOCUS step 1-3.	Risk acceptable for all scenarios in FOCUS step 1-3.	Risk acceptable for all scenarios in FOCUS step 1-3.	Risk acceptable for some FOCUS scenarios, if risk mitigation is applied (FOCUS Step 4).
Step 9: anatagonism assessment	Measured mixture toxicity not plausible: Go to 8	Measured mixture toxicity plausible: Go to 3	Measured mixture toxicity plausible: Go to 3	Measured mixture toxicity plausible: Go to 3
Step 10: synergism assessment				

zRMS comment:

Mixture toxicity assessment:

An acceptable mixture toxicity risk after application of Zeppos on fish, aquatic invertebrates and algae for all FOCUS scenarios at step 1-3 were obtained. Aquatic plants are the most sensitive species without requiring measures for exposure mitigation in regions other than those represented by scenario D2 (not applied on artificially drained soils in areas represented by the D2 scenario, when topsoil clay content is 45%).
The risk assessment performed for each individual active substance is sufficient to conclude acceptable risk.

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9.5.3 Overall conclusions

ZRMS comments:

zRMS agrees with the risk assessment based on the Tier 1 study with RAC=0.129 µg a.s./L (for the most sensitive species) and FOCUS STEP 1-4 calculations for application winter cereals at rate 15 g a.s./L (covering the risk for application rate of 9 g a.s./ha) and for spring cereals.

The conclusion of the risk assessment was summarised below:

Tier 1 data (RAC =0.129 µg a.s./L)

The risk for winter cereals at rate 15 g a.s./ha is considered acceptable when:

- A vegetative filter strip of 10 m for **R3 scenario** and a vegetative buffer strip of 20 m for **R4 scenario** will be applied to surface water bodies
- For D1 scenarios – further refinement is required.
- For member states where D2 is relevant, a national specific risk mitigation measures should be applied.

The risk for winter cereals at rate 9 g a.s./ha is considered acceptable when:

- A vegetative filter strip of 10 m for **R3 scenario** and a vegetative buffer strip of 20 m for **R4 scenario** will be applied to surface water bodies
- For member states where D2 is relevant, a national specific risk mitigation measure should beap-plied.

The risk for spring cereals at rate 9 g a.s./ha is considered acceptable except For D1 scenario – further refinement is required.

Refinment based on geomean approach Tier 2A (**RAC=0.196 µg a.s./L)**)

Refinement of the Lemna Tier 1 based on the geomean of the growth rate endpoints (Tier 2A) based on frond number from the Lemna EU-reviewed studies (1.71, 1.61 µg a.s./L) and the applicant's study (2.72 µg a.s./L). This is a justified approach as frond number is the primary measurement variable according to the OECD 221-guideline and the same measured variable should be used for a geomean calculation.
This results in a geomean ErC_{50} of 1.96 µg a.s./L. Using an AF of 10, this results in a RAC for risk as-sessment of 0.196 µg/L (Table 9.5-37).

Based on this approach the following risk mitigation measures can be concluded:

Winter cereals 15 g a.s./ha:

- A risk mitigation of 10m + DVP 10m for R3 and R4 scenarios.
- For D1 (stream) scenario the risk is acceptable with STEP 3. For D1 (ditch) further refinement is required)
- For member states where D2 is relevant, a national specific risk mitigation measure should beap-plied

Winter cereals at rate 9 g a.s./ha

- A risk mitigation of 10m + DVP 10m for R3 and R4 scenarios
- For D1 scenarios the risk is considered acceptable with FOCUS Step 3.
- For member states where D2 is relevant, a national specific risk mitigation measure should beap-plied

Spring cereals:

- Acceptable risk for all scenarios

Refinment based on geomean approach Tier 2A based on the value agreed at EU level (RAC=0.149 µg a.s./L)

During commenting period process furter refinement based on **Tier 2A based on the value agreed at EU level** was considered by the applicant

EU agreed ErC_{50} endpoints for Lemna were considered as a weight of evidence (i.e, 1.717-frond number, 1.29-frond area) the resulting **RAC of 0.149 µg a.s. /L** from the geomean approach (Tier 2A).
The following risk mitigation measures are proposed:

Winter cereals at rate 15 g a.s./ha:

- A risk mitigation of 10m + DVP 10m for R3 and R4 scenarios.
- For D1 (stream) scenario the risk is acceptable with STEP 3. For D1 (ditch further refinement is required)
- For member states where D2 is relevant, a national specific risk mitigation measures should be applied

Winter cereals at rate 9 g a.s./ha

- A risk mitigation of 10m + DVP 10m for R3 and R4 scenarios
- For D1 scenarios the risk is considered acceptable with FOCUS Step 3.
- For member states where D2 is relevant, a national specific risk mitigation measure should be applied

Spring cereals:

- For D1 scenarios the risk further needs further refinement

Refinment based on SSD approach :

The one of the refinement options was SSD approach with HC_5 of 0.90 µg a.s./L value for mesosulforon methyl obtained from the new data for the active substance .
The HC_5 of 0.90 µg a.s./L value was not considered by some MSs to use in the risk assessment.
Therefore, the applicant provided the justification of using the additional data for the a.s. and provided the alternative approach which may be considered at MSs level, if relevant

Applicant's approach during commenting period process for using by MSs and their national level, if relevant:

Taking into account that the new active substance data submitted has been already assessed under Data Matching process resulting in a non-change of agreed EU endpoints of toxicological reference values, residue definitions or relevance of metabolites, the applicant proposes to use an SSD-approach (Tier 2B) using the Lemna Tier 1 endpoint from the EU-review together with the endpoints of the 9 additional aquatic species tested by the applicant according to "Guidance on tiered risk assessment for edge-of-field surface waters, (EFSA Journal 2013;11(7):3290).

The HC5 calculated can then be used to refine the Tier 1 Lemna endpoint from the EU-review. This SSD-approach leads to an HC5 of 0.83 µg/L. Using an AF of 3, as advised in the EFSA Journal 2013; 11(7):3290 for the risk assessment for primary producers, the RAC will be 0.28 µg a.s./L. This would result in an acceptable risk for aquatic organisms when respecting a risk mitigation measure of 10m + DVP 10m.

In conclusion:

The final decision of the refinement for scenarios D1, R3 and R4, D2 scenarios should be decided at MSs level.

Formulation GLOB389H/SAP63H:

For acute risk assessment of the formulation, PEC_{sw} based on drift events were calculated using the SWASH Drift Calculator tool in ditch, pond and stream surface waters. The resulting PEC_{sw} were used for comparison with the measured mixture toxicity for aquatic plants. The risk for aquatic invertebrates and algae is covered by the risk assessment for aquatic plants, as *lemna gibba* is by far the most sensitive species (more than factor 10 difference).
The PEC/RAC ratio is <1 for product if a 5m buffer zone is considered. An acceptable risk for the formulated product GLOB289H can be concluded based on the proposed use pattern, further consideration is not required.

Based on the above risk assessment, following risk mitigation measures are required for a safe use of GLOB289H:

Winter cereals (0.5 kg PPP/ha)		
Scenario	**Water body**	**Mitigation measure requested**
D1	Ditch	No
D1	Stream	No
D2	Ditch	Yes
D2	Stream	Yes
D3	Ditch	No
D4	Pond	No
D4	Stream	No
D5	Pond	No
D5	Stream	No
R1	Pond	No
R1	Stream	No
R3	Stream	No
R4	Stream	No
Winter cereals (0.3 kg PPP/ha)		
Scenario	**Water body**	**Mitigation measure requested**
D1	Ditch	No
D1	Stream	No
D2	Ditch	Yes
D2	Stream	Yes
D3	Ditch	No
D4	Pond	No
D4	Stream	No
D5	Pond	No
D5	Stream	No
R1	Pond	No
R1	Stream	No
R3	Stream	No
R4	Stream	No
Spring cereals (0.3 kg PPP/ha)		
Scenario	**Water body**	**Mitigation measure requested**
D1	Ditch	No
D1	Stream	No
D3	Ditch	No
D4	Pond	No
D4	Stream	No
D5	Pond	No
D5	Stream	No
R4	Stream	No

9.5.1 Overall conclusions

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 both active substances iodosulfuron-methyl-sodium and mesosulfuron-methyl. Full details of these studies are provided in the respective EU DAR and related documents. No bee toxicity endpoints are available for the pure safener mefenpyr-diethyl. The potential effect of the safener is inherently addressed via the toxicity studies performed on the preparation.

To improve efficacy of the product, an adjuvant can be added in tank mix. As non-target organisms will be exposed to the combination of the formulation and the adjuvants, the combined acute effect on bees was addressed. Two different adjuvants were tested for acute effects; a non-esterified rapeseed oil

(Actirob) and a non-ionic surfactant (Pottok). According to the EFSA GD, a study with the active substance will be sufficient if there is no indication from the acute oral study that the formulation is more toxic than the active substance. If the difference between the formulation endpoint and the active substance endpoint is less than a factor of 5, then the adult chronic toxicity study and larval study should only be carried out on the active substance. In this specific case, the difference between the endpoints of the formulation and the formulation + Actirob/Pottok in the acute tests is less than a factor of 5. Therefore, no additional effect of the adjuvant is expected for the chronic studies.

Effects on bees of GLOB289H or GLOB289H + adjuvant weren't evaluated as part of the EU assessment of iodosulfuron-methyl-sodium or mesosulfuron-methyl. New data submitted with this application are listed in Table 9.6-1 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 9.6-1: Endpoints and effect values relevant for the risk assessment for bees

Species	Substance	Exposure System	Results	Reference
Apis mellifera	Iodosulfuron-methyl-sodium	Oral, 72h	LD_{50} > 70 µg/bee	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Apis mellifera	Iodosulfuron-methyl-sodium	Contact, 72h	LD_{50} > 131 µg/bee	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Apis mellifera	Iodosulfuron-methyl-sodium	Oral, 48h	LD_{50} > 107.6 µg/bee	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Apis mellifera	Iodosulfuron-methyl-sodium	Contact, 48h	LD_{50} > 100.0 µg/bee	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Bombus terrestris	Iodosulfuron-methyl-sodium	Contact, 48h	LD_{50} > 100.0 µg/bee	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Apis mellifera	Iodosulfuron-methyl-sodium	Chronic, 10d	LC_{50} > 111.6 a.a./kg diet LD_{50} > 4.4 µg a.i./bee NOEC = 116 mga.s./kg diet NOEL = 4.4 µg a.s./bee/d	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Apis mellifera	Iodosulfuron-methyl-sodium WG 10	21d Feeding test Bee brood development	$NOEC_{mort,\ adult}$ < 25 mg a.s./L $NOEC_{mort,\ pupae}$ < 25 mg a.s./L $NOEC_{brood\ developm.}$ < 25 mg a.s./L[1] $NOEC_{behaviour}$ < 25 mg a.s./L	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016

Species	**Substance**	**Exposure System**	**Results**	**Reference**
Apis mellifera	Mesosulfuron-methyl	Oral	LD_{50} > 105.6 µg a.i./bee	EFSA Conclusion Mesosulfuron-methyl, 2016
Apis mellifera	Mesosulfuron-methyl	Contact	LD50 > 100 µg a.i. /bee	EFSA Conclusion Mesosulfuron-methyl, 2016
Apis mellifera	Mesosulfuron-methyl	Chronic, 10d	LD_{50} > 120 mg a.i./kg food, esquivalent to > 4.85 µg a.s./bee/d**	EFSA Con-clusion Mesosulfuron-methyl, 2016
Apis mellifera	GLOB289H	Oral	**> 194.95 µg fp/bee**	Sipos K., 2018
Apis mellifera	GLOB289H + Actirob[#]	Oral	> 198.9 µg fp/bee	Franke M., 2019a
Apis mellifera	GLOB289H + Pottok[#]	Oral	> 196.1 µg fp/bee	Franke M., 2019b
Apis mellifera	GLOB289H	Contact	**> 200.00 µg fp/bee**	Sipos K., 2018
Apis mellifera	GLOB289H + Actirob[#]	Contact	> 200 µg fp/bee	Franke M., 2019c
Apis mellifera	GLOB289H + Pottok[#]	Contact	> 200 µg fp/bee	Marin M., 2019
Apis mellifera	GLOB289H	Adult, oral, 10d	LDD_{50} = 115 µg consumed product/bee/day (4.14 µg consumed a.s./bee/d) NOEDD = 45.7 µg consumed product/bee/d (1.65 µg con-sumed a.s./bee/d) NOEC = 1.326 g product/kg food (= 0.048 g a.s./kg food)	Ruhland S., 2018
Apis mellifera	GLOB289H	Larval, oral, 22d	NOED = 32,5 µg product/larva (= 1.17 µg a.s./larva) NOEC = 206 mg/kg food (= 7.4 mg a.s./kg food)	Kleebaum K., 2018
Higher-tier studies (tunnel test, field studies)				
-				

* corresponding to concentration of mesosulfuron-methyl present in the spray tank with a standard water volume of 400 l/ha.
** There was no mortality at the LDD_{50}
[1] Endpoint based on Brood termination rate. No significant effects on Brood index or Compensation index
[#] Concentrations are related to GLOB289H only. The adjuvant was applied at the ratio advised on the label.

9.6.1.1 Justification for new endpoints

As GLOB289H is was not the reference formulation evaluated in the EFSA review, toxicity to bees from the formulation and the formulation in combination with two different adjuvants was also tested. From the different studies, the lowest endpoint was used in the risk assessment.

9.6.2 Risk assessment

Although the guidance document is not yet approved, the evaluation of the risk for honeybees was performed in accordance with the recommendations of the "EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera, Bombis* spp. and solitary bees)", EFSA Journal 2013;11(7):3295.

9.6.2.1 Hazard Quotients and Exposure Toxicity Ratios for bees

Acute risk assessment – contact exposure

The acute risk to honeybees from contact to GLOB289H was assessed using the maximum application rate and the LD50 values to calculate hazard quotients as follows:

$HQ_{contact} = AR\ (g/ha) / LD_{50\ contact}$

Table 9.6-2: Screening assessment of the acute contact risk for bees due to the use of GLOB289H in cereals

Intended use **Product** **Application rate (kg/ha)**	Cereals GLOB289H + ADJUVANT 1 × 0.5		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 42$**
Contact toxicity	> 200.00	500	2.50

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

A hazard quotient of less than 42 indicates a low risk to bees in the field.

Acute risk assessment – oral exposure

The acute risk to honeybees from oral exposure to GLOB289H was assessed using the maximum application rate and the LD50 value to calculate the Exposure Toxicity Rate (ETR) as follows:

$ETR\ acute\ adult\ oral = AR * SV\ / LD_{50\ oral}$

Table 9.6-3: Screening assessment of the acute oral risk for bees due to the use of GLOB289H in cereals

Intended use	Cereals				
Product	GLOB289H + ADJUVANT				
Application rate (kg/ha)	1 × 0.5				
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (kg/ha)**	**SV**	**ETR**	**Trigger value**

Oral toxicity	> 194.95	0.5	7.6	0.02	0.2

The trigger value of 0.2 was not exceeded with an ETR of 0.02, which indicates a low risk to bees in the field.

Chronic risk assessment – adult bees

The chronic risk to honeybees from oral exposure to GLOB289H was assessed using the maximum application rate and the LDD50 value to calculate the Exposure Toxicity Rate (ETR) as follows:

ETR chronic adult oral = AR * SV / 10d $LDD_{50\ oral}$

Table 9.6-4: Screening assessment of the chronic oral risk for bees due to the use of GLOB289H in cereals

Intended use	Cereals				
Product	GLOB289H				
Application rate (kg/ha)	1 × 0.5				
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (kg/ha)**	**SV**	**ETR**	**Trigger value**
Oral toxicity	115	0.5	7.6	0.033	0.03

The trigger value of 0.03 was not exceeded with an ETR of 0.033, which indicates a low risk to bees in the field.

Chronic risk assessment – bee larvae

The risk to honeybee larvae from oral exposure to GLOB289H was assessed using the maximum application rate and the NOEL value to calculate the Exposure Toxicity Rate (ETR) as follows:

ETR chronic adult oral = AR * SV / $NOEL_{larvae}$

Table 9.6-5: Screening assessment of the chronic oral risk for bee larvae due to the use of GLOB289H in cereals

Intended use	Cereals				
Product	GLOB289H				
Application rate (kg/ha)	1 × 0.5				
Test design	**NOEL (lab.) (µg/bee)**	**Single application rate (kg/ha)**	**SV**	**ETR**	**Trigger value**
Oral toxicity	32.5	0.5	4.4	0.07	0.2

The trigger value of 0.2 was not exceeded with an ETR of 0.07, which indicates a low risk to bees in the field.

ZRMS comments:

The risk assessment for bees for the active substances and the formulation GLOB289H/SAP63H provided with consideration of "EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera, Bombis* spp. and solitary bees)", EFSA Journal 2013;11(7):3295. which is not yet approved was accepted by zRMS-PL. The toxicity to bees from the formulation and the formulation in combination with two different adjuvants was also tested. From the different studies, the lowest endpoint was used in the risk assessment. The acute and chronic risk risk is considered as acceptable from exposure of GLOB289H?SAP63H.

First-tier assessment of the risk for bees due to the use of GLOB289H in cereals according to SANCO/10329/2002 rev.2 (final), October 17, 2002).

Intended use	Cereals		
Product	GLOB289H		
Application rate (g/ha)	500 g /ha		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	>194.95	500	<2.56
Contact toxicity	>200		<2.5

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

zRMS comment:

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).
The acute risk assessment for adult bees based on laboratory studies for GLOB289H indicated acceptable oral and acute risk with the HQ value <50.

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

Not relevant.

9.6.3 Effects on bumble bees

No information available.

9.6.4 Effects on solitary bees

No information available.

9.6.5 Overall conclusions

All the hazard quotients are acceptable, indicating that GLOB289H poses a low acute and chronic risk to bees.

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

For the Annex I renewal, studies on the toxicity to non-target arthropods have been carried out with a representative formulation for both active substances iodosulfuron-methyl sodium and mesosulfuron-methyl, however our formulation was not the representative formulation.

To improve efficacy of the product, an adjuvant can be added in tank mix. As non-target organisms will be exposed to the combination of the formulation and the adjuvants, both the effect of the solo product and the combined effect on non-target arthropods was addressed. Two different adjuvants were tested; a non-esterified rapeseed oil (Actirob) and a non-ionic surfactant (Pottok).
New data submitted with this application are listed in Table 9.7-1 and summarised in Appendix 2.

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

Species	Substance	Exposure System	Results	Reference
Iodosulfuron-methyl sodium				
Typhlodromus pyri (protonymphs)	IMS + MPR OD 400	Laboratory test glass plates (2D)	LR_{50} = 30.3 g a.s./ha ER_{50} = 30.3 g a.s./ha	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
Aphidius rhopalosiphi (adults)	IMS + MPR OD 400	Laboratory test glass plates (2D)	LR_{50} = 30.3 g a.s./ha ER_{50} = 30.3 g a.s./ha	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
Mesosulfuron-methyl				
Typhlodromus pyri (protonymphs)	Atlantis OD*	Laboratory test glass plates (2D)	LR_{50} > 1500 ml/ha	EFSA Conclusion Mesosulfuron-methyl, 2016
Aphidius rhopalosiphi (adults)	Atlantis OD*	Laboratory test glass plates (2D)	LR_{50} = 877.3 ml/ha	EFSA Conclusion Mesosulfuron-methyl, 2016
Chrysoperla carnea	Atlantis OD*	Extended lab test	LR_{50} > 1500 ml/ha ER_{50} > 1500 ml/ha	EFSA Conclusion Mesosulfuron-methyl, 2016
Aphidius rhopalosiphi	Atlantis OD*	Extended lab test	LR_{50} > 1500 ml/ha ER_{50} > 1500 ml/ha	EFSA Conclusion Mesosulfuron-methyl, 2016
PPP				
Typhlodromus pyri (protonymphs)	GLOB289H	Laboratory test glass plates (2D)	**ER_{50} > 1 kg/ha**	Röhlig U., 2017a
Aphidius rhopalosiphi (adults)	GLOB289H	Laboratory test glass plates (2D)	ER_{50} > 1 kg/ha	Röhlig U., 2017b
Typhlodromus pyri (protonymphs)	GLOB289H + Actirob#	Laboratory test glass plates (2D)	ER_{50} > 1 kg GLOB289H/ha + 2 L Actirob B/ha	Röhlig U., 2019a
Aphidius rhopalosiphi (adults)	GLOB289H + Actirob#	Extended lab test	**ER_{50} > 0.833 kg GLOB289H/ha** + 1 L Actirob B/ha.	Röhlig U., 2019b
Chrysoperla carnea	GLOB289H + Actirob#	Extended lab test	**ER_{50} > 0.833 kg GLOB289H/ha** + 1 L Actirob B/ha	Röhlig U., 2019c
Aleochara bilineata	GLOB289H + Actirob#	Extended lab test	**ER_{50} > 0.833 kg GLOB289H/ha** + 1 L Actirob B/ha.	Röhlig U., 2019d
Aphidius	GLOB289H + Pot-	Extended lab test	ER_{50} > 0.833 kg	Luna F., 2019

Species	Substance	Exposure System	Results	Reference
rhopalosiphi (adults)	tok#		GLOB289H/ha + 0.2 L Pottok/ha.	
Field or semi-field tests				
-				

* 10 g/L mesosulfuron-methyl, 2 g/L iodosulfuron-methyl-sodium and 30 g/L mefenpyr-diethyl
# Concentrations are related to GLOB289H only. The adjuvant was applied at the ratio advised on the label.

9.7.1.1 Justification for new endpoints

The risk assessment was performed using the lowest endpoint obtained from studies with GLOB289H and GLOB289H + adjuvants as presented in Table 9.7-1.

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

Non-target arthropods living in the crop can be exposed to residues from GLOB289H by direct contact either as a result of overspray or through contact with residues on plants and soil or in food items. To achieve a concise risk assessment, the risk envelope approach is applied with the critical GAP (1 x 0.5 kg/ha).

Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of GLOB 289H in cereals

Intended use	Cereals		
Product	GLOB289H + ADJUVANT		
Application rate (g/ha)	1 x 500		
MAF	1		
Test species Tier I	**LR_{50} (lab.) (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**HQ**
Typhlodromus pyri	1000	500	0.5
Aphidius rhopalosiphi	833	500	0.6
Chrysoperla carnea	833		0.6
Aleochara bilineata	833		0.6

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 in-field HQ values falls below the trigger of 2 for the four different species tested. Based on these results can be concluded that GLOB289H does not pose an unacceptable risk to non-target arthropods in in-field areas.

Aphidius appeared to be the most sensitive species in the RAR of mesosulfuron-methyl and in the studies performed with the adjuvant Actirob. Since no effects were observed at the highest rate tested and there is not any specific requirement and/or any recommended ecotox studies for mixtures of adjuvants with PPP, no additional species are considered needed to be tested with SAP63H+POTTOK. In addition, a safe use on NTA after application of GLOB289H/SAP63H along with the adjuvants Actirob/Pottok can be demonstrated.

zRMS comments:

zRMS verified the risk assessment in -field for all tested species according to recommendations given in the ESCORT 2 GD. The zRMS's in - field risk assessment is provided in the Table below:

First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of GLOB 289H in cereals

Intended use	Cereals		
Product	GLOB289H + ADJUVANT		
Application rate (g/ha)	1 x 500		
MAF	1		
Test species Tier I	**LR_{50} (lab.) (g/ha)**	**$PER_{in-field}$ (g/ha)**	**HQ<2**
Typhlodromus pyri	1000	500	0.5
Test species Tier I	**LR_{50} (lab.)/ER50 (g/ha)**	**$PER_{in-field}$ (g/ha)**	**$PER_{in-field}$ below rate with ≤ 50 % effect?**
Aphidius rhopalosiphi	833	500	Yes
Chrysoperla carnea	833		Yes
Aleochara bilineata	833		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 in-field HQ values falls below the trigger of 2 for T. pyri indicating an acceptable risk in field for this species.
For remained species tested in extended laboratory condition the PER $_{in-field}$ was below rate with <50% effects indicating an acceptable risk.
Based on these results can be concluded that GLOB289H does not pose an unacceptable risk to non-target arthropods in in-field areas.

9.7.2.2 Risk assessment for off-field exposure

To achieve a concise risk assessment, the risk envelope approach is applied with the critical GAP (1 x 0.5 kg/ha).

$PER_{off\text{-}field}$ = application rate x MAF x (drift factor/VDF)
= 500 x 1 x (0.0277/10)
= 1.385

MAF: As only 1 application is intended, the MAF is 1.
Drift rate: 0.0277 (recommended by ESCORT II)
CF: since the species sensitivity analysis are mainly based on a comparison of in-field species which represent a lower species diversity than expected within off-field habitats, an uncertainty factor of 10 is included.

Off-field HQ = $PER_{off\text{-}field}$ (g/ha) / LR_{50} (g/ha) x Correction factor

Table 9.7-3: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use ofGLOB289H in cereals

Intended use	Cereals				
Product	GLOB289H				
Application rate (g/ha)	1 x 500				
MAF	1				
Vdf	10 (Tier 1)				
Test species Tier I	**LR_{50} (lab.) (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	1000	0.0277	1.385	10	0.01385
Aphidius rhopalosiphi	833				0.01663
Chrysoperla carnea	833				0.01663
Aleochara bilineata	833				0.01663

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.
* If an LR_{50} or ER_{50} from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

The off-field HQ values for the four different tested species fall below the trigger of 2. Based on these results can be concluded that GLOB289H does not pose an unacceptable risk to non-target arthropods in off-field areas.

zRMS comments:

ZRMS verified the off – field risk assessment for all tested species according to recommendation given in the ESCORT 2GD. The zRMS's off - field risk assessment is provided in the Table below:

First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of GLOB 289H in cereals

Intended use	Cereals
Product	GLOB289H
Application rate (g/ha)	1 x 500

MAF	1				
Vdf	10 (2D) , CF 10 (2D), CF=5 (3D)				
Test species Tier I	**LR_{50} (lab.) (g/ha)**	**Drift rate**	**$PER_{off\text{-}field\ corr}$ (g/ha)**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	1000 (2D)	0.0277	1.385	10	0.01385
Test species Tier I	**LR_{50} (lab.)/ER50 (g/ha)**	**Drift rate**	**$PER_{off\text{-}field\ corr}$ (g/ha)**	**CF**	**PERin-field below rate with ≤ 50 % effect?**
Aphidius rhopalosiphi	833 (3D)	0.0277	69.25	5	Yes
Chrysoperla carnea	833 (2D)		6.925	5	Yes
Aleochara bilineata	833 (2D)		6.925	5	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.

The off -field HQ values falls below the trigger of 2 for T. pyri indicating an acceptable risk in field for this species.
For remained species tested in extended laboratory condition the PER $_{off\text{-}field}$ was below rate with <50% effects indicating an acceptable risk.
Based on these results can be concluded that GLOB289H does not pose an unacceptable risk to non-target arthropods off-field areas.

9.7.2.3 Additional higher-tier risk assessment

Not relevant.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

Considering the acceptable risk to *Aphidius rhopalosiphi*, *Typhlodromus pyri*, *Crysoperla carnea* and *Aleochara bilineata* from the glass plate and extended laboratory studies, GLOB289H complies with the trigger values recommended by ESCORT 2. It is therefore concluded that the risk to non-target arthropods following the recommended uses of GLOB289H will be negligible. No mitigation measures are necessary.

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

9.8.1 Toxicity data

Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with active substances iodosulfuron-methyl-sodium, mesosulfuron-methyl, safener mefenpyr-diethyl and their relevant metabolites. Full details of these studies are provided in the respective

EU RAR and related documents.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of GLOB289H not evaluated as part of the EU assessment of the active substances.
To improve efficacy of the product, an adjuvant can be added in tank mix. As non-target organisms will be exposed to the combination of the formulation and the adjuvants, both the effect of the solo product and the combined effect on earthworms was addressed. Two different adjuvants were tested; a non-esterified rapeseed oil (Actirob) and a non-ionic surfactant (Pottok). As no effects of the adjuvants were observed in NTA and earthworms, no effect of the adjuvants is expected on other non-target soil organisms. Therefore, studies with the adjuvant on *Hypoaspis aculeifer* and *Folsomia candida* were not considered necessary.
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. For the formulation, the lowest endpoint of the different studies (solo PPP or PPP + adjuvant) was used for the risk assessment.

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
Iodosulfuron-methyl sodium				
Eisenia fetida	Iodosulfuron-methyl-sodium	Mixed into substrate 56 d, 10 % peat content	EC_{10} = 7 mg/kg dw	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
Eisenia fetida	AE F145741	Mixed into substrate 56 d, 10 % peat content	NOEC = 94.4 mg/kg dw	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
Eisenia fetida	AE F145740	Mixed into substrate 56 d, 10 % peat content	NOEC = 97.5 mg/kg dw	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
Eisenia fetida	AE 0002166	Mixed into substrate 56 d, 10 % peat content	NOEC = 95.0 mg/kg dw	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
		Worst-case assumption	NOEC = 0.7 mg/kg dw	10x more toxic than parent
Eisenia fetida	BCS-CW81253	Mixed into substrate 56 d, 10 % peat content	NOEC = 99.0 mg/kg dw	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
Eisenia fetida	AE 0000119	Mixed into substrate 56 d, 10 % peat content	NOEC = 97.8 mg/kg dw	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
Eisenia fetida	AE F059411	Mixed into substrate 56 d, 10 % peat content	NOEC = 30 mg/kg dw	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
Folsomia candida	Iodosulfuron-methyl sodium	Mixed into substrate 28 d, 5 % peat content	NOEC = 316 mg/kg dw	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
Folsomia candida	AE F075736	Mixed into substrate 28 d, 5 % peat content	NOEC = 9.86 mg/kg dw	EFSA Conclusion Iodosulfuron-methyl sodium, 2016

Species	Substance	Exposure System	Results	Reference
Folsomia candida	BCS-CW81253	Mixed into substrate 28 d, 5 % peat content	NOEC = 99.0 mg/kg dw	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
		Worst-case assumption	NOEC = 31.6 mg/kg dw	10x more toxic than parent
Folsomia candida	AE F059411	Mixed into substrate 28 d, 5 % peat content	NOEC = 99.7 mg/kg dw	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
Folsomia candida	AE 0000119	Mixed into substrate 28 d, 5 % peat content	NOEC = 97.8 mg/kg dw	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
		Worst-case assumption	NOEC = 31.6 mg/kg dw	
Hypoaspis aculeifer	Iodosulfuron-methyl sodium	Mixed into substrate 15 d, 5 % peat content	NOEC = 1000 mg/kg dw	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
Hypoaspis aculeifer	AE F075736	Mixed into substrate 14 d, 5 % peat content	NOEC = 9.86 mg/kg dw	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
Hypoaspis aculeifer	AE F145741	Mixed into substrate 14 d, 5 % peat content	NOEC = 100 mg/kg dw	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
		Worst-case assumption	NOEC = 100 mg/kw dw	
Hypoaspis aculeifer	AE F145740	Mixed into substrate 14 d, 5 % peat content	NOEC = 97.5 mg/kg dw	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
		Worst-case assumption	NOEC = 100 mg/kg dw	10x more toxic than parent
Hypoaspis aculeifer	AE 0002166	Mixed into substrate 14 d, 5 % peat content	NOEC = 95.2 mg/kg dw	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
		Worst-case assumption	NOEC = 100 mg/kg dw	10x more toxic than parent
Hypoaspis aculeifer	BCS-CW81253	Mixed into substrate 14 d, 5 % peat content	NOEC = 99.0 mg/kg dw	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
		Worst-case assumption	NOEC = 100 mg/kg dw	10x more toxic than parent
Hypoaspis aculeifer	AE F059411	Mixed into substrate 14 d, 5 % peat content	NOEC = 98.7 mg/kg dw	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
Hypoaspis aculeifer	AE 0000119	Mixed into substrate 14 d, 5 % peat content	NOEC = 97.8 mg/kg dw	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
		Worst-case assumption	NOEC = 100 mg/kg dw	

Species	Substance	Exposure System	Results	Reference
Mesosulfuron-methyl				
Eisenia fetida	Mesosulfuron-methyl	Mixed into substrate 56 d, 10 % peat content	NOEC = 125 mg/kg dw	EFSA Conclusion Mesosulfuron-methyl, 2016
Eisenia fetida	AE F160459	Mixed into substrate 56 d, 5 % peat content	NOEC = 90 mg/kg dw	EFSA Conclusion Mesosulfuron-methyl, 2016
		Worst-case assumption	NOEC = 12.5 mg/kg dw	
Eisenia fetida	AE F099095	Mixed into substrate 56 d, 10 % peat content	NOEC = 100 mg/kg dw	EFSA Conclusion Mesosulfuron-methyl, 2016
		Worst-case assumption	NOEC = 12.5 mg/kg dw	
Eisenia fetida	AE F092944	Mixed into substrate 56 d, 10 % peat content	NOEC = 10 mg/kg dw	EFSA Conclusion Mesosulfuron-methyl, 2016
		Worst-case assumption	NOEC = 12.5 mg/kg dw	
Eisenia fetida	AE F160460	Mixed into substrate 56 d, 10 % peat content	NOEC = 100 mg/kg dw	EFSA Conclusion Mesosulfuron-methyl, 2016
		Worst-case assumption	NOEC = 12.5 mg/kg dw	
Eisenia fetida	AE F140584	Mixed into substrate 56 d, 10 % peat content	NOEC = 117 mg/kg dw	EFSA Conclusion Mesosulfuron-methyl, 2016
		Worst-case assumption	NOEC = 12.5 mg/kg dw	
Eisenia fetida	AE F147447	Mixed into substrate 56 d, 5 % peat content	NOEC = 90 mg/kg dw	EFSA Conclusion Mesosulfuron-methyl, 2016
		Worst-case assumption	NOEC = 12.5 mg/kg dw	
Folsomia candida	Mesosulfuron-methyl	Mixed into substrate 28 d, 5 % peat content	NOEC = 1000 mg/kg dw	EFSA Conclusion Mesosulfuron-methyl, 2016
Folsomia candida	mesosulfuron	Mixed into substrate 28 d, 5 % peat content	NOEC = 100 mg/kg dw	EFSA Conclusion Mesosulfuron-methyl, 2016
		Worst-case assumption	NOEC = 100 mg/kg dw	
Folsomia candida	AE F160459	Mixed into substrate 28 d, 5 % peat content	NOEC = 100 mg/kg dw	EFSA Conclusion Mesosulfuron-methyl, 2016
		Worst-case assumption	NOEC = 100 mg/kg dw	

Species	Substance	Exposure System	Results	Reference
Folsomia candida	AE F092944	Mixed into substrate 28 d, 5 % peat content	NOEC = 100 mg/kg dw	EFSA Conclusion Mesosulfuron-methyl, 2016
		Worst-case assumption	NOEC = 100 mg/kg dw	
Folsomia candida	AE F092944 (IN-J0290)	Mixed into substrate 28 d, 5 % peat content	NOEC = 50 mg/kg dw	EFSA Conclusion Mesosulfuron-methyl, 2016
Folsomia candida	AE F147447	Mixed into substrate 28 d, 5 % peat content	NOEC = 100 mg/kg dw	EFSA Conclusion Mesosulfuron-methyl, 2016
		Worst-case assumption	NOEC = 100 mg/kg dw	
Hypoaspis aculeifer	Mesosulfuron-methyl	Mixed into substrate 14 d, 5 % peat content	NOEC = 1000 mg/kg dw	EFSA Conclusion Mesosulfuron-methyl, 2016
Hypoaspis aculeifer	AE F092944	Mixed into substrate 14 d, 5 % peat content	NOEC = 100 mg/kg dw	EFSA Conclusion Mesosulfuron-methyl, 2016
		Worst-case assumption	NOEC = 100 mg/kg dw	
Mefenpyr-diethyl				
Eisenia fetida	Mefenpyr-diethyl	Acute, 14d	LC_{50} > 500 mg a.s./kg dw* NOEC > 50 mg a.s./kg dw[2]	DAR of mefenpyr-diethyl, 2011
Eisenia fetida	AE F113225	Acute, 14d	LC_{50} > 1000 mg/kg dw (NOEC > 100 mg/kg dw[2])	DAR of mefenpyr-diethyl, 2011
Eisenia fetida	AE F094270	Acute, 14d	LC_{50} > 500 mg/kg dw*	DAR of mefenpyr-diethyl, 2011
		Chronic, 14d	NOEC = 50 mg/kg gw*	
Eisenia fetida	AE 2211046	-	LC_{50} > 50 mg a.s./kg dw*[3] (NOEC > 5 mg a.s./kg dw[2])	DAR of mefenpyr-diethyl, 2011
GLOB289H				
Eisenia fetida	GLOB289H	Mixed into substrate 56 d 10 % peat content	**NOEC = 325 mg/kg dw**	Friedrich S., 2018a
Eisenia fetida	GLOB289H + Actirob#	Mixed into substrate 56 d 10 % peat content	NOEC = 325 mg/kg dw	Friedrich S., 2019a
Eisenia fetida	GLOB289H + Pottok#	Mixed into substrate 56 d 10 % peat content	NOEC = 325 mg/kg dw	Friedrich S., 2019b
Folsomia candida	GLOB289H	Mixed into substrate 28 d 5 % peat content	**NOEC = 100 mg/kg dw**	Friedrich S., 2018b

Species	Substance	Exposure System	Results	Reference
Hypoaspis aculeifer	GLOB289H	Mixed into substrate 14 d 5 % peat content	**NOEC = 325 mg/kg dw**	Schulz L., 2018a
Field studies				
-				
Litter bag test				
-				

* Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002.
[2] Acute endpoint divided by 10 as a worst-case assumption for the chronic risk assessment
[3] Metabolite considered 10 times more toxic than mefenpyr-diethyl
[#] Concentrations are related to GLOB289H only. The adjuvant was applied at the ratio advised on the label.

9.8.1.1 Justification for new endpoints

For the active substances and most metabolites, EU agreed endpoints were used in the risk assessment. For iodosulfuron-methyl-sodium metabolites AE 0002166, BCS-CW81253 and AE F145740, 10 times the toxicity of the parent was assumed. This is considered acceptable as this is a worst-case scenario. For the formulation, the endpoints presented in Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with active substances iodosulfuron-methyl-sodium, mesosulfuron-methyl, safener mefenpyr-diethyl and their relevant metabolites. Full details of these studies are provided in the respective EU RAR and related documents.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of GLOB289H not evaluated as part of the EU assessment of the active substances.
To improve efficacy of the product, an adjuvant can be added in tank mix. As non-target organisms will be exposed to the combination of the formulation and the adjuvants, both the effect of the solo product and the combined effect on earthworms was addressed. Two different adjuvants were tested; a non-esterified rapeseed oil (Actirob) and a non-ionic surfactant (Pottok). As no effects of the adjuvants were observed in NTA and earthworms, no effect of the adjuvants is expected on other non-target soil organisms. Therefore, studies with the adjuvant on *Hypoaspis aculeifer* and *Folsomia candida* were not considered necessary.
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. For the formulation, the lowest endpoint of the different studies (solo PPP or PPP + adjuvant) was used for the risk assessment.
Table 9.8-1 were used.

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 (En-

vironmental Fate), Chapter 8.7.2, Table 8.7-3. According to the assessment of environmental-fate data, multi-annual accumulation in soil is considered for iodosulfuron-methyl-sodium, mesosulfuron-methyl and mefenpyr-diethyl.
To achieve a concise risk assessment, the risk envelope approach is applied with a critical GAP of 1 x 0.5 kg/ha

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 GLOB289H in cereals

Intended use			
Chronic effects on earthworms			
Product/active substance	**LC_{50} (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_a (criterion TER ≥ 5)**
Iodosulfuron-methyl sodium	7.0	0.0032	2187.5
AE F075736	0.7 *	0.0020	350 *
AE F161778	0.7 *	0.0003	2333.3 *
AE F059411	30.0	0.0005	60000.0
AE F145740	97.5	0.0003	325000.0
AE F145741	94.4	0.0002	472000.0
AE 0000119	97.8	0.0002	489000.0
BCS-CW81253	99.0	0.0008	123750.0
AE 0002166	**0.7**	**0.0005**	**1400.0**
Mesosulfuron-methyl	125.0	0.0199	6281.4
Mesosulfuron	12.5 *	0.0036	3472.2 *
AE F160459	12.5 *	0.0017	7352.94
AE F099095	12.5 *	0.0030	4166.67
AE F092944	12.5 *	0.0005	25000
AE F160460	12.5 *	0.0013	9615.38
AE F140584	12.5 *	0.0007	17857.14
AE F1447447	12.5 *	0.0023	5434.78
Mefenpyr-diethyl	> 50.0[1]	0.04800	1041.67
AE F113225	> 100.0[1]	0.01958	5107.25
AE F094270	50.0	0.0777	643.50
AE 2211046	> 5.0[*1]	0.00579	863.56
GLOB289H	325	0.5333	609.4
Chronic effects on other soil macro- and mesofauna			
Folsomia candida			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Iodosulfuron-methyl soduim	316.0	0.0032	98750.0
AE F075736	9.86	0.0020	4930.0
AE F161778	31.6 *	0.0003	105333.3 *

AE F059411	99.7	0.0005	199400.0
AE F145740	31.6 *	0.0003	105333.3 *
AE F145741	31.6 *	0.0002	158000 *
AE 0000119	31.6	0.0002	158000.0
BCS-CW81253	31.6	0.0008	93500.0
AE 0002166	31.6 *	0.0005	63200.0 *
Mesosulfuron-methyl	1000.0	0.0199	50251.3
Mesosulfuron	100.0	0.0036	27777.8
AE F160459	100.0	0.0017	58823.5
AE F099095	100.0 *	0.0030	33333.3 *
AE F092944	100	0.0005	200000.0
AE F160460	100.0 *	0.0013	76923.1 *
AE F140584	100.0 *	0.0007	142857.1 *
AE F1447447	100.0	0.0023	43478.3
GLOB289H	100.0	0.5333	187.5
Hypoaspis Aculeifer			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Iodosulfuron-methyl soduim	1000.0	0.0032	312500.0
AE F075736	9.86	0.0020	4930.0
AE F161778	100.0 *	0.0003	333333.3 *
AE F059411	98.7	0.0005	197400.0
AE F145740	100.0 *	0.0003	333333.33
AE F145741	100.0	0.0002	500000.0
AE 0000119	100	0.0002	500000.0
BCS-CW81253	100.0 *	0.0008	125000.00
AE 0002166	100.0 *	0.0005	200000.00
Mesosulfuron-methyl	1000.0	0.0199	50251.3
Mesosulfuron	100.0 *	0.0036	27777.8 *
AE F160459	100.0 *	0.0017	58823.6 *
AE F099095	100.0 *	0.0030	33333.3 *
AE F092944	100.0	0.0005	200000.0
AE F160460	100.0 *	0.0013	76923.1 *
AE F140584	100.0 *	0.0007	142857.1 *
AE F1447447	100.0 *	0.0023	43478.3 *
GLOB289H	325.0	0.5333	609.4

TER values shown in bold fall below the relevant trigger.
* When no valid experimental data were available for the metabolites, a screening risk assessment was carried out by considering the metabolites as ten times more toxic than the parent.

ZRMS comments:

The risk assessment for earthworms and other soil macroorganism for both a.s., safner and the product GLOB289 H was accepted by zRMS. However, in case of the risk assessment for mesosulfuron metabolites the risk was recalculated by zRMS with consideration avalible endpoints for earthworms, included in EFSA Journal 2016;14(10):4584.
In case of iodosulfuron – methyl metabolites the risk assessment for Folsomia candidia and Hypoapsis acc. due to the fact that the data was available for AE 0000119, BCS-CW81253 in the risk was considered these data instead of proposed by the applicant. For earthworm for metabolite AE 0002166 the endpoits was also available.
In case of mefenpyr-methyl metabolites acute toxicity data are available for mefenpyr-diethyl and the soil metabolites AE F113225 and AE F094270 were avalible in the DAR.
Chronic toxicity data were available only for the metabolite AE F094270 (single compound for which such data are required).
The soil metabolite AE F2211046 is considered 10 times more toxic than mefenpyr-diethyl since no toxicity data are available for this metabolite.
The TER values estimated for all the compounds are above the trigger values (10 for acute and 5 for chronic), indicating acceptable risks.

Table 9.8-3: 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 in cereals

Intended use			
Chronic effects on earthworms			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{LT} (criterion TER ≥ 5)**
Mesosulfuron			
AE F160459	90	0.0017	5294.11
AE F099095	100	0.0030	33333.33
AE F092944	10	0.0005	20000
AE F160460	100	0.0013	76923.07
AE F140584	117	0.0007	167142.85
AE F1447447	90	0.0023	39130.43
Iodosulfuron metabolites			
AE 0002166	95	0.0005	190000
Chronic effects on other soil macro- and mesofauna			
Folsomia candida			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Iodosulfuron-methyl metabolites			
AE 0000119	97.8	0.0002	489000
BCS-CW81253	99.0	0.0008	123750
Hypoaspis Aculeifer			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Iodosulfuron-methyl			

AE F145740	97.5	0.0003	325000
AE 0000119	97.8	0.0002	489000
BCS-CW81253	99	0.0008	123750
AE 0002166	95.2	0.0005	190400

TER values shown in bold fall below the relevant trigger.

Combined toxicity assessment for soil organisms:
An assessment of combination toxicity for soil organisms is provided here below, according to the tiered assessment scheme explained above (see combined toxicity assessment for birds, mammals and aquatic organisms).
The first step proceeds as a screening to check whether the TER for each single a.s. exceeds the regulatory trigger multiplied by the number of a.s. (here: trigger x 3).

Species	**TER values**			**Trigger all compounds**	**Trigger x n**	**TER ≥ trigger x n?**
	ISMS	**MSM**	**MDE**			
Eisenia fetida (reproduction)	2187.5	6281.4	1041.67	5	15	Yes
Folsomia candida	98750.0	50251.3	N/A*	5	15	Yes
Hypoaspis aculeifer	312500.0	50251.3	N/A*	5	15	Yes

* No EU reviewed data available for this endpoint, nevertheless no indication for concern based on results from the formulated product testing.

It can be concluded that the acute and long-term risk for soil organisms is acceptable, if the application of the product is done according to the proposed use pattern. This is also confirmed by the risk assessment with the formulation endpoint.

zRMS comments:

Combined toxicity assessment for soil organisms was not agreed approach at EU level.

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

All the long-term TER values calculated in the earthworms and other non-target soil organisms risk assessment largely exceed the trigger value of 5. Based on these results can be concluded that GLOB289H (+ adjuvant) poses low long-term risk to earthworms, collembola and predatory mites.

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 both active substances iodosulfuron-methyl sodium and mesosulfuron-methyl and their relevant metabolites. No data for mefenpyr-diethyl are available. However, studies on the effects on soil microbial activity were conducted with the terminal metabolite AE F094270 that will result from the biological breakdown of mefenpyr-diethyl in soils. Full details of these studies are provided in the respective EU DAR/RAR and related documents.

Effects on soil microorganisms of GLOB289H were not evaluated as part of the EU assessment of the active substances.
To improve efficacy of the product, an adjuvant can be added in tank mix. Although the adjuvants are not persistent in soil and no exposure to the adjuvants is expected, both the effect of the solo product and the combined effect on soil micro-organisms was addressed. Two different adjuvants were tested; a non-esterified rapeseed oil (Actirob) and a non-ionic surfactant (Pottok).
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
Iodosulfuron-methyl sodium				
N-mineralisation	Iodosulfuron-methyl sodium	28 d, aerobic soil type	Treatment caused < 25% deviation from control; 0.067 mg/kg dry soil	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
N-mineralisation	AE F075736 (as formulated product 20 DP)	28 d, aerobic soil type	NOAEC = 0.2 mg/kg dry soil	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
N-mineralisation	AE F145741	28 d, aerobic soil type	16% effect at day 28 at rated up to 0.063 mg/kg soil dw	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
		Worst-case assumption	0.0067 mg/kg dry soil	10x more toxic than parent
N-mineralisation	AE F145740	28 d, aerobic soil type	14.2% effect at day 28 at rated up to 0.063 mg/kg soil dw	EFSA Conclusion Iodosulfuron-methyl sodium, 2016

Endpoint	Substance	Exposure System	Results	Reference
		Worst-case assumption	0.0067 mg/kg dry soil	10x more toxic than parent
N-mineralisation	AE 0002166	28 d, aerobic soil type	17.3% effect at day 28 at rated up to 0.053 mg/kg soil dw	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
		Worst-case assumption	0.0067 mg/kg dry soil	10x more toxic than parent
N-mineralisation	AE F161778	28 d, aerobic soil type	16.3% effect at day 28 at rated up to 0.049 mg/kg soil dw	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
		Worst-case assumption	0.0067 mg/kg dry soil	10x more toxic than parent
N-mineralisation	BCS-CW81253	28 d, aerobic soil type	17.1% effect at day 28 at rated up to 0.043 mg/kg soil dw	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
		Worst-case assumption	0.0067 mg/kg dry soil	10x more toxic than parent
N-mineralisation	AE 0000119	28 d, aerobic soil type	2.9% effect at day 28 at rated up to 0.4 mg/kg soil dw	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
		Worst-case assumption	0.0067 mg/kg dry soil	
N-mineralisation	AE F059411	42 d, aerobic soil type	22.9% effect at day 42 at rated up to 0.204 mg/kg soil dw	EFSA Conclusion Iodosulfuron-methyl sodium, 2016
Mesosulfuron-methyl				
N-mineralisation	Mesosulfuron-methyl	28 d, aerobic soil type	< 25% effect at day 28 at 0.1 mg/kg soil dw	EFSA Conclusion Mesosulfuron-methyl, 2016
N-mineralisation	Mesosulfuron	28 d, aerobic soil type	< 25% effect at day 28 at 0.1 mg/kg soil dw	EFSA Conclusion Mesosulfuron-methyl, 2016
N-mineralisation	AE F160459	42 d, aerobic soil type	< 25% effect at day 42 at 0.1 mg/kg soil dw	EFSA Conclusion Mesosulfuron-methyl, 2016
N-mineralisation	AE F099095	28 d, aerobic soil type	< 25% effect at day 28 at 0.1 mg/kg soil dw	EFSA Conclusion Mesosulfuron-methyl, 2016
N-mineralisation	AE F092944 (SSRE-002)	28 d, aerobic soil type	< 25% effect at day 28 at 0.06 mg/kg soil dw	EFSA Conclusion Mesosulfuron-methyl, 2016 (original from EFSA conclusion Flazasulfuron 2016)
N-mineralisation	AE F092944	28 d, aerobic soil type	< 25% effect at day 28 at 0.137 mg/kg soil dw	EFSA Conclusion Mesosulfuron-methyl, 2016
N-mineralisation	AE F147447	28 d, aerobic	< 25% effect at day 28 at 0.057 mg/kg	EFSA Conclusion

Endpoint	Substance	Exposure System	Results	Reference
		soil type	soil dw	Mesosulfuron-methyl, 2016
Mefenpyr-diethyl				
Carbon transformation	AE F094270	28d	No influence 0.67 mg/kg; 500g/ha	DAR mefenpyr-diethyl, 2011
Nitrogen transformation	AE F094270	28d	No influence 0.67 mg/kg; 500g/ha	DAR mefenpyr-diethyl, 2011
GLOB289H				
N-mineralisation	GLOB289H	28 d, aerobic soil type	< 25% effect at day 28 at 5.3 mg/kg soil dw	Schulz L., 2018b
N-mineralisation	GLOB289H + Actirob B#	56d, aerobic soil type	< 25% effect at day 56 at 5.3 mg/kg soil dw	Persdorf M., 2019a
N-mineralisation	GLOB289H + Pottok#	28 d, aerobic soil type	**< 25% effect at day 28 at 3.35 mg/kg soil dw**	Persdorf M., 2019b

Concentrations are related to GLOB289H only. The adjuvant was applied at the ratio advised on the label.

ZRMS comments:

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 assessment covering the proposed use pattern are taken from Section 8 (Environmental Fate), No risk for soil micro-organisms is expected after the application GLOB289H + Pottok and GLOB289H + Pottok and according to the proposed GAP as the <25% effects were observed f 5.3 and 3.35 mg product/kg dws respectively.

9.9.1.1 Justification for new endpoints

For the active substances and most metabolites, EU agreed endpoints were used in the risk assessment. For iodosulfuron-methyl-sodium metabolites AE 0002166, BCS-CW81253, AE F145740, AE F145741 and AE F161778, 10 times the toxicity of the parent was assumed. This is considered acceptable as this is a worst-case assumption. For the formulation, the endpoints presented in Table 9.9-1 were used.

9.9.2 Risk assessment

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

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.8).

To achieve a concise risk assessment, the risk envelope approach is applied with the critical GAP of 1 x

0.5 kg/ha.

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of GLOB289H in cereals

Intended use	Cereals		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
Iodosulfuron-methyl-sodium	0.067 (at 28 d)	0.0032	yes
AE F075736 (as formulated product 20 DP)	0.2 (at 28 d)	0.0020	Yes
AE F145741	0.0067	0.0002	Yes
AE F145740	0.0067	0.0003	Yes
AE 0002166	0.0067	0.0005	Yes
AE F161778	0.0067	0.0003	Yes
BCS-CW81253	0.0067	0.0008	Yes
AE 0000119	0.4 (at 28 d)	0.0002	Yes
AE F059411	0.204 (at 42 d)	0.0005	Yes
Mesosulfuron-methyl	0.1 (at 28 d)	0.0199	Yes
Mesosulfuron	0.1 (at 28 d)	0.0036	Yes
AE F160459	0.1 (at 42 d)	0.0017	Yes
AE F099095	0.1 (at 28 d)	0.0030	Yes
AE F092944 (SSRE-002)	0.06 (at 28 d)	0.0005	Yes
AE F147447	0.57 (at 28 d)	0.0023	Yes
GLOB289H	3.35 (at 28 d)	0.5333	Yes

9.9.3 Overall conclusions

The maximum concentrations with an effect ≤ 25% are higher than the maximum PEC_{soil} of iodosulfuron-methyl-sodium, mesosulfuron-methyl, their relevant metabolites and GLOB289H (+ adjuvant) from the intended use so the risk is acceptable.
The use of GLOB289H according to the proposed use pattern in cereals will not have unacceptable effects on soil micro-organisms.

zRMS comments:

The maximum concentrations with an effect ≤ 25% are higher than the maximum PEC_{soil} of iodosulfuron-methyl-sodium, mesosulfuron-methyl, their relevant metabolites and GLOB289H (+ adjuvant) from the intended use . The risk is acceptable.

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 iodosulfuron-methyl-sodium, mesosulfuron-methyl and their relevant metabolites. No data on mefenpyr-diethyl are available. However, as mefenpyr-diethyl is a safener, no effects to non-target plants are expected. In addition, mefenpyr-diethyl is covered by the risk assessment of the formulated product. Full details of the available studies are provided in the respective EU DAR and related documents.

Effects on non-target terrestrial plants of GLOB289H were not evaluated as part of the EU assessment of iodosulfuron-methyl-sodium or mesosulfuron-methyl.
To improve efficacy of the product, an adjuvant can be added in tank mix. As the non-target plants will be exposed to the tank-mix, both the effect of the solo product and the combined effect with the adjuvants on non-target plants was addressed. Two different adjuvants were tested; a non-esterified rapeseed oil (Actirob) and a non-ionic surfactant (Pottok). As the adjuvants are not persistent in soil, only vegetative vigour was addressed and not seedling emergence.
New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

Species	Substance	Exposure System	Results	Reference
Iodosulfuron-methyl-sodium				
Allium cepa *Lolium perenne* *Zea mays* *Lycopersicon esculentum* *Pisum sativum* *Sinapis alba*	Iodosulfuron-methyl-sodium (formulated as WG20)	21d vegetative vigour	ER_{50} plant height = 0.042 g/ha	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Allium cepa *Brassica oleracea* *Lolium perenne* *Zea mays* *Cucumis sativus* *Lactuca sativa* *Avena sativa* *Glycine max* *Lycopersicon esculentum* *Brassica rapa*	Iodosulfuron-methyl-sodium (formulated as WG20)	21 d vegetative vigour	ER_{50} shoot weight = 0.036 g/ha	EFSA Conclusion Iodosulfuron-methyl-sodium, 2016
Mesosulfuron-methyl-sodium				
Sunflower (most sensitive species)	Preparation	21 d Vegetative vigour	ER_{50} = 27 mL prod./ha	EFSA Conclusion Mesosulfuron-methyl, 2016
Onion (most sensitive species	Preparation	21 d Seedling emergence	ER_{50} = 64 mL prod/ha	EFSA Conclusion Mesosulfuron-methyl, 2016
Mean from data for 8 species	Preparation	21 d Vegetative vigour	HC5 = 16 mL prod/ha	EFSA Conclusion Mesosulfuron-methyl, 2016

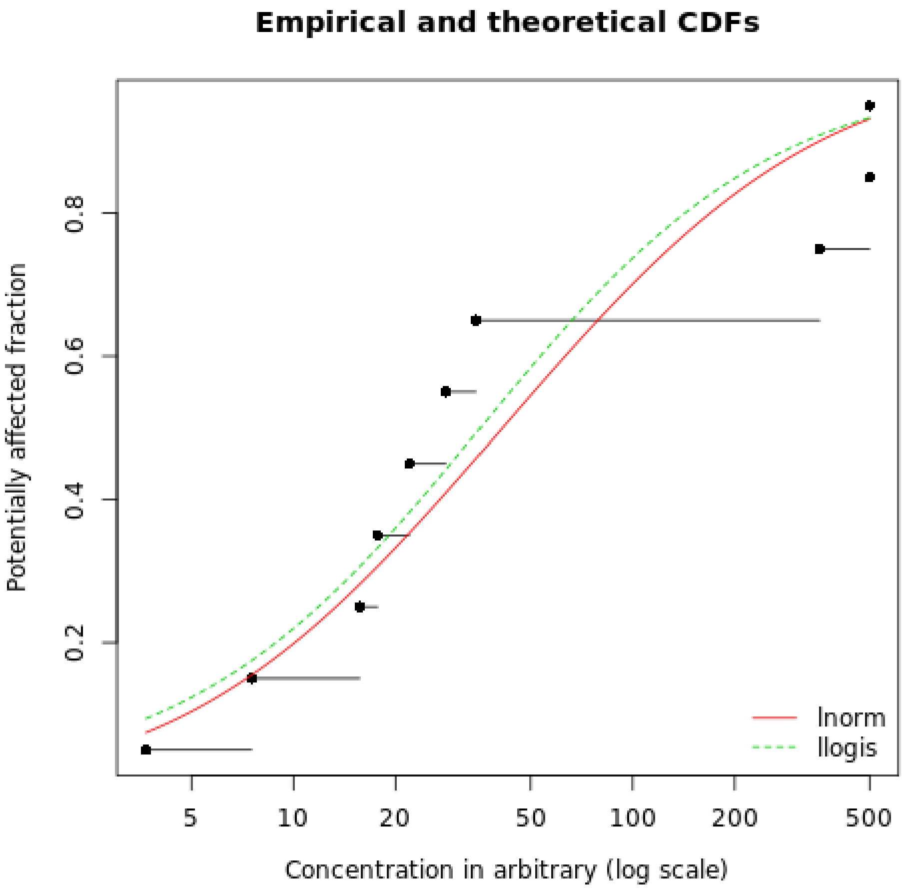
Species	Substance	Exposure System	Results	Reference
GLOB289H				
Zea mays (m) *Avena sativa (m)* *Allium cepa (m)* *Cucumis sativus (d)* *Brassica napus (d)* *Raphanus sativus (d)* *Glycine max (d)* *Beta vulgaris (d)* ***Helianthus annuus (d)*** *Lycopersicon esculentum (d)*	GLOB289H	Vegative vigour	ER_{50} shoot weight = 43.393 g prod./ha (value for the most sensitive species, ~~*Helianthus annuus (d))*~~ radish (d)	Davies C.; 2018
Zea mays (m) *Avena sativ (m)* *Allium cepa (m)* *Cucumis sativus (d)* *Brassica napus (d)* *Raphanus sativus (d)* *Glycine max (d)* *Beta vulgaris (d)* ***Helianthus annuus (d)*** *Lycopersicon esculentum (d)*	GLOB289H + Actirob	Vegetative vigour	ER_{50} shoot weight = 3.67 g prod./ha (value for the most sensitive species, *Helianthus annuus (d))* HC5= **1.9 g prod/ha** (based on an SSD of 10 species)	Davies C., 2019
Raphanus sativus (d) *Helianthus annuus (d)* *Lycopersicon (d) esculentum* *Beta vulgaris (d)* *Glycine max (d)* ***Allium cepa (m)***	GLOB289H + Pottok	Vegetative vigour	ER_{50} shoot weight = 16.014 g prod./ha (value for the most sensitive species, *Allium cepa (m))*	Huerta F., 2019
Zea mays (m) *Avena sativa (m)* *Allium cepa (m)* *Cucumis sativus (d)* *Brassica napus (d)* ***Raphanus sativus (d)*** *Glycine max (d)* *Beta vulgaris (d)* *Helianthus annuus (d)* *Lycopersicon esculentum (d)*	GLOB289H	Seedling emergene	ER_{50} fresh weight = 51.483 g prod./ha (value for the most sensitive species, *Rophanus sativus (d))*	Stead A.; 2018

m: monocotyledonous; d: dicotyledonous

9.10.1.1 Justification for new endpoints

Since GLOB289H was not the representative formulation during the EU review, a new risk assessment for vegetative vigour and seedling emergence is provided here and is considered adequate. The risk assessment was performed using the lowest endpoint obtained from studies with GLOB289H and GLOB289H + adjuvants as presented in Table 9.10-1Table 9.7-1. The most sensitive endpoint was observed for the formulation in combination with Actirob, with an ER_{50} = 3.67 g PPP/ha. As 10 species were available, the HC5 was calculated. The Species Sensitivity Distribution has been calculated using the **Mosaic tool.** Based on ER_{50} values from 10 species, the median HC5 was calculated to be 1.9 g/ha.

Species	E_rC_{50} (g/ha)	
Zea mays (corn)	> 500	Median HC_5 = 1.9 g/ha n = 10
Avena sativa (oats)	17.70	
Allium cepa (onion)	> 500	
Cucumis sativus (cucumber)	355.74	
Brassica napus (oilseed rape)	7.52	
Raphanus sativus (radish)	15.69	
Glycine max (soybean)	28.15	
Beta vulgaris (sugar beet)	34.53	
Helianthus annuus (sunflower)	3.67	
Lycopersicon esculentum (tomato)	21.97	



Log normal distribution (log-likelihood = -56.6)
meanlog: 3.7 [2.6 ; 4.8]
sdlog: 1.7 [0.86 ; 2.3]
Log logistic distribution (log-likelihood = -57.0)
shape: 36 [12 ; 1e+02]
scale: 1 [0.67 ; 2]
Figure 9.10.1: Species Sensitivity Distribution for non-target plants exposed to GLOB289H + Actirob, based on EC_{50} values (10 species – MOSAIC tool)

Table 9.10-2: HC5 with specified confidence interval

HC	Log-normal	Log-logistic
HC5	2.6 [0.62 ; 15]	**1.9 [0.3 ; 12]**
HC10	4.8 [1.3 ; 22]	3.9 [0.85 ; 19]
HC20	10 [3.2 ; 37]	8.9 [2.5 ; 32]
HC50	41 [14 ; 1.2e+02]	36 [12 ; 1e+02]

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.

Maximum dose in winter cereals (500 g/ha)
The $PER_{off\text{-}field}$ is calcualated as: Application rate x MAF x drift factor
1m buffer zone: $PER_{off\text{-}field}$ = 500 g/ha x 1 x 0.0277 = 13.85 g/ha
5m buffer zone: $PER_{off\text{-}field}$ = 500 g/ha x 1 x 0.0057 = 2.85 g/ha
10 m buffer zone $PER_{off\ field}$ = 500g/ha x 1 x 0.0029=1.44 g/ha
Lower dose in winter cereals and spring cereal (300 g/ha)
1m buffer zone: $PER_{off\text{-}field}$ = 300 g/ha x 1 x 0.0277 = 8.31 g/ha
5m buffer zone: $PER_{off\text{-}field}$ = 300 g/ha x 1 x 0.0057= 1.71 g/ha
10 m buffer zone $PER_{off\ field}$ = 300g/ha x 1 x 0.0029= 0.87 g/ha

For the risk assessment, only the most sensitive species was used as a worst-case approach.

Table 9.10-3: Assessment of the risk for non-target plants due to the use of GLOB289H in cereals – 500 g/ha

Intended use	Cereals			
Active substance/product	GLOB289H			
Application rate (g/ha)	1 × 500			
MAF	1			
Test species	**HC5 (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**TER criterion: TER ≥ 1**
Helianthus annuus (d)	~~2.04*~~ 1.9	2.77	13.85	**~~0.15~~** **0.13**

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

Table 9.10-4: Assessment of the risk for non-target plants due to the use of GLOB289H in cereals – 300 g/ha

Intended use	Cereals			
Active substance/product	GLOB289H			
Application rate (g/ha)	1 × 300			
MAF	1			
Test species	**HC5* (g/ha)**	**Drift rate**	**PER$_{off-field}$ (g/ha)**	**TER criterion: TER ≥ 1**
Helianthus annuus (d)	~~2.04~~ 1.9	2.77	8.31	~~**0.25**~~ **0.22**

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

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

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

Table 9.10-5: Risk assessment for non-target terrestrial plants due to the use of GLOB289H in cereals considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles) – winter cereals 0.5kg GLOB289H/ha

Intended use		Winter cereals			
Product		GLOB289H			
Application rate (g/ha)		1 x 500			
MAF		1			
Buffer strip (m)	**Drift rate (%)**	**PER$_{off-field}$ (g/ha)**	**PER$_{off-field}$ 50 % drift red. (g/ha)**	**PER$_{off-field}$ 75 % drift red. (g/ha)**	**PER$_{off-field}$ 90% drift red. (g/ha)**
1	2.77	13.85	6.925	3.4625	1.385
5	0.57	2.85	1.425	0.7125	0.285
10	0.29	1.45	0.725	0.3625	0.145
Toxicity value ~~ER$_{50}$~~ HC$_5$=1.9 g PPP/ha		**TER Criterion: TER ≥ 1**			
1		**0.14**	**0.27**	**0.55**	1.37
5		**0.67**	1.33	2.67	6.67
10		1.31	2.62	5.24	13.10

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

Table 9.10-6: Risk assessment for non-target terrestrial plants due to the use of GLOB289H in cereals considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles) – winter and spring cereals 0.3kg GLOB289H/ha

Intended use		Winter cereals			
Product		GLOB289H			
Application rate (g/ha)		1 x 300			
MAF		1			
Buffer strip (m)	**Drift rate (%)**	**$PER_{off-field}$ (g/ha)**	**$PER_{off-field}$ 50 % drift red. (g/ha)**	**$PER_{off-field}$ 75 % drift red. (g/ha)**	**$PER_{off-field}$ 90% drift red. (g/ha)**
1	2.77	8.31	4.155	2.0775	0.831
5	0.57	1.71	0.855	0.4275	0.171
10	0.29	0.87	0.435	0.2175	0.087
Toxicity value ~~ER_{50}~~ **HC_5 = 1.9 g PPP/ha**		**TER Criterion: TER ≥ 1**			
1		**0.23**	**0.46**	**0.91**	2.29
5		1.11	2.22	4.44	11.11
10		2.18	4.37	8.74	21.84

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

9.10.3 Overall conclusions

The use of GLOB289H according to the proposed use pattern in cereals will not have unacceptable effects on considering risk mitigation measures are used. Following combinations offer an acceptable risk:
Winter cereals – 0.5 kg/ha

- 1m buffer zone combined with 90% drift reducing nozzles
- 5m buffer zone combined with 50% drift reducing nozzles
- 10m buffer zone

Winter and spring cereals – 0.3 kg/ha

- 1m buffer zone combined with 90% drift reducing nozzles
- 5 m buffer zone

ZRMS comments:

The probabilistic risk assessment h was accepted by zRMS with consideration of HC5 value of 1.9 g product/ha and AF of 1.
It is the position of the ZRMS-PL that a trigger value of 1 should be used in the probabilistic risk assessment with a HR5 value.
However, it is noted that this is not a Central Zone harmonised position and other member states may consider the use of a different trigger value at National Registration.
Based on the probabilistic risk assessment it is concluded that the use of the product will not produce unacceptable effects on terrestrial non-target plants growing near treated fields, when considering the following mitigation measures:

Winter cereals – 0.5 kg/ha
- 1m buffer zone combined with 90% drift reducing nozzles
- 5m buffer zone combined with 50% drift reducing nozzles
- 10m buffer zone
-

Winter and spring cereals – 0.3 kg/ha
- 1m buffer zone combined with 90% drift reducing nozzles
- 5 m buffer zone

The determistic risk assessment was provided by zRMS for completeness and is presented in the Tables below:

Table 9.10-7-1: Determistic risk assessment of the risk for non-target plants due to the use of GLOB289H in cereals – 500 g/ha.

Intended use	Cereals			
Active substance/product	GLOB289H			
Application rate (g/ha)	1 × 500			
MAF	1			
Test species	**ER50 (g/ha)**	**Drift rate**	**$PER_{off-field}$ (g/ha)**	**TER criterion: TER ≥ 1**
Helianthus annuus (d)	3.67	2.77	13.85	**0.26**

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

Table 9.10-8-1: Determistic risk assessment of the risk for non-target plants due to the use of GLOB289H in cereals – 300 g/ha.

Intended use	cereals			
Active substance/product	GLOB289H			
Application rate (g/ha)	1 × 300			
MAF	1			
Test species	**ER50 (g/ha)**	**Drift rate**	**$PER_{off-field}$ (g/ha)**	**TER criterion: TER ≥ 1**
Helianthus annuus (d)	3.67	2.77	8.31	**0.44**

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

Table 9.10-9-1: Risk assessment for non-target terrestrial plants due to the use of GLOB289H in cereals considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles) – winter cereals 0.5kg GLOB289H/ha.

Intended use		Winter cereals			
Product		GLOB289H			
Application rate (g/ha)		1 x 500			
MAF		1			
Buffer strip (m)	**Drift rate (%)**	**$PER_{off-field}$ (g/ha)**	**$PER_{off-field}$ 50 % drift red. (g/ha)**	**$PER_{off-field}$ 75 % drift red. (g/ha)**	**$PER_{off-field}$ 90% drift red. (g/ha)**
1	2.77	13.85	6.925	3.4625	1,385
5	0.57	2.85	1.425	0.7125	0,285
10	0.29	1.45	0.725	0.3625	0,145
Toxicity value ER_{50} =3.67 g PPP/ha		**TER Criterion: TER ≥ 5**			
1		0.26	0.53	1.06	2.65
5		1.29	2.57	**5.11**	**12.87**
10		2.53	**5.06**	**10.12**	**25.31**

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

Table 9.10-10-1: Risk assessment for non-target terrestrial plants due to the use of GLOB289H in cereals considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles) – winter and spring cereals 0.3kg GLOB289H/ha.

Intended use		Winter cereals			
Product		GLOB289H			
Application rate (g/ha)		1 x 300			
MAF		1			
Buffer strip (m)	**Drift rate (%)**	**$PER_{off-field}$ (g/ha)**	**$PER_{off-field}$ 50 % drift red. (g/ha)**	**$PER_{off-field}$ 75 % drift red. (g/ha)**	**$PER_{off-field}$ 90% drift red. (g/ha)**
1	2.77	8.31	4.155	2.0775	0.831
5	0.57	1.71	0.855	0.4275	0.171
10	0.29	0.87	0.435	0.2175	0.087
Toxicity value ER_{50} = 3.67 g PPP/ha		**TER Criterion: TER ≥ 5**			
		0.44	0.88	1.77	4.42
		2.15	4.29	**8.58**	**21.46**
		4.22	**8.44**	**16.87**	**42.18**

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

Based on the determistic risk assessment it is concluded that the use of the product will not produce unacceptable effects on terrestrial non-target plants growing near treated fields, when considering the following mitigation measures:

Winter cereals – 0.5 kg/ha

- 10m buffer zone combined with 50% drift reducing nozzles or 5 m buffer zone combined with with 75 % drift reducing nozzles

Winter and spring cereals – 0.3 kg/ha

- 10m buffer zone combined with 50% drift reducing nozzles or 5 m buffer zone combined with with 75 % drift reducing nozzles

The final risk mitigation measures should be decided at MSs level.

ZRMS comments:

The probabilistic risk assessment h was accepted by zRMS with consideration of HC5 value of 1.9 g product/ha and AF of 1.
It is the position of the ZRMS-PL that a trigger value of 1 should be used in the probabilistic risk assessment with a HR5 value.
However, it is noted that this is not a Central Zone harmonised position and other member states may consider the use of a different trigger value at National Registration.
Based on the probabilistic risk assessment it is concluded that the use of the product will not produce unacceptable effects on terrestrial non-target plants growing near treated fields, when considering the following mitigation measures:

Winter cereals – 0.5 kg/ha
- 1m buffer zone combined with 90% drift reducing nozzles
- 5m buffer zone combined with 50% drift reducing nozzles
- 10m buffer zone

Winter and spring cereals – 0.3 kg/ha
- 1m buffer zone combined with 90% drift reducing nozzles
- 5 m buffer zone

The determistic risk assessment was provided by zRMS for completeness and is presented in the Tables below:

Table 9.10-11-1: Determistic risk assessment of the risk for non-target plants due to the use of GLOB289H in cereals – 500 g/ha.

Intended use	Cereals			
Active substance/product	GLOB289H			
Application rate (g/ha)	1 × 500			
MAF	1			
Test species	**ER50 (g/ha)**	**Drift rate**	**$PER_{off-field}$ (g/ha)**	**TER criterion: TER ≥ 5**
Helianthus annuus (d)	3.67	2.77	13.85	**0.26**

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

Table 9.10-12-1: Determistic risk assessment of the risk for non-target plants due to the use of GLOB289H in cereals – 300 g/ha.

Intended use	cereals			
Active substance/product	GLOB289H			
Application rate (g/ha)	1 × 300			
MAF	1			
Test species	**ER50 (g/ha)**	**Drift rate**	**PER$_{off-field}$ (g/ha)**	**TER criterion: TER ≥ 5**
Helianthus annuus (d)	3.67	2.77	8.31	**0.44**

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

Table 9.10-13-1: Risk assessment for non-target terrestrial plants due to the use of GLOB289H in cereals considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles) – winter cereals 0.5kg GLOB289H/ha.

Intended use		Winter cereals			
Product		GLOB289H			
Application rate (g/ha)		1 x 500			
MAF		1			
Buffer strip (m)	**Drift rate (%)**	**PER$_{off-field}$ (g/ha)**	**PER$_{off-field}$ 50 % drift red. (g/ha)**	**PER$_{off-field}$ 75 % drift red. (g/ha)**	**PER$_{off-field}$ 90% drift red. (g/ha)**
1	2.77	13.85	6.925	3.4625	1,385
5	0.57	2.85	1.425	0.7125	0,285
10	0.29	1.45	0.725	0.3625	0,145
Toxicity value ER$_{50}$ =3.67 g PPP/ha		**TER Criterion: TER ≥ 5**			
1		0.26	0.53	1.06	2.65
5		1.29	2.57	**5.11**	**12.87**
10		2.53	**5.06**	**10.12**	**25.31**

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

Table 9.10-14-1: Risk assessment for non-target terrestrial plants due to the use of GLOB289H in cereals considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles) – winter and spring cereals 0.3kg

GLOB289H/ha.

Intended use		Winter cereals			
Product		GLOB289H			
Application rate (g/ha)		1 x 300			
MAF		1			
Buffer strip (m)	**Drift rate (%)**	**PER$_{off-field}$ (g/ha)**	**PER$_{off-field}$ 50 % drift red. (g/ha)**	**PER$_{off-field}$ 75 % drift red. (g/ha)**	**PER$_{off-field}$ 90% drift red. (g/ha)**
1	2.77	8.31	4.155	2.0775	0.831
5	0.57	1.71	0.855	0.4275	0.171
10	0.29	0.87	0.435	0.2175	0.087
Toxicity value ER$_{50}$ = 3.67 g PPP/ha		**TER Criterion: TER ≥ 5**			
		0.44	0.88	1.77	4.42
		2.15	4.29	**8.58**	**21.46**
		4.22	**8.44**	**16.87**	**42.18**

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

Based on the determistic risk assessment it is concluded that the use of the product will not produce unacceptable effects on terrestrial non-target plants growing near treated fields, when considering the following mitigation measures:

Winter cereals – 0.5 kg/ha

- 10m buffer zone combined with 50% drift reducing nozzles or 5 m buffer zone combined with with 75 % drift reducing nozzles

Winter and spring cereals – 0.3 kg/ha

- 10m buffer zone combined with 50% drift reducing nozzles or 5 m buffer zone combined with with 75 % drift reducing nozzles

The final risk mitigation measures should be decided at MSs level.

Commenting period process:

It should highlight that the data was analysed through the tool MOSAIC which uses the log-likelihood method for the evaluation of the goodness-of-fit, different method than the one used in other statistical programs as ETX.
In King *et al*. (2013) - available at https://arxiv.org/ftp/arxiv/papers/1311/1311.5772.pdf - goodness-of-fit methods are explained in high detail, but in summary there are three main ones: likelihood function (used in MOSAIC), the least-square regression on the empirical Cumulative Distribution Function (used in CADDIS_SSD) and moment matching (used in ETX). As the author’s state: “Apart from maximum likelihood, there is no straightforward approach for a non-expert in statistics to make use of all types of censored data. Indeed, all of the available turn-key software for SSD fitting require the use of non-censored data. Yet, there is a possibility to use the R-package fitdistrplus [10] to fit censored data using maximum likelihood”.

Considering that the data set used to derive the SSD contained two unbounded values, MOSAIC was indeed the best tool to use attending at the arguments above. The derived SSD curves presented similar but slightly different log-likelihood values. The highest log-likelihood value (-56.6) came from the log-normal distribution indicating that this is the function that best suits the data. Even so, the Applicant used the lowest HC5 value that came from the log-logistic distribution for the risk assessment as a conservative approach.

Since MOSAIC does not allow the check of the data normality (because its acceptability is done on the likelihood function), the Applicant run the SSD with and without the unbounded values on ETX just to prove the goodness-of-fit and that the approach presented in this Core Dossier is reliable and the correct one.

For reference, the lowest HC5 derived in the Core Dossier was 1.9 g/ha.

ETX 2.3 - SSD only with bounded values

HC5 = 2.223 g/ha

N=8

Goodness-of-fit

Anderson-Darling test for normality		
Sign. level	Critical	Normal?
0.1	0.631	Accepted
0.05	0.752	Accepted
0.025	0.873	Accepted
0.01	1.035	Accepted
Kolmogorov-Smirnov test for normality		
Sign. level	Critical	Normal?
0.1	0.819	Accepted
0.05	0.895	Accepted
0.025	0.995	Accepted
0.01	1.035	Accepted
Cramer von Mises test for normality		
Sign. level	Critical	Normal?
0.1	0.104	Accepted
0.05	0.126	Accepted
0.025	0.148	Accepted
0.01	0.179	Accepted

ETX 2.3 - SSD with all values (bounded and unbounded)

HC5 = 2.048 g/ha

N=10

Goodness-of-fit

Anderson-Darling test for normality		
Sign. level	Critical	Normal?
0.1	0.631	Rejected
0.05	0.752	Accepted
0.025	0.873	Accepted
0.01	1.035	Accepted
Kolmogorov-Smirnov test for normality		
Sign. level	Critical	Normal?
0.1	0.819	Rejected
0.05	0.895	Accepted
0.025	0.995	Accepted
0.01	1.035	Accepted
Cramer von Mises test for normality		
Sign. level	Critical	Normal?
0.1	0.104	Accepted
0.05	0.126	Accepted
0.025	0.148	Accepted
0.01	0.179	Accepted

At the significance level of 5% as normally accepted in statistics, both SSDs run in ETX are acceptable. The SSD with the 10 values (2 unbounded) failed to pass at the significance level of 10 % in two tests, especially since ETX is not designed to include these data in the analysis. Even so, it passes at the last test for normality.

To be noted that all HC5 values derived in ETX (2.223 and 2.048 g/ha) are above the value used in the risk assessment coming from the analysis run in MOSAIC (1.9 g/ha).

Attending at all the arguments and additional analyses provided above, the approach delivered in the is the correct one and these calculations provided above should only be regarded as additional information.

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

Tests on other non-target species are not required.

9.12 Monitoring data (KCP 10.8)

Not relevant

9.13 Classification and Labelling

Pictogram:	GHS09
Signal word:	Warning

H-statements

H400	Very toxic to aquatic life.
H410	Very toxic to aquatic life, lasting the long-term effect to aquatic organism

P-statements

P273	Avoid release into the environment.
P391	Collect spillage.
P501	Dispose of contents/container to … in accordance with local/regional/national/international regulations (to be specified).

Other safety/precautionary phrases:

SP1
SPe3
EUH401

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 10.2-01	Renner P.	2018a	Acute toxicity of GLOB289H to *Daphnia magna* in a 48-hour static test 18 48 ADL 0008 Biochem agrar GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A
KCP 10.2-02	Renner P.	2018b	Effects of GLOB289H on *Pseudokirchneriella subcapitata* in an algal growth inhibition test 18 48 ADL 0019 Biochem agrar GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A
KCP 10.2-03	Renner P.	2018c	Effects of GLOB289H on *Lemna gibba* in a growth rate inhibition test under semi-static test conditions. 18 48 ALE 0006 Biochem agrar GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A
KCP 10.2-04	Renner P.	2019a	Effects of GLOB289H and Actirob B on *Lemna gibba* in a growth inhibition test under semi-static test conditions. 19 48 ALE 0004 Biochem agrar GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A
KCP 10.2-05	Renner P.	2019b	Effects of Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (6 + 30 + 90) g/kg WG (SAP63H) and the adjuvant (Pottok) on *Lemna gibba* in a growht inhibition test under semi-static test conditions 19 48 ALE 0007 Biocem agrar GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A
KCP 10.2-06_01 (also submitted as KCA 8.2.7_08-01)	Dill, M.	2018a	Mesosulfuron-methyl technical: Growth inhibition of *Nasturtium officinale* in a water/sediment system S18-00146 Eurofins GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A Helm A.G

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 10.2-06_02 (also submitted as KCA 8.2.7_08-02)	Dill, M.	2018b	Mesosulfuron-methyl technical: Growth inhibition of *Hottonia palustris* in a water/sediment system S18-00147 Eurofins GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A Helm A.G
KCP 10.2-06_03 (also submitted as KCA 8.2.7_08-03)	Dill, M.	2018c	Mesosulfuron-methyl technical: Growth inhibition of *Myriophyllum sibiricum* in a water/sediment system S18-00148 Eurofins GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A Helm A.G.
KCP 10.2-06_04 (also submitted as KCA 8.2.7_08-04)	Dill, M.	2018d	Mesosulfuron-methyl technical: Growth inhibition of *Ceratophyllum demersum* in a water/sediment system S18-00149 Eurofins GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A Helm A.G.
KCP 10.2-06_05 (also submitted as KCA 8.2.7_08-05)	Dill, M.	2018e	Mesosulfuron-methyl technical: Growth inhibition of *Vallisneria spiralis* in a water/sediment system S18-00150 Eurofins GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A Helm A.G.
KCP 10.2-06_06 (also submitted as KCA 8.2.7_08-0f)	Dill, M.	2018f	Mesosulfuron-methyl technical: Growth inhibition of *Glyceria maxima* in a water/sediment system S18-00151 Eurofins GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A Helm A.G.
KCP 10.2-06_07 (also submitted as KCA 8.2.7_08-07)	Dill, M.	2018g	Mesosulfuron-methyl technical: Growth inhibition of *Elodea canadensis* in a water/sediment system S18-00152 Eurofins GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A Helm A.G.
KCP 10.2-06_08	Dill, M.	2018h	Mesosulfuron-methyl technical: Growth inhibition of *Wolffia arrhiza* in a water/sediment system S18-00153	N	Globachem N.V. Ascenza

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
(also submitted as KCA 8.2.7_08-08)			Eurofins GLP Unpublished		Agro S.A Helm A.G.
KCP 10.2-06_09 (also submitted as KCA 8.2.7_08-09)	Dill, M.	2018i	Mesosulfuron-methyl technical: Growth inhibition of *Spirodela polyrhiza* in a water/sediment system S18-00154 Eurofins GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A Helm A.G
KCP 10.2-07 (also submitted as KCA 8.2.7_09)	Lang, C.	2018	Mesosulfuron-methyl technical: Toxicity to the Duckweed *Lemna gibba* under laboratory conditions (acute test – static) S18-00164 Eurofins GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A Helm A.G
KCP 10.3.1-01	Sipos K.	2018	Acute contact and oral toxicity of Iodosulfuron + Mesosulfuron (0.6% + 3%) WG on honey bees (*apis mellifera*) 17/173-116MT Citoxlab GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A
KCP 10.3.1-02	Franke M.	2019a	Acute contact toxicity of GLOB289H + Actirob (adjuvant) to the honeybee *Apis mellifera* L. under laboratory conditions. 19 48 BAA 0019 Biochem agrar GLP Unpublised	N	Globachem N.V. Ascenza Agro S.A
KCP 10.3.1-03	Marin M.	2019b	Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (6 + 30 + 90) g/kg WG (SAP63H) + adjuvant (Pottok) – Acute contact toxicity to the honey bee, *Apis mellifera* L. under laboratory conditions S19-00809 Eurofins GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A
KCP 10.3.1-04	Franke M.	2019c	Acute oral toxicity of GLOB289H + Actirob (adjuvant) to the honeybee *Apis mellifera* L. under laboratory conditions. 19 48 BAA 0089 Biochem agrar GLP Unpublised	N	Globachem N.V. Ascenza Agro S.A
KCP 10.3.1-05	Franke M.	2019d	Acute oral toxicity of GLOB289H + Pottok (adjuvant) to the honeybee *Apis mellifera* L. under laboratory conditions.	N	Globachem N.V.

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**
			19 48 BAA 0088 Biochem agrar GLP Unpublised		Ascenza Agro S.A
KCP 10.3.1.2-01	Kleebaum K.	2018	GLOB289H – Repeated exposure of honey bee (*Apis mellifera* L.) larvae under laboratory conditions (*in vitro*) 17 48 BLC 0089 Biochem agrar GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A
KCP 10.3.1.2-02	Ruhland S.	2018	Chronic toicity of Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (0.6+3+9)% WG to the honey bee *Apis mellifera L* under laboratory conditions. 17 48 BAC 0055 Biochem Agrar GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A
KCP 10.3.2-01	Röhlig U.	2017a	Effects of Iodosulfuron + Mesosulfuron (0.6+3) % WG on predatory mite *Typhlodromus pyri* scheuten in a laboratory test 17 48 NTL 0008 Biochem agrar GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A
KCP 10.3.2-02	Röhlig U.	2017b	Effects of Iodosulfuron + Mesosulfuron (0.6+3) % WG on the parasitic wasp *Aphidius rhopalosiphi* (destefani-perez) in a laboratory test 17 48 NAL 0009 Biochem Agrar GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A
KCP 10.3.2-03	Röhlig U.	2019a	Effects of GLOB289H + Actirob B on the predatory mite *Typhlodromus pyri* Scheuten in a laboratory test 19 48 NTL 0002 Biochem Agrar GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A
KCP 10.3.2-04	Röhlig U.	2009b	Effects of GLOB289H + Actirob B on the parasitic was *Aphidius rhopalosihpi* (DeStefani-Perez) in an extended laboratory test 19 48 NAE 0009 Biochem Agrar GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A
KCP 10.3.2-05	Röhlig U.	2009c	Effects of GLOB289H + Actirob B on the green lacewing *Chrysoperla carnea* Steph. in an extended laboratory test 19 48 NCE 0010 Biochem Agrar GLP	N	Globachem N.V. Ascenza Agro S.A

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Unpublished		
KCP 10.3.2-06	Röhlig U.	2019d	Effects of GLOB289H + Actirob B on the rove beetle *Aleochara bilineata* Gyll. in an extended laboratory test 19 48 NKE 0006 Biochem Agrar GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A
KPC 10.3.2-07	Luna F.	2019	Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (6 + 30 + 90) g/kg WG (SAP63H) + adjuvant (Pottok) – Toxicity to the parasitoid *Aphidius rhopalosiphi* De Stefani Perez (Hymenoptera, Braconidae) under extended laboratory conditions S19-02623 Eurofins GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A
KCP 10.4-01	Friedrich S.	2018a	Effects of Iodosulfuron + Mesosulfuron (0.6+3)% WG on the reproduction of the earthworm *Eisenia andrei* in artificial soil 17 48 TEC 0043 Biochem agrar GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A
KCP 10.4-02	Friedrich S.	2019a	Effects of GLOB289H + Actirob B on the reproduction of the earthworm *Eisenia andrei* in artificial soil 19 48 TEC 0063 Biochem Agrar GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A
KCP 10.4-03	Friedrich S.	2019b	Effects of GLOB289H + Pottok in the reproduction of the earthworm *Eisenia andrei* in artificial soil 19 48 TEC 0064 Biochem Agrar GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A.
KCP 10.4-04	Friedrich S	2018b	Effects of Iodosulfuron + Mesosulfuron (0.6+3) % WG on the reporduction of the collembolan *Folsomia candida* 17 48 TCC 0043 Biochem agrar GLP Unpublised	N	Globachem N.V. Ascenza Agro S.A
KCP 10.4-05	Schulz L.	2018a	Effects of Iodosulfuron + Mesosulfuron (0.6+3) % WG on the reproduction of the predatory mite *Hypoaspis aculeifer* 17 48 THC 0038 Biochem agrar GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A
KCP 10.5-01	Schulz L.	2018b	Effects of Iodsulfuron + Mesosulfuron (0.6 + 3) % WG on the activity of soil microflora (Nitrogen transformation test)	N	Globachem N.V.

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
			17 48 SMN 0050 Biochem agrar GLP Unpublished		Ascenza Agro S.A
KCP 10.5-02	Persdorf M.	2019a	Effects of Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (6 + 30 + 90) g/kg WG (GLOB289H) and the adjuvat (Pottok) on the activitiy of soil microflora (Nitrogen transformation test) 19 48 SMN 0054 Biochem agrar GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A
KCP 10.5-03	Persdorf M.	2019b	Effects of GLOB289H + Actirob B on the activity of soil microflora (Nitrogen transformation test) 19 48 SMN 0053 Biochem agrar GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A
KCP 10.6-01	Davies C.	2018	GLOB289H GLP Vegetative vigour test terrestrial non-target plants STC/17/E1118 Stockbridge technology centre Ltd GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A
KCP 10.6-02	Stead A.	2018	GLP Seedling emergence and seedling growth test terrestrial non-target plants STC/17/E1119 Stockbridge technology centre Ltd GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A
KCP 10.6-03	Davies C.	2019	GLOB289H + Actirob B GLP Vegetative Vigour test terrestrial non-target plants (based on OECD guideline 227) – 2019 STC/19/E1233 Stockbridge technology centre Ltd GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A
KCP 10.6-04	Huerta F.	2019	Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (6 + 30 + 90) g/kg WG (SAP63H) + adjuvant (Pottok): Effects on the Vegetative Vigour of six non-target terrestrial plant species under greenhouse conditions S19-00811 Eurofins GLP Unpublished	N	Globachem N.V. Ascenza Agro S.A

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

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

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

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 Aquatic invertebrates

Comments of zRMS:	The study is considered valid. All validity criteria were met. • Immobilised *daphnia* in controls were not found (criteria: ≤ 10 %) • Dissolved oxygen concentration was ≥ 8.01 mg/L O_2 (criteria: ≥ 3 mg/L) in all treatments groups • Daphnia were not trapped at the water surface **Agreed endpoints:** **48 h EC_{50} = 21.97 mg/L test item (nom)** **48 h LOEC = 15.02 mg/L test item (nom)** **48 h NOEC = 7.47 mg/L test item (nom)**

Reference:	KCP 10.2-01
Report	Acute toxicity of GLOB289H to *Daphnia magna* in a 48-hour static test, Renner P, 2018, 18 48 ADL 0008.
Guideline(s):	Yes, OECD 202 (2004)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	N/A

Materials and methods

Test item:	GLOB289H, batch no.: R-BAA content of a.i.: Iodosulfuron-methyl-sodium: 5.6 g/kg Mesosulfuron-methyl: 29.2 g/kg Mefenpyr-diethyl: 90 g/kg (nominal)
Test species:	*Daphnia magna* STRAUS
Test system:	exposure of *Daphnia* to the test item applied in test medium (dilution water)
Test conditions:	Temperature: 19.5 – 19.9 °C Photoperiod: 20 µEm-2s-1
Treatments:	control (untreated test medium), test item (GLOB289H)
Number of test vessels/concentration:	4
Number of *Daphnia*/concentration:	20
Test concentrations (nominal):	3.77, 7.48, 15.02, 30.03 and 60.0 mg/L test item, equivalent to: 0.021, 0.042, 0.084, 0.168, 0.336 mg/L iodosulfuron-methyl-sodium 0.110, 0.218, 0.438, 0.877, 1.752 mg/L mesosulfuron-methyl
Exposure time:	48 hours (static test procedure)
Biological observations:	number of immobilized *Daphnia*: after 24 and 48 hours
Dates of work:	experimental start date: 08.05.2018 experimental completion date: 10.05.2018

Results and discussions

Recoveries of iodosulfuron-methyl-sodium were within 84-90% of nominal concentrations. For mesosulfuron-methyl, recoveries were within a range of 81 and 88%. These values were determined covering fresh taken at test start and spend samples taken at test end.

Number of immobilised *Daphnia magna* and percentage immobility

treatment group mg/L test item nominal	immobilised *Daphnia* (number)			immobility of *Daphnia* (%)		
	3 h	24 h	48 h	3 h	24 h	48 h
control	0	0	0	0.00	0.00	0.00
3.77	0	0	0	0.00	0.00	0.00
7.48	0	0	0	0.00	0.00	0.00
15.02	0	0	3	0.00	0.00	15.00+
30.03	0	7	16	0.00	35.00+	80.00+
60.00	0	20	20	0.00	100.00+	100.00+

+ significantly different from the control, Step-down Cochran-Armitage Test Procedure, $p \leq 0.05$, one-sided greater

Effects of GLOB289H: summary of effect concentrations

effect concentration	GLOB289H (mg/L)					
	24 h			48 h		
	after application					
LOEC test item nominal	30.03			15.02		
NOEC test item nominal	15.02			7.47		
EC$_x$	**EC$_{10}$**	**EC$_{20}$**	**EC$_{50}$**	**EC$_{10}$**	**EC$_{20}$**	**EC$_{50}$**
test item nominal (CI)	26.38 (n.d.)	28.11 (n.d.)	31.75 (n.d.)	13.94 (9.57 – 16.92)	16.30 (12.20 – 19.32)	21.97 (18.41 – 26.22)

CI - confidence intervals, lower – upper; calculations performed using unrounded values; n.d. not determined due to mathematical issues

No abnormal behaviour or appearance was observed.

All validity criteria were met.

- Immobilised *daphnia* in controls were not found (criteria: ≤ 10 %)
- Dissolved oxygen concentration was ≥ 8.01 mg/L O_2 (criteria: ≥ 3 mg/L) in all treatments groups
- Daphnia were not trapped at the water surface

Conclusion

In a static test in which *Daphnia magna* was exposed to GLOB289H, significant effects were observed at LOEC = 15.02 mg/L test item nominal. The corresponding NOEC was 7.47 mg/L test item nominal. An EC$_{50}$ of 21.97 mg/L test item nominal was determined. These values were determined considering the time point 48 hours.

A 2.2.1.2 Algae

Comments of zRMS:	The study is considered valid. All validity criteria were met. • Biomass in controls increased by a factor of 69.3 (criteria: 16). • The mean coefficient of variation for section-by-section specific growth rates in control cultures was 31.4 % (criteria: 35 %) • The coefficient of variation of average specific growth rates was 0.5 % (critera: 7%) **Agreed endpoints:** **72 h E_rC_{50} =17.95 mg/L test item** **72 h E_yC_{50} of 7.53 mg/L test item** **72 h LOEC = 4.38 mg/L test item.** **72 h NOEC was 2.19 mg/L test item.**

Reference:	KCP 10.2-02
Report	Effects of GLOB289H on *Pseudokirchneriella subcapitata* in an algal growth inhibition test, Renner P, 2018, 18 48 AAL 0019.
Guideline(s):	Yes, OECD 201 (2011)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	N/A

Materials and methods

Test item:	GLOB289H, batch no.: R-BAA content of a.i.: Iodosulfuron-methyl-sodium: 5.6 g/kg Mesosulfuron-methyl: 29.2 g/kg Mefenpyr-diethyl: 90 g/kg (nominal)
Test species:	*Pseudokirchneriella subcapitata*, HILSE
Test system:	exposure of *Pseudokirchneriella subcapitata* to the test item applied in test medium (static conditions)
Test conditions:	Temperature: 22.8 – 23.5 °C Photoperiod: continuous illumination (on average 66 $\mu Em^{-2}s^{-1}$)
Treatments:	control (untreated test medium), test item (GLOB289H)
Number of test vessels/concentration:	control group:6 Treated group: 3
Initial biomass:	5×10^3 cells/mL test solution

Test concentrations (nominal): 2.19, 4.38, 8.75, 17.50, 35.0 mg/L test item, equivalent to: 0.012, 0.025, 0.049, 0.098, 0.196 mg/L iodosulfuron-methyl-sodium
0.064, 0.128, 0.256, 0.511, 1.022 mg/L mesosulfuron-methyl

Exposure time: 72 hours (static test procedure)

Biological observations: Biomass (number of cells): after 24, 48 and 72 hours

Dates of work: experimental start date: 08.05.2018
experimental completion date: 11.05.2018

Results and discussions

Recoveries of iodosulfuron-methyl-sodium were within 111 to 120% of nominal concentrations. For mesosulfuron-methyl, recoveries were within a range of 104 to 116%. These values were determined covering fresh taken at test start and spend samples taken at test end.

Biomass (mean) determined at test start, at 24, 48 and 72 hours after test start

treatment group mg/L test item nominal	biomass (x 10^4 cells/mL)			
	0 h	24 h	48 h	72 h
control	0.50	3.17	13.08	34.67
2.19	0.50	3.25	12.83	33.50
4.38	0.50	2.42	9.33	26.17
8.75	0.50	1.50	5.92	14.83
17.50	0.50	1.08	1.67	4.00
35.00	0.50	0.58	1.08	1.50

Growth rate and percentage inhibition

treatment group mg/L test item nominal	growth rates and % Inhibition					
	0 - 24 h		0 - 48 h		0 - 72 h	
	µ	%[1]	µ	%	µ	%
control	1.842		1.632		1.413	
2.19	1.870	-1.5	1.622	0.6	1.401	0.8
4.38	1.571	14.7+	1.461	10.5+	1.319	6.7+
8.75	1.089	40.9+	1.235	24.3+	1.130	20.0+
17.50	0.732	60.2+	0.597	63.4+	0.691	51.1+
35.00	0.135	92.7+	0.384	76.5+	0.363	74.3+

+ significantly different from the control (24 h and 72 h: Williams t-test, $p \leq 0.05$, one-sided smaller; 48 h: Welch`s t-test, $p \leq 0.05$, one-sided smaller); [1] negative values indicate an increase in growth relative to control

Growth rate inhibition was significant at LOEC 4.38 mg/L test item nominal at 24, 48 and 72 hours after test start. A NOEC of 2.19 mg/L test item nominal was determined.

Effect concentration E_rC_x of growth rate inhibition

effect concentration	GLOB289H (mg/L) 0 – 24 h		
E_rC_x	E_rC_{10}	E_rC_{20}	E_rC_{50}
test item nominal (CI)	n.d.	n.d.	n.d.

CI – confidence intervals (lower – upper); n.d. – not determined due to mathematical issues (LU factorisation)

effect concentration	GLOB289H (mg/L) 0 – 48 h		
E_rC_x	E_rC_{10}	E_rC_{20}	E_rC_{50}
test item nominal (CI)	4.20 (3.28 – 5.05)	6.67 (5.60 – 7.62)	14.70 (13.29 – 16.15)
	0 – 72 h		
E_rC_x	E_rC_{10}	E_rC_{20}	E_rC_{50}
test item nominal (CI)	5.16 (4.56 – 5.72)	8.17 (7.49 – 8.81)	17.95 (17.07 – 18.86)

CI – confidence intervals (lower – upper)

Yield y and percent inhibition of y

treatment group mg/L test item nominal	yield y and % Inhibition of y					
	24 h		48 h		72 h	
	y	%[1]	y	%[1]	y	%[1]
control	2.667		12.583		34.167	
2.19	2.750	-3.1	12.333	2.0	33.000	3.4
4.38	1.917	28.1+	8.833	29.8+	25.667	24.9+
8.75	1.000	62.5+	5.417	57.0+	14.333	58.0+
17.50	0.583	78.1+	1.167	90.7+	3.500	89.8+
35.00	0.083	96.9+	0.583	95.4+	1.000	97.1+

+ significantly different from control (Williams t-test, $p \leq 0.05$, one-sided smaller); [1] negative values in % inhibition indicate an increase in growth relative to that of control

Yield was significantly inhibited at LOEC = 4.38 mg/L test item nominal at 24, 48 and 72 hours after test start. A NOEC of 2.19 mg/L test item nominal was determined.

Effect concentration E_yC_{10}, E_yC_{20} and E_yC_{50}

effect concentration	GLOB289H (mg/L) 24 h after application		
E_yC_x	E_yC_{10}	E_yC_{20}	E_yC_{50}
test item nominal (CI)	2.54 (1.48 – 3.45)	3.68 (2.52 – 4.65)	6.96 (5.62 – 8.34)
	48 h after application		
E_yC_x	E_yC_{10}	E_yC_{20}	E_yC_{50}
test item nominal (CI)	2.85 (2.37 – 3.31)	4.07 (3.54 – 4.56)	7.45 (6.73 – 8.20)

Effect concentration	GLOB289H (mg/L) 72 h after application		
E_yC_x	E_yC_{10}	E_yC_{20}	E_yC_{50}
test item nominal (CI)	3.02 (2.77 – 3.26)	4.23 (3.96 – 4.48)	7.53 (7.19 – 7.87)

CI – confidence intervals (lower – upper)

All validity criteria were met.

- Biomass in controls increased by a factor of 69.3 (criteria: 16).
- The mean coefficient of variation for section-by-section specific growth rates in control cultures was 31.4 % (criteria: 35 %)
- The coefficient of variation of average specific growth rates was 0.5 % (critera: 7%)

Conclusion

In a 72 hours *Pseudokirchneriella subcapitata* algal growth inhibition with GLOB289H, significant effects were found at LOEC = 4.38 mg/L test item. The corresponding NOEC was 2.19 mg/L test item. An E_rC_{50} of 17.95 mg/L test item and an E_yC_{50} of 7.53 mg/L test item was determined.

A 2.2.1.3 Aquatic macrophytes

Comments of zRMS:	The study is considered valid. All validity criteria were met. • The doubling time of frond number in controls must be less than 2.5 d (60 h), corresponding to approximately a 7-fold increase in 7 days and an average specific growth rate of 0.275 d-1. **Agreed endponits:** **72 h E_yC_{50} =23.20 µg/L test item nominal** **(119.11 ng/L Iodosulfuron-methyl sodium mean measured).** **72 h E_rC_{50} =41.27 µg/L test item nominal** **(236.21 ng/L Iodosulfuron-methyl sodium mean measured).**

Reference:	KCP 10.2-03
Report	Effects of GLOB289H on *Lemna gibba* in a growth inhibition test under semi-static conditions, Renner P, 2018, 18 48 ALE 0006.
Guideline(s):	Yes, OECD 221 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	N/A

Materials and methods

Test item:	GLOB289H, batch no.: R-BAA content of a.i.: Iodosulfuron-methyl-sodium: 5.6 g/kg Mesosulfuron-methyl: 29.2 g/kg Mefenpyr-diethyl: 90 g/kg (nominal)
Test species:	Duckweed – *Lemna gibba* L.
Test system:	exposure of *Lemna gibba* to the test item applied in test medium (semi-static conditions), no vehicles used
Test conditions:	Temperature: 23.1 – 24.4 °C

	Photoperiod: continuous illumination (on average 118 $\mu Em^{-2}s^{-1}$)
Treatments:	control (untreated test medium), test item (GLOB289H)
Number of test vessels/concentration:	control group:6 Treated group: 3
Test concentrations:	4.38, 8.75, 17.50, 35.00, 70.00 µg/L test item nominal 42.52, 48.99, 97.97, 196.01, 392.01 ng/L Iodosulfuron-methyl-sodium nominal 23.92, 23.92, 82.78, 204.99, 417.70 ng/L Iodosulfuron-methyl-sodium mean measured (in order to compute mean measured concentration for concentrations below the LOQ, LOQ/2 was applied)
Exposure time:	7 days (semi-static test procedure)
Biological observations:	frond number: day 0, 2, 5 and 7 changes in plant development: day 0, 2, 5 and 7 dry weight: day 0 and 7
Statistics	LOEC: William's t-test ($p \leq 0.05$, one-sided smaller) EC_x: sigmoidal 3 parameter Statistical program: ToxRat Professional Version 3.2.1
Dates of work:	experimental start date: 13.06.2018 experimental completion date: 27.06.2018

Analytical findings

Recoveries of Mesosulfuron-methyl were within a range of 94 to 120 % of nominal concentrations in fresh and spent samples. It can be stated, that GLOB289H was correctly dosed during the test. Analysis of Iodosulfuron-methyl sodium was successful at concentrations ≥ 17.50 µg/L test item nominal (97.97 ng/L Iodosulfuron-methyl sodium). Concentrations below were found below the LOQ of 47.85 ng/L. Recoveries of Iodosulfuron-methyl sodium at concentrations ≥ 17.50 µg/L test item nominal were within a range of 80 to 117 % of nominal concentrations.

Based on these findings, toxicity results are based on test item nominal concentrations and Iodosulfuron-methyl sodium mean measured. Notably, mean measured concentrations were calculated as concentrations of Iodosulfuron-methyl sodium could not be analysed rather than degradation. Chemical analysis was performed at concentrations of interest (lowest EyC50 = 23.20 µg/L test item nominal) and mean measured concentrations at these point estimates are very similar.

Effects of GLOB289H on *Lemna gibba* applied under test conditions (7d)

effect concentration	GLOB289H			
	growth rate inhibition		yield inhibition	
	frond number	biomass	frond number	biomass
LOEC test item nominal (µg/L)	8.75	35.00	8.75	35.00
Iodosulf nominal (ng/L)	49.00	196.00	49.00	196.00
Iodosulf m.m. (ng/L)	23.92*	204.99	23.92*	204.99
NOEC test item nominal (µg/L)	4.38	17.50	4.38	17.50
Iodosulf nominal (ng/L)	24.53	98.00	24.53	98.00
Iodosulf m.m. (ng/L)	23.92*	82.78	23.92*	82.78
EC_{10} test item nominal (µg/L) (CI)	11.25 (9.41 – 13.44)	17.58 (11.57 – 26.71)	7.90 (7.85 – 7.95)	12.38 (6.69 – 22.90)
Iodosulf nominal (ng/L) (CI)	63.00 (52.70 – 75.26)	98.45 (64.79 – 149.58)	44.24 (43.96 – 44.52)	69.33 (37.46 – 128.24)
Iodosulf m.m. (ng/L) (CI)	52.06 (42.76 – 63.38)	90.00 (55.84 – 145.05)	31.48 (23.79 – 41.66)	57.12 (26.63 – 122.52)
EC_{20} test item nominal (µg/L) (CI)	17.57 (14.84 – 20.86)	28.25 (18.65 – 42.66)	11.44 (11.37 – 11.51)	17.80 (9.97 – 31.96)
Iodosulf nominal (ng/L) (CI)	98.39 (83.10 – 116.82)	158.20 (104.44 – 238.90)	64.06 (63.67 – 64.46)	99.68 (55.83 – 178.98)
Iodosulf m.m. (ng/L) (CI)	87.49 (72.59 – 105.73)	153.54 (95.51 – 246.06)	49.71 (38.17 – 64.63)	87.77 (81.81. – 181.08)
EC_{50} test item nominal (µg/L) (CI)	41.27 (33.26 – 50.85)	69.99 (39.26 – 120.94)	23.20 (23.02 – 23.37)	35.67 (17.32 – 72.14)
Iodosulf nominal (ng/L) (CI)	231.11 (186.26 – 284.76)	391.94 (219.86 – 677.26)	129.92 (128.91 – 130.87)	199.75 (96.99 – 403.98)
Iodosulf m.m. (ng/L) (CI)	236.21 (186.31 – 297.21)	426.60 (218.79 – 803.06)	119.11 (87.01 – 163.30)	199.63 (81.81 – 476.95)

Calculations preformed using unrounded values; CI – 95 % confidence intervals, upper – lower; Iodosulf m.m = Iodosulfuron-methyl sodium mean measured; * the two lowest concentrations are identical due to mean measured calculations

Conclusion

In a semi-static test in which *Lemna gibba* was exposed to 4.38, 8.75, 17.50, 35.00, 70.00 µg/L GLOB289H nominal, the most sensitive EyC50 was 23.20 µg/L GLOB289H nominal (119.11 ng/L Iodosulfuron-methyl sodium mean measured). The most sensitive ErC_{50} was 41.27 µg/L test item nominal (236.21 ng/L Iodosulfuron-methyl sodium mean measured). Both of these values were determined based on the assessment of frond numbers. All validity criteria were met.

Comments of zRMS:	The study is considered valid. All validity criteria were met. • The doubling time of frond number in controls was on average 1.9 corresponding to a 12.6-fold increase (113/9). The average specific growth rate in the control was 0.361 d-1 (required ≥ 0.275 d-1). **Agreed enpoints:** **7 d $E_rC_{50\ \text{frond number}}$ = 30.26 µg/L nominal** **(21.47 µg/L test item adjusted; 0.736 µg/L mesosulfuron-methyl mean measured)** **7 d $E_yC_{50\ \text{frond nmber}}$ = 15.25 µg/L nominal (9.64 µg/L test item adjusted; 0.363 µg/L mesosulfuron-methyl mean measured)**

Reference:	KCP 10.2-04
Report	Effects of GLOB289H and Actirob B on *Lemna gibba* in a growth inhibition test under semi-static conditions, Renner P, 2019, 19 48 ALE 0004.
Guideline(s):	Yes, OECD 221 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	N/A

Materials and methods

Test item:	GLOB289H, batch no.: R-BAA content of a.i.: Iodosulfuron-methyl-sodium: 0.5604% (authenticated) Mesosulfuron-methyl: 3.001% (authenticated) Mefenpyr-diethyl: 8.804% (authenticated) Actirob B Rapeseed oil methyl ester: actual content not available
Test species:	Duckweed – *Lemna gibba* L.
Test system:	exposure of *Lemna gibba* to the GLOB289H and Actirob B applied in test medium (semi-static conditions), no vehicles used
Test conditions:	Temperature: 22.7 – 23.2 °C Photoperiod: continuous illumination (on average 119 $\mu Em^{-2}s^{-1}$)
Treatments:	control (untreated test medium), test item (GLOB289H and Actirob B, in a 1:3.33 ratio)
Number of test vessels/concentration:	control group:6 Treated group: 3

Test concentrations:

GLOB289H and Actirob B[1] (µg/L + µL/L nominal)	Test item adjusted (based on geo. mean of recoveries)	Iodosulfuron-methyl-sodium (µg/L nominal)	Mesosulfuron-methyl (µg/L nominal)	Mesosulfuron-methyl (µg/L mean measured)
adjuvant control 0 + 0.17	-	-	-	-
3.12 + 0.01	2.58 + 0.008	0.017	0.094	0.077
6.24 + 0.02	5.09 + 0.016	0.035	0.187	0.154
12.51 + 0.04	9.51 + 0.03	0.070	0.375	0.287
24.99 + 0.08	12.20 + 0.04	0.140	0.750	0.614
50.01 + 0.17	41.36 + 0.14	0.280	1.501	1.246

Exposure time: 7 days (semi-static test procedure)

Biological observations:
frond number: day 0, 3, 5 and 7
changes in plant development: day 0, 3, 5 and 7
dry weight: day 0 and 7

Statistics
LOEC: William's t-test ($p \leq 0.05$, one-sided smaller)
EC_x: non-linear regression, sigmoidal 3 parameter
Statistical program: ToxRat Professional Version 3.2.1

Dates of work:
experimental start date: 17.05.2019
experimental completion date: 28.05.2019

Analytical findings

Recoveries of mesosulfuron-methyl were within 80 to 89 % of nominal concentrations in fresh water samples. In spent samples, recoveries were within 64 to 89 % of nominal concentration. Nominal concentrations were analytically confirmed in fresh samples. Recoveries of iodosulfuron-methyl sodium were within 91 to 112 % on nominal concentrations in fresh samples. In spent samples, recoveries within 86 to 115 % of nominal concentrations were found. Nominal concentrations were analytically confirmed in fresh and spent samples.
Based on these finding, test item nominal concentrations are reported along with test item adjusted concentrations and mesosulfuron-methyl mean measured concentrations.

Effects of GLOB289H and Actirob B on Lemna gibba

Table 1: Effects of GLOB289H and Actirob B on Lemna gibba (7 d)

effect concentration	GLOB289H and Actirob B# (µg/L)			
	growth rate inhibition		yield inhibition	
	frond number	biomass	frond number	biomass
LOEC				
test item nominal	6.24	12.51	6.24	12.51
test item adjusted	5.09	9.51	5.09	9.51
mesosulfuron-methyl mean measured	0.154	0.287	0.154	0.287
NOEC				
test item nominal	3.12	6.24	3.12	6.24
test item adjusted	2.58	5.09	2.58	5.09
mesosulfuron-methyl mean measured	0.077	0.154	0.07	0.154
EC_{10}				
test item nominal (CI)	7.35 (4.73 – 11.32)	9.62 (6.58 – 13.95)	4.72 (2.69 – 8.18)	5.08 (2.97 – 8.64)
test item adjusted (CI)	3.83 (1.83 – 7.94)	5.28 (3.13 – 8.89)	3.50 (1.77 – 6.82)	3.46 (1.87 – 6.33)
mesosulfuron-methyl mean measured (CI)	0.170 (0.111 – 0.261)	0.224 (0.153 – 0.329)	0.111 (0.066 – 0.191)	0.118 (0.070 – 0.201)
EC_{20}				
test item nominal (CI)	11.95 (8.66 – 16.34)	16.25 (12.34 – 21.18)	7.06 (4.68 – 10.75)	7.92 (5.35 – 11.75)
test item adjusted (CI)	6.92 (4.04 – 11.87)	9.89 (6.74 – 14.49)	4.95 (3.02 – 7.98)	5.26 (3.37 – 8.19)
mesosulfuron-methyl mean measured (CI)	0.281 (0.207 – 0.384)	0.387 (0.295 – 0.510)	0.167 (0.113 – 0.252)	0.186 (0.126 – 0.278)

Table 1 (continued): Effects of GLOB289H and Actirob B on *Lemna gibba* (7 d)

effect concentration	GLOB289H and Actirob B# (µg/L)			
	growth rate inhibition		yield inhibition	
	frond number	biomass	frond number	biomass
EC_{50}				
test item nominal (CI)	30.26 (25.07 – 36.54)	44.23 (37.92 – 51.59)	15.25 (11.44 – 20.33)	18.52 (14.24 – 24.10)
test item adjusted (CI)	21.47 (15.11 – 30.51)	32.78 (25.61 – 41.97)	9.64 (7.30 – 12.74)	11.77 (8.88 – 15.59)
mesosulfuron-methyl mean measured (CI)	0.736 (0.609 – 0.889)	1.096 (0.935 – 1.285)	0.363 (0.273 – 0.481)	0.445 (0.339 – 0.583)

Calculations preformed using unrounded values; CI – 95 % confidence intervals, upper – lower; # concentrations related to GLOB289H only

Conclusion

In a semi static test in which Lemna gibba was exposed to the test item at nominal concentrations of 3.12, 6.24, 12.51, 14.99, 50.01 µg/L, the most sensitive ErC50 was 30.26 µg/L nominal (21.47 µg/L test item adjusted; 0.736 µg/L mesosulfuron-methyl mean measured). The most sensitive EyC50 was 15.25 µg/L nominal (9.64 µg/L test item adjusted; 0.363 µg/L mesosulfuron-methyl mean measured). Both of these values were determined based on the assessment of frond number.

Phytotoxic effects
Noticeable chlorotic effects of around 10 were observed at concentrations ≥ 12.51 mg/L test item nominal. These effects were first observed at day 3. Effects on roots were found at concentrations ≥ 12.51 mg/L test item nominal. Evidently, there are time- and concentration-related effects. The adjuvant control inhibited the root length.

Discussion
The inhibition of root growth by Actirob B in the adjuvant control most likely contributes to the effect observed in the highest treatment group as the concentrations of Actirob B were identical in both of these treatment groups.

Validity criteria
All validity criteria were met.

Comments of zRMS:	The study is considered valid. All validity criteria were met. • The measured doubling time of frond number in controls was on average 2.2 days (required: < 2.5) corresponding to an 8.88-fold increase over the 7 days. Average specific growth rate in the control treatment was 0.312 d-1 (required: > 0.275 d-1) **Agreed endpoints:** **7 d $E_rC_{50\ \text{fromd number}}$ =18.08 µg/L test item nominal** **7 d $E_yC_{50\ \text{frond number}}$ =9.80 µg/L test item nominal**

Reference:	KCP 10.2-05
Report	Effects of Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (6 + 30 + 90) g/kg WG (SAP63H) and the adjuvant (POTTOK) on *Lemna gibba* in a growth inhibition test under semi-static test conditions, Renner P, 2019, 19 48 ALE 0007.
Guideline(s):	Yes, OECD 221 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	N/A

Materials and methods

Test item:	Iodosulfuron-methyl-sodium + Mesosulfuron-methyl +Mefenpyr-diethyl (6 + 30 + 90) g/kg WG (SAP63H) and the adjuvant (POTTOK); batch no.: F-DBA analysed content: Iodosulfuron-methyl-sodium: 7.36 g/kg Mesosulfuron-methyl: 27.2 g/kg Mefenpyr-diethyl: 89.9 g/kg

Test species: Duckweed – *Lemna gibba* L.

Test system: Exposure of *Lemna gibba* to Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (6 + 30 + 90) g/kg WG (SAP63H) and the adjuvant (POTTOK) applied in test medium (semi-static conditions), no vehicles used

Test conditions: Temperature: 22.5 – 22.9 °C
Photoperiod: continuous illumination (on average 119 $\mu Em^{-2}s^{-1}$)

Treatments: control (untreated test medium),
test item – Iodosulfuron-methyl-sodium + Mesosulfron-methyl + Mefenpyr-diethyl (6 + 30 + 90) g/kg WG (SAP63H) and the adjuvant (POTTOK)

Nominal test concentrations:

SAP63H and POTTOK (µg/L)	Iodosulfuron-methyl-sodium (µg/L)	Mesosulfuron-methyl (µg/L)	POTTOK (µL/L)
2.49	0.011	0.068	0.002
5.01	0.023	0.136	0.003
9.99	0.046	0.272	0.007
20.01	0.092	0.544	0.013
39.99	0.184	1.088	0.027

Exposure time: 7 days (semi-static test procedure)

Biological observations:

frond number:	day 0, 2, 5 and 7
changes in plant development:	day 0, 2, 5 and 7
dry weight:	day 0 and 7

Statistics: LOEC: William's t-test ($p \leq 0.05$, one-sided smaller)
EC_x: Probit analysis using linear max. likelihood regression
Statistical program: ToxRat Professional Version 3.3.0

Dates of work: experimental start date: 12.06.2019
experimental completion date: 21.06.2019

The study was performed in compliance with the principles of GLP.

Analytical findings

Recoveries of iodosulfuron-methyl sodium and mesosulfuron-methyl were within a range of 80 to 120 % of nominal concentrations at test start, at test solution renewal processes and at test end in 'fresh' and 'spent' test solutions. Nominal concentrations were analytically confirmed.
Therefore, toxicity results are based on test item nominal concentrations.

Effects of SAP63H and POTTOK on Lemna gibba applied under test conditions

Table 1: Effects of SAP63H and POTTOK on *Lemna gibba* applied under test conditions (7 d)

effect concentration	SAP63H and POTTOK (µg/L)[#]			
	growth rate inhibition		yield inhibition	
	frond number	biomass	frond number	biomass
LOEC test item nominal	9.99	n.d.	9.99	5.01
NOEC test item nominal	5.01	n.d.	5.01	2.49
EC_{10} test item nominal (CI)	4.84 (4.46 – 5.26)	7.99 (4.00 – 15.80)	4.24 (2.99 – 5.95)	4.10 (2.81 – 5.71)
EC_{20} test item nominal (CI)	7.61 (7.16 – 8.09)	15.70 (9.48 – 25.79)	5.65 (4.37 – 7.35)	6.78 (5.23 – 8.78)
EC_{50} test item nominal (CI)	18.08 (17.37 – 18.82)	57.27* (41.99 – 78.10)	9.80 (8.17 – 11.75)	18.52 (15.72 – 21.82)

Calculations preformed using unrounded values; CI – 95 % confidence intervals, upper – lower; * extrapolated (concentration outside the range of applied concentrations); n.d. – not determined; LOEC: determined related to untreated control; [#] concentration related to SAP63H only (mixing ratio 300 g SAP63H : 0.2 L Pottok)

Conclusion

In a semi-static test in which *Lemna gibba* was exposed to nominal concentrations of 2.49, 5.01, 9.99, 20.01, 39.99 µg/L SAP63H and POTTOK, the most sensitive ErC50 was 18.08 µg/L test item nominal. The most sensitive EyC50 was 9.80 µg/L test item nominal. Both of these values were determined based on theassessment of frond numbers.

Validity criteria

All validity criteria were met.

Analysis of test solution

Recoveries of iodosulfuron-methyl sodium and mesosulfuron-methyl were within a range of 80 to 120 % of nominal concentrations at test start, at test solution renewal processes and at test end in ‘fresh’ and ‘spent’test solutions. Nominal concentrations were analytically confirmed.

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 – mesosulfuron-methyl

A 2.2.3.1 Lemna gibba

<table>
<tr><td>Comments of zRMS:</td><td>The study is considered valid. All validity criteria were met.

• The doubling time of frond numbers in the solvent control was 1.670 days (corresponding to 40.1 hours).

Agreed endpoints:</td></tr>
</table>

	Test item [µg/L] nominal							
Endpoint	EC_{10}	95 % confidence limit	EC_{20}	95 % confidence limit	EC_{50}	95 % confidence limit	NOEC	LOEC
Yield of frond numbers	0.273[1)]	0.202-0.345	0.430[1)]	0.340-0.520	1.02[1)]	0.866-1.20	0.391[3)]	1.25[3)]
Growth rate of frond numbers	0.549[2)]	0.358-0.738	1.04[2)]	0.780-1.29	2.72[2)]	2.29-3.28	0.391[4)]	1.25[4)]
Yield of dry weight	0.372[1)]	$1.28E^{-32}$-1.10	0.756[1)]	$2.37E^{-14}$-2.95	2.93[1)]	0.976-$4.25E^{22}$	1.25[3)]	4.00[3)]
Growth rate of dry weight	1.53[2)]	0.923-2.19	3.43[2)]	2.40-5.76	> 4.00[5)]	-	1.25[4)]	4.00[4)]
Yield of fresh weight	0.346[1)]	0.256-0.436	0.545[1)]	0.432-0.658	1.30[1)]	1.10-1.55	1.25[4)]	4.00[4)]
Growth rate of fresh weight	0.743[2)]	0.491-0.983	1.35[2)]	1.03-1.65	3.30[2)]	2.77-4.04	0.391[4)]	1.25[4)]

Reference: KCP 10.2-07

Report: Mesosulfuron-methyl technical: Toxicity to the duckweed *Lemna gibba* under laboratory conditions (acute test – static)

Guideline(s): Yes, OECD 221 (2006)

Deviations: No major (the pH of the control increased by more than 1.5 units during the test. At test start, the pH was only measured in the control and the solvent control. The test is still valid, as it was shown that validity criteria are met. As the test item concentrations remained stable during the test duration of 7 d, it is assumed that the pH has no impact on test item stability.).

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): N/A

Materials and methods

Test item: Mesosulfuron-methyl technical, Batch No.: 20160408; purity (analysed): 96.78 % w/w

Test species: *Lemna gibba* G3 (duckweed)

Test design: In a static dose-response test vegetative growth was observed for a test period of 7 days by measuring the increase of frond numbers, dry weight and fresh weight.

Endpoints: Inhibition of growth was assessed by the determination of NOEC/LOEC and $EC_{10,20,50}$ for following response variables:

- Growth rates and frond numbers, dry weight and fresh weight
- Yield of frond numbers, dry weight and fresh weight

Additionally the doubling time of frond numbers was determined.

Test rates: A static main test with nominal concentrations of 4.00, 1.25, 0.391, 0.122, 0.0381 µg/L, solvent control and control was performed. Dimethyl formamide (DMF) was used as solvent.

Test conditions: Six replicates were employed for the control as well as the solvent control and three for each test item concentration. The test was performed in 250 mL glass beakers each containing ~ 150 mL test solution. The pH was recorded in the controls at test start and in the controls and all treatments at test end. Temperature was measured continuously over the whole test period and recorded after 0, 2, 5 and 7 days. Light intensity of the continuous illumination was measured at test start.

Samples analysed: Analytical samples were taken in the main test from control, solvent control and all test item concentrations at test start (fresh) and after two, five and seven days from aged solutions. Samples were additionally taken from the stock solutions used for application at day 0. Samples of all test item concentrations, solvent control and control were analysed at t= 0d fresh and 7d aged.

Statistics: NOEC and LOEC were determined by using a multiple comparison method, EC10, 20, 50-values were determined by probit analysis where possible.

Dates of work: 09 May 2018 – 31 Aug 2018

Results and discussions

Findings:

Validity criteria: The doubling time of frond numbers in the control should be less than 2.5 days (< 60 hours). The test is valid as the doubling time of frond numbers in the solvent control was 1.670 days (corresponding to 40.1 hours).

Test conditions: The pH-value of fresh test solutions (controls) was 7.60, the temperature was measured to be 23.7 – 25.3 °C during the test and the mean light intensity at the beginning of the test was 7620 lux.

Analytical Results: The measured initial concentration of mesosulfuron-methyl ranged from 97 % to 103 % of nominal with a mean initial concentration of 101 % of nominal. In the aged samples the measured content was between 99 % and 115 % of nominal with a mean measured concentration of 106 % of nominal. Therefore toxicological endpoints were evaluated using the nominal concentrations of the test item.

Statistical Results: EC10, 20, 50- and NOEC/LOEC-values of Lemna gibba exposed to the test item evaluated using nominal concentrations

	Mesosulfuron-methyl technical [µg/L] nominal				
Endpoint	EC_{10}	EC_{20}	EC_{50}	NOEC	LOEC
Yield of frond numbers	0.273[1)]	0.430[1)]	1.02[1)]	0.391[3)]	1.25[3)]
Growth rate of frond numbers	0.549[2)]	1.04[2)]	2.72[2)]	0.391[4)]	1.25[4)]
Yield of dry weight	0.372[1)]	0.756[1)]	2.93[1)]	1.25[3)]	4.00[3)]
Growth rate of dry weight	1.53[2)]	3.43[2)]	> 4.00[5)]	1.25[4)]	4.00[4)]
Yield of fresh weight	0.346[1)]	0.545[1)]	1.30[1)]	1.25[4)]	4.00[4)]
Growth rate of fresh weight	0.743[2)]	1.35[2)]	3.30[2)]	0.391[4)]	1.25[4)]

1) Probit analysis following normal distribution
2) Probit analysis following Gompertz distribution
3) Following Welch Bonferroni-Holmes corrected test ((left-sided, p<0.05)
4) Following Dunnetts-t-test (left-sided, p<0.05)
5) Due to an inhibition below 50% the database was inappropriate for probit analysis, which hence was not performed

Conclusions

Significant inhibitory effects were determined for yield of frond numbers, growth rate of frond numbers and growth rate of fresh weight at test item concentrations of 1.25 µg/L (nominal) and above. For yield of dry weight, growth rate of dry weight and yield of fresh weight significant inhibitory effects were determined at 4.00 µg/L (nominal). The overall LOEC was therefore determined to be 1.25 µg/L (nominal), the corresponding NOEC was set at 0.391 µg/L (nominal).
The EC10-value for yield of frond numbers was determined to be 0.273 µg/L (nominal). The EC10-value for growth rate of frond numbers was determined to be 0.549 µg/L (nominal). The EC10-value for yield of dry weight was determined to be 0.372 µg/L (nominal). The EC10-value for growth rate of dry weight was determined to be 1.53 µg/L (nominal). The EC10-value for yield of fresh weight was determined to be 0.346 µg/L (nominal). The EC10- value for growth rate of fresh weight was determined to be 0.743 µg/L (nominal).
The EC20-value for yield of frond numbers was determined to be 0.430 µg/L (nominal). The EC20-value for growth rate of frond numbers was determined to be 1.04 µg/L (nominal). The EC20-value for yield of dry weight was determined to be 0.756 µg/L (nominal). The EC20-value for growth rate of dry weight was determined to be 3.43 µg/L (nominal). The EC20-value for yield of fresh weight was determined to be 0.545 µg/L (nominal). The EC20- value for growth rate of fresh weight was determined to be 1.35 µg/L (nominal).
The EC50-value for yield of frond numbers was determined to be 1.02 µg/L (nominal), the EC50-value for growth rate of frond numbers was determined to be 2.72 µg/L (nominal). The EC50-value for yield of dry weight was determined to be 2.93 µg/L (nominal), the EC50-value for growth rate of dry weight was assumed to be > 4.00 µg/L (nominal). The EC50-value for yield of fresh weight was determined to be 1.30 µg/L (nominal), the EC50-value for growth rate of fresh weight was determined to be 3.30 µg/L (nominal).

A 2.2.3.2 Nasturtium officinale

Comments of zRMS:	The study is considered valid. All validity criteria were met. • Increase in shoot length of control plants: The shoot length increased by a factor of 6.3 after 14 days of exposure, • Increase in shoot fresh weight of control plants: The shoot fresh weight increased by a factor of 5.6 after 14 days of exposure, • Coefficient of variation for yield (based on shoot fresh weight in Control): 33.0 % **Agreed endpoints:** 14 d E_rC_{50} = 1.60 µg/L and E_yC_{50} = 1.21 µg/L (nominal), (based on total shoot length) NOEC =1.17 µg/L (nominal) for growth rate NOEC=0.366 µg/L (nominal) for yield. (based on total shoot length) 14 d E_rC_{50} = 1.18 µg/L and E_yC_{50} = 0.670 µg/L (based on biomass shoot fresh weight) 14 d NOEC ==0.366 µg/L (nominal for growth rate and yield based on biomass (based on shoot fresh weight)) 14 d E_rC_{50}= 3.99 µg/L and E_yC_{50} = 1.12 µg/L (nominal) (based on biomass shoot dry weight) 14 d NOEC based on biomass (shoot dry weight) = 0.366 µg/L (nominal) for growth rate and 0.114 nominal for yield.

Reference: KCP 10.2-06_01

Report: Mesosulfuron-methyl technical: Growth inhibition of *Nasturtium officinale* in water/sediment system. Maren D, 2018, S18-00146.

Guideline(s): Yes, OECD 239 (2014)

Deviations: In addition to the guideline recommendation which only evaluates the shoot fresh weight and dry weight, the total plant fresh weight and dry weight, comprising roots and shoots was assessed as well. Evaluating total plant fresh weight and dry weight avoids underestimating effects on rooted aquatic macrophytes, especially for test items which may affect root development.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): N/A

Materials and methods

Test item:	Mesosulfuron-methyl technical, Batch No.: 20160408; purity (analysed): 96.78 % w/w
Test species:	*Nasturtium officinale*
Test design:	Five replicates per test item concentration and ten replicates for the control and solvent control were used. There was one plant per replicate. The duration of the test was 14 days. The test was performed under static test conditions. On day 14 plants were harvested from each treatment group for assessment of shoot length, shoot fresh weight, shoot dry weight, root fresh and dry weight and number and length of side shoots.
Endpoints:	Endpoints reported are the $EC_{10,\ 20,\ 50}$ values for yield ($E_yC_{10,\ 20,\ 50}$) and growth rate ($E_rC_{10,\ 20,\ 50}$) based on the increase in total shoot length, shoot fresh weight and shoot dry weight respectively after 14 days of exposure. The NOEC and LOEC values for yield and growth rate were also determined. Endpoints based on total plant fresh weight and dry weight are reported for completeness in the Appendices.
Test rates:	The nominal concentrations of the test item during the test were 0.114, 0.366, 1.17, 3.75 and 12.0 µg/L. An untreated control and a solvent control were tested in parallel. The test item was spiked to the water.
Test conditions:	Temperature, pH-value and oxygen saturation of the test solutions measured after 0, 7 and 14 days are reported.
Samples analysed:	Test item concentrations in the definitive test were verified by analyses of mesosulfuron-methyl at all concentration levels by analysing the overlaying water from samples taken at test start and test end and wet sediment from samples taken at test termination on day 14. The pore water from the highest concentration level was analysed from samples taken at test termination on day 14.
Dates of work:	15 Feb 2018 – 28 Apr 2018

Results and discussions

Findings:

The measured concentration of the test item in the test vessels based on the mesosulfuron-methyl content in the freshly prepared test solution ranged between 89 and 112% of nominal in the overlaying water. The mean measured content for all concentrations in the freshly prepared test solutions was 99% of nominal for mesosulfuron-methyl. In the aged test solutions the measured concentration of the test item based on the mesosulfuron-methyl content in the test vessels ranged between 82 and 88% of nominal in the overlaying water. The mean measured concentration of the test item in aged test solutions based on the mesosulfuron-methyl content in the test vessels was 85% of nominal in the overlaying water. In the sediment, concentrations of mesosulfuron-methyl above the LOQ were detectable at a concentration of 12.0 with a recovery of 6% of the applied amount at test end after 14 days. In pore water < 1 % of the applied amount was measured after 14 days at the highest nominal concentration level of 12.0 µg test item/L.

Since all measured concentrations of mesosulfuron-methyl were between 80% and 120% of nominal, all toxicological endpoints were evaluated using nominal concentrations of the test item. For completeness, endpoints based on geometric mean measured concentrations of fresh and aged test solutions are also reported in the Appendices.

Following exposure to Mesosulfuron-methyl technical, inhibition effects on *Nasturtium officinale* for shoot fresh weight were found to be more sensitive than for shoot dry weight and total shoot length when comparing the E_yC_{50} values as indicated in the tables below.

Summary of Effects on Total Shoot Length following a 14-day exposure of *Nasturtium officinale* to Mesosulfuron-methyl technical

	Growth rate (total shoot length in cm) [µg Mesosulfuron-methyl technical/L]	Yield (total shoot length in cm) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
14-day EC_{50}	1.60	1.21
95% Conf. Limits	1.36 – 1.87	1.04 – 1.39
14-day EC_{20}	0.715	0.603 [1)]
95% Conf. Limits	0.565 – 0.865	(0.485 - 0.719)
14-day EC_{10}	0.469 [1)]	0.420 [1)]
95% Conf. Limits	(0.348 - 0.591)	(0.319 - 0.518)
14-day NOEC	1.17	0.366
14-day LOEC	3.75	1.17

[1)] Values not reliable. Pooled control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.

Summary of Effects on Shoot Fresh Weight following a 14-day exposure of *Nasturtium officinale* to Mesosulfuron-methyl technical

	Growth rate (shoot fresh weight in g) [µg Mesosulfuron-methyl technical/L]	Yield (shoot fresh weight in g) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
14-day EC_{50}	1.18	0.670
95% Conf. Limits	0.972 - 1.45	0.549 – 0.818
14-day EC_{20}	0.396	0.211 [1)]
95% Conf. Limits	0.302 - 0.492	(0.154 - 0.270)
14-day EC_{10}	0.224 [1)]	0.115 [1)]
95% Conf. Limits	(0.156 - 0.294)	(0.0763 - 0.157)
14-day NOEC	0.366	0.366
14-day LOEC	1.17	1.17

[1)] Values not reliable. Pooled control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.

Summary of Effects on Shoot Dry Weight following a 14-day exposure of *Nasturtium officinale* to Mesosulfuron-methyl technical

	Growth rate (shoot dry weight in g) [µg Mesosulfuron-methyl technical/L]	Yield (shoot dry weight in g) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
14-day EC_{50}	3.99	1.12
95% Conf. Limits	2.79 – 6.24	0.808 – 1.55
14-day EC_{20}	0.380	0.115 [1)]
95% Conf. Limits	0.220 – 0.568	(0.0577 - 0.187)
14-day EC_{10}	0.111 [1)]	0.0349[1)]
95% Conf. Limits	(0.0486 - 0.195)	(0.0134 - 0.0674)
14-day NOEC	0.366	0.114
14-day LOEC	1.17	0.366

[1)] Values not reliable. Pooled control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.

The average pH-value was determined to be 7.26 ± 0.32, the average temperature was measured to be 18.7 ± 0.5 °C and the oxygen saturation was determined to be 95 ± 5 %. The test item had no influence on the pH-value of the test solutions.

Conclusions:

Following exposure of the aquatic rooted eudicotyledon macrophyte *Nasturtium officinale* to Mesosulfuron-methyl technical for 14 days the E_rC_{50} and E_yC_{50} values based on total shoot length were 1.60 µg/L and 1.21 µg/L (nominal) respectively. The NOEC based on total shoot length was 1.17 µg/L (nominal) for growth rate and 0.366 µg/L (nominal) for yield.

The E_rC_{50} and E_yC_{50} values based on biomass (shoot fresh weight) were 1.18 µg/L and 0.670 µg/L (nominal) respectively. The NOEC for growth rate and yield based on biomass (shoot fresh weight) was 0.366 µg/L (nominal) respectively.

The E_rC_{50} and E_yC_{50} values based on biomass (shoot dry weight) were 3.99 µg/L and 1.12 µg/L (nominal). The NOEC based on biomass (shoot dry weight) was 0.366 µg/L (nominal) for growth rate and 0.114 µg/L (nominal) for yield.

A 2.2.3.3 Hottonia palustris

Comments of zRMS:	The study is considered valid. All validity criteria were met. For the tested species no validity criteria could be given. However test design was chosen to be as close as possible to the available Myriophyllum guideline (OECD 239). • Increase in shoot length of control plants: Mean total shoot length must at least double during the exposure period. • Increase in shoot fresh weight of control plants Mean shoot fresh weight must at least double during the exposure period. • Coefficient of variation for yield (based on shoot fresh weight of control plants): Must not exceed 35 % between replicates. **In the current study:** Increase in shoot length of control plants: The shoot length increased by a factor of 3.3 after 16 days of exposure, in shoot fresh weight of control plants: The shoot fresh weight increased by a factor of 4.1after 16 days of exposure, Coefficient of variation for yield (based on shoot fresh weight in Control): 30.2 %, Sublethal effects in Control: Control plants did not show any sign of chlorosis and were visibly free from contamination by other organisms such as algae and/or bacterial film, **Agreed endpoints:** **16 days E_rC_{50}= 15.6 µg/L** **16 days E_yC_{50} =10.3 µg/L** **(based on total shoot length)** **NOEC based on could not be determined for growth rate and yield.** **E_rC_{50} = 26.2 µg/L and E_yC_{50} 9.81 µg/L** **(based on biomass shoot fresh weight)** **NOEC for growth rate and yield based on biomass (shoot fresh weight) could not be determined.** **E_rC_{50} and E_yC_{50} values based on biomass (shoot dry weight) were estimated to be > 1000 µg/L (nominal).** **NOEC for growth rate and yield based on biomass (shoot dry weight) was 30.5 µg/L (nominal), whereas not considered to be reliable as no dose response could be observed for these parameters.**

Reference:	KCP 10.2-06_02
Report	Mesosulfuron-methyl technical: Growth inhibition of *Hottonia palustris* in water/sediment system. Maren D, 2018, S18-00147.
Guideline(s):	Yes, OECD 239 (2014)
Deviations:	In addition to the guideline recommendation which only evaluates the shoot fresh weight and dry weight, the total plant fresh weight and dry weight, comprising roots and shoots was assessed as well. Evaluating total plant

	fresh weight and dry weight avoids underestimating effects on rooted aquatic macrophytes, especially for test items which may affect root development.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	N/A

Materials and methods

Test item:	Mesosulfuron-methyl technical, Batch No.: 20160408; purity (analysed): 96.78 % w/w
Test species:	Hottonia palustris
Test design:	Five replicates per test item concentration and ten replicates for the control and solvent control were used. There was one plant per replicate. The duration of the test was 16 days. The test was performed under static test conditions. On day 16 plants were harvested from each treatment group for assessment of shoot length, shoot fresh weight, shoot dry weight, root fresh and dry weight and number and length of side shoots.
Endpoints:	Endpoints reported are the EC10, 20, 50 values for yield (EyC10, 20, 50) and growth rate (ErC10, 20, 50) based on the increase in total shoot length and shoot fresh weight and shoot dry weight respectively after 16 days of exposure. The NOEC and LOEC values for yield and growth rate were also determined, where possible. Endpoints based on total plant fresh weight and dry weight are reported for completeness in the Appendices.
Test rates:	The nominal concentrations of the test item during the test were 9.54, 30.5, 97.7, 313 and 1000 µg/L. An untreated control and a solvent control were tested in parallel. The test item was spiked to the water.
Test conditions:	Temperature, pH-value and oxygen saturation of the test solutions measured after 0, 7, 14 and 16 days are reported.
Samples analysed:	Test item concentrations in the definitive test were verified by analyses of mesosulfuron-methyl at all concentration levels by analysing the overlaying water from samples taken at test start and test end and wet sediment from samples taken at test termination on day 16. The pore water from the highest concentration level was analysed from samples taken at test termination on day 16.
Dates of work:	15 Feb 2018 – 28 Apr 2018

Results and discussions

Findings:	The measured concentration of the test item in the test vessels based on the mesosulfuron-methyl content in the freshly prepared test solution ranged between 103 and 118 % of nominal in the overlaying water. The mean measured content for all concentrations in the freshly prepared test solutions was 107 % of nominal for mesosulfuron-methyl. In the aged test solutions the measured concentration of the test item based on the mesosulfuron-methyl content in the test vessels ranged between 89 and 105 % of nominal in the overlaying water. The mean measured concentration of the test item in aged test solutions based on the mesosulfuron-methyl content in the test vessels

was 95 % of nominal in the overlaying water. In the sediment, concentrations of mesosulfuron-methyl above the LOQ were detectable at all nominal concentrations with recoveries between 4 and 7 % of the applied amount at test end after 16 days. In pore water 19 % of the applied amount was measured after 16 days at the highest nominal concentration level of 1000 µg test item/L.

Since mean measured concentrations of mesosulfuron-methyl were between 80% and 120% of nominal, all toxicological endpoints were evaluated using nominal concentrations of the test item. For completeness, endpoints based on geometric mean measured concentrations of fresh and aged test solutions are also reported in the Appendices.

Following exposure to Mesosulfuron-methyl technical, inhibition effects on *Hottonia palustris* for total shoot length and shoot fresh weight were found to be more sensitive than for shoot dry weight for the EC_{50} as indicated in the tables below.

Summary of Effects on Total Shoot Length following a 16-day exposure of *Hottonia palustris* to Mesosulfuron-methyl technical

	Growth rate (total shoot length in cm) [µg Mesosulfuron-methyl technical/L]	Yield (total shoot length in cm) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
16-day EC_{50}	15.6	10.3
95% Conf. Limits	11.9 – 19.5	7.39 – 13.2
16-day EC_{20}	5.09	3.50 [1)]
95% Conf. Limits	3.13 – 7.18	(1.93 - 5.21)
16-day EC_{10}	2.83 [1)]	1.99 [1)]
95% Conf. Limits	(1.53 - 4.35)	(0.939 - 3.25)
16-day NOEC	n.d.	n.d.
16-day LOEC	9.54	9.54

1) Values not reliable. Pooled control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.
(n.d.) not determined (no NOEC observed)

Summary of Effects on Shoot Fresh Weight following a 16-day exposure of *Hottonia palustris* to Mesosulfuron-methyl technical

	Growth rate (shoot fresh weight in g) [µg Mesosulfuron-methyl technical/L]	Yield (shoot fresh weight in g) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
16-day EC_{50}	26.2	9.81
95% Conf. Limits	18.0 – 35.7	5.32 – 15.1
16-day EC_{20}	3.83 [1)]	1.19 [1)]
95% Conf. Limits	(1.83 - 6.47)	(0.376 - 2.54)
16-day EC_{10}	1.40 [1)]	0.398 [1)]
95% Conf. Limits	(0.538 - 2.74)	(0.0923 - 1.02)
16-day NOEC	n.d.	n.d.
16-day LOEC	9.54	9.54

1) Values not reliable. Pooled control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.
(n.d.) not determined (no NOEC observed)

Summary of Effects on Shoot Dry Weight following a 16-day exposure of *Hottonia palustris* to Mesosulfuron-methyl technical

	Growth rate (shoot dry weight in g) [µg Mesosulfuron-methyl technical/L]	Yield (shoot dry weight in g) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
16-day EC_{50}	> 1000	> 1000
95% Conf. Limits	n.a.	n.a.
16-day EC_{20}	n.r.[1)]	n.r.[1)]
95% Conf. Limits	n.r.[1)]	n.r.[1)]
16-day EC_{10}	n.r.[1)]	n.r.[1)]
95% Conf. Limits	n.r.[1)]	n.r.[1)]
16-day NOEC	30.5 [2)]	30.5 [2)]
16-day LOEC	97.7 [2)]	97.7 [2)]

(n.a.) not applicable
(n.r. [1)]) estimates not reliable as no clear dose response was obtained and pooled control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.
[2)] values not reliable; no dose response could be observed for this parameter

The average pH-value was determined to be 7.72 ± 0.21, the average temperature was measured to be 18.9 ± 0.4 °C and the oxygen saturation was determined to be 105 ± 4 %. The test item had no influence on the pH-value of the test solutions.

Conclusions:

Following exposure of the aquatic rooted macrophyte *Hottonia palustris* to Mesosulfuron-methyl technical for 16 days the E_rC_{50} and E_yC_{50} values based on total shoot length were 15.6 µg/L and 10.3 µg/L (nominal) respectively. The NOEC based on total shoot length could not be determined for growth rate and yield.

The E_rC_{50} and E_yC_{50} values based on biomass (shoot fresh weight) were 26.2 µg/L and 9.81 µg/L (nominal) respectively. The NOEC for growth rate and yield based on biomass (shoot fresh weight) could not be determined.

The E_rC_{50} and E_yC_{50} values based on biomass (shoot dry weight) were estimated to be > 1000 µg/L (nominal). The NOEC for growth rate and yield based on biomass (shoot dry weight) was 30.5 µg/L (nominal), whereas not considered to be reliable as no dose response could be observed for these parameters.

A 2.2.3.4 Myriophyllum sibiricum

Comments of zRMS:	The study is considered valid. All validity criteria were met. • Increase in shoot length of control plants: Mean total shoot length must at least during the exposure period • Increase in shoot fresh weight of control plants Mean shoot fresh weight must at lea during the exposure period • Coefficient of variation for yield (based on shoot fresh weight of control plants): Must not exceed 35 % between replicates **In the current study:** • Increase in shoot length of control plants: The shoot length increased by a factor of 8.2 after 14 days of exposureIncrease in fresh weight of control plants • The fresh weight increased by a factor of 6.0 after 14 days of exposure • Coefficient of variation for yield (based on fresh weight in Control): 25.6 % • Sublethal effects in Control: Control plants did not show any sign of chlorosis and were visibly free from contamination by other organisms such as algae and/or bacterial film **Agreed endpoints:** 14 **d E_rC_{50} = 32.1 µg/L** **14 d E_yC_{50} = 13.9 µg/L** **Based on based total shoot length** **NOEC = 1.91 µg/L for growth rate and yield based on total shoot length** **14 d E_rC_{50} =20.7 µg/L** **14 d E_yC_{50} = 12.4 µg/L** **Based on biomass (shoot fresh weight).** **NOEC for growth rate and yield based on biomass (shoot fresh weight) was 6.10 µg/L (nominal)** **E_rC_{50} =101 µg/L and E_yC_{50} = 51.2 µg/L values based on biomass (shoot dry weight)** **NOEC based on biomass (shoot dry weight) was 6.10 µg/L (nominal) for growth rate and yield.**

Reference:	KCP 10.2-06_03
Report	Mesosulfuron-methyl technical: Growth inhibition of *Myriophyllum sibiricum* in water/sediment system. Maren D, 2018, S18-00148.
Guideline(s):	Yes, OECD 239 (2014)
Deviations:	In addition to the guideline recommendation which only evaluates the shoot fresh weight and dry weight, the total plant fresh weight and dry weight, comprising roots and shoots was assessed as well. Evaluating total plant fresh weight and dry weight avoids underestimating effects on rooted aquatic macrophytes, especially for test items which may affect root development.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	N/A

Materials and methods

Test item: Mesosulfuron-methyl technical, Batch No.: 20160408; purity (analysed): 96.78 % w/w

Test species: *Myriophyllum sibiricum*

Test design: Five replicates per test item concentration and ten replicates for the control and solvent control were used. There was one plant per replicate. The duration of the test was 14 days. The test was performed under static test conditions. On day 14 plants were harvested from each treatment group for assessment of shoot length, shoot fresh weight, shoot dry weight, root fresh and dry weight and number and length of side shoots.

Endpoints: Endpoints reported are the $EC_{10,\ 20,\ 50}$ values for yield ($E_yC_{10,\ 20,\ 50}$) and growth rate ($E_rC_{10,\ 20,\ 50}$) based on the increase in total shoot length and shoot fresh weight and shoot dry weight respectively after 14 days of exposure. The NOEC and LOEC values for yield and growth rate were also determined. Endpoints based on total plant fresh weight and dry weight are reported for completeness in the Appendices.

Test rates: The nominal concentrations of the test item during the test were 1.91, 6.10, 19.5, 62.5 and 200 µg/L. An untreated control and a solvent control were tested in parallel. The test item was spiked to the water.

Test conditions: Temperature, pH-value and oxygen saturation of the test solutions measured after 0, 7 and 14 days are reported.

Samples analysed: Test item concentrations in the definitive test were verified by analyses of mesosulfuron-methyl at all concentration levels by analysing the overlaying water from samples taken at test start and test end and wet sediment from samples taken at test termination on day 14. The pore water from the highest concentration level was analysed from samples taken at test termination on day 14.

Dates of work: 22 Feb 2018 – 10 Apr 2018

Results and discussions

Findings**:** The measured concentration of the test item in the test vessels based on the mesosulfuron-methyl content in the freshly prepared test solution ranged between 83 and 109 % of nominal in the overlaying water. The mean measured content for all concentrations in the freshly prepared test solutions was 98 % of nominal for mesosulfuron-methyl. In the aged test solutions the measured concentration of the test item based on the mesosulfuron-methyl content in the test vessels ranged between 81 and 103 % of nominal in the overlaying water. The mean measured concentration of the test item in aged test solutions based on the mesosulfuron-methyl content in the test vessels was 89 % of nominal in the overlaying water. In the sediment, concentrations of mesosulfuron-methyl above the LOQ were detectable at nominal concentrations of 6.10 µg/L and higher with respective recoveries between 4 and 7% of the applied amount at test end after 14 days. In pore water < 1 % of the applied amount was measured after 14 days at the highest nominal concentration level of 200 µg test item/L.

Since mean measured concentrations of mesosulfuron-methyl were between 80% and 120% of nominal, all toxicological endpoints were evaluated using nominal concentrations of the test item. For completeness, endpoints based on geometric mean measured concentrations of fresh and aged test solutions are also reported in the Appendices.

Following exposure to Mesosulfuron-methyl technical, inhibition effects on *Myriophyllum sibiricum* for total shoot length and shoot fresh weight were found to be more sensitive than for shoot dry weight for the EC_{50} as indicated in the tables below.

Summary of Effects on Total Shoot Length following a 14-day exposure of *Myriophyllum sibiricum* to Mesosulfuron-methyl technical

	Growth rate (total shoot length in cm) [µg Mesosulfuron-methyl technical/L]	Yield (total shoot length in cm) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
14-day EC_{50}	32.1	13.9
95% Conf. Limits	26.1 – 39.8	11.4 – 16.8
14-day EC_{20}	8.65	4.29 [1)]
95% Conf. Limits	6.40 – 11.1	(3.18 - 5.48)
14-day EC_{10}	4.35	2.32 [1)]
95% Conf. Limits	2.94 – 5.93	(1.58 - 3.13)
14-day NOEC	1.91	1.91
14-day LOEC	6.10	6.10

[1)] Values not reliable. Pooled control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.

Summary of Effects on Shoot Fresh Weight following a 14-day exposure of *Myriophyllum sibiricum* to Mesosulfuron-methyl technical

	Growth rate (shoot fresh weight in g) [µg Mesosulfuron-methyl technical/L]	Yield (shoot fresh weight in g) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
14-day EC_{50}	20.7	12.4
95% Conf. Limits	17.4 – 24.7	10.3 – 14.8
14-day EC_{20}	7.62	4.35 [1)]
95% Conf. Limits	5.96 – 9.36	(3.33 - 5.42)
14-day EC_{10}	4.52 [1)]	2.52 [1)]
95% Conf. Limits	(3.32 - 5.80)	(1.80 - 3.30)
14-day NOEC	6.10	6.10
14-day LOEC	19.5	19.5

[1)] Values not reliable. Pooled control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.

Summary of Effects on Shoot Dry Weight following a 14-day exposure of *Myriophyllum sibiricum* to Mesosulfuron-methyl technical

	Growth rate (shoot dry weight in g) [µg Mesosulfuron-methyl technical/L]	Yield (shoot dry weight in g) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
14-day EC_{50}	101	51.2
95% Conf. Limits	76.0 – 142	40.4 – 66.5
14-day EC_{20}	20.6	11.3 1)
95% Conf. Limits	15.0 – 26.9	(8.10 - 14.7)
14-day EC_{10}	8.99 1)	5.11 1)
95% Conf. Limits	(5.71 - 12.6)	(3.25 - 7.21)
14-day NOEC	6.10	6.10
14-day LOEC	19.5	19.5

1) Values not reliable. Pooled control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.

The average pH-value was determined to be 7.96 ± 0.54, the average temperature was measured to be 19.4 ± 0.7 °C and the oxygen saturation was determined to be 109 ± 10 %. The test item had no influence on the pH-value of the test solutions.

Conclusions: Following exposure of the aquatic rooted eudicotyledon macrophyte *Myriophyllum sibiricum* to Mesosulfuron-methyl technical for 14 days the E_rC_{50} and E_yC_{50} values based on total shoot length were 32.1 µg/L and 13.9 µg/L (nominal) respectively. The NOEC based on total shoot length was 1.91 µg/L (nominal) for growth rate and yield.

The E_rC_{50} and E_yC_{50} values based on biomass (shoot fresh weight) were 20.7 µg/L and 12.4 µg/L (nominal) respectively. The NOEC for growth rate and yield based on biomass (shoot fresh weight) was 6.10 µg/L (nominal) respectively.

The E_rC_{50} and E_yC_{50} values based on biomass (shoot dry weight) were 101 µg/L and 51.2 µg/L (nominal). The NOEC based on biomass (shoot dry weight) was 6.10 µg/L (nominal) for growth rate and yield.

A 2.2.3.5 Ceratophyllum demersum

Comments of zRMS:	The study is considered valid. All validity criteria were met. • Increase in shoot length of control plants: Mean total shoot length must at least double during the exposure period • Increase in shoot fresh weight of control plants: Mean shoot fresh weight must at least double during the exposure period • Coefficient of variation for yield (based on shoot fresh weight of control plants): Must not exceed 35 % between replicates

In the current study:

Increase in shoot length of control plants:	The shoot length increased by a factor of 2.4 after 14 days of exposure,
Increase in shoot fresh weight of control plants:	The shoot fresh weight increased by a factor of 3.0 after 14 days of exposure,
Coefficient of variation for yield (based on shoot fresh weight in Control):	32.5 %

Sublethal effects in Control: Control plants did not show any sign of chlorosis and were visibly free from contamination by other organisms such as algae and/or bacterial film

Agreed endpoints:

Effects on Total Shoot Length following a 14-day exposure of *Ceratophyllum demersum* to Mesosulfuron-methyl technical

	Growth rate (total shoot length in cm) [µg Mesosulfuron-methyl technical/L]	**Yield (total shoot length in cm) [µg Mesosulfuron-methyl technical/L]**
Nominal concentrations		
14-day EC_{50}	8.66	8.20
95% Conf. Limits	7.41 – 10.1	6.98 – 9.61
14-day EC_{20}	3.89 [1)]	3.54 [1)]
95% Conf. Limits	(3.13 - 4.66)	(2.81 - 4.27)
14-day EC_{10}	2.56 [1)]	2.28 [1)]
95% Conf. Limits	(1.95 - 3.18)	(1.71 - 2.86)
14-day NOEC	3.05	3.05
14-day LOEC	9.77	9.77
Geometric mean measured concentrations		
14-day EC_{50}	7.77	7.37
95% Conf. Limits	6.60 - 9.16	6.22 - 8.73
14-day EC_{20}	3.32 [1)]	2.98 [1)]
95% Conf. Limits	(2.65 - 4.00)	(2.35 - 3.63)
14-day EC_{10}	2.12 [1)]	1.86 [1)]
95% Conf. Limits	(1.60 - 2.66)	(1.38 - 2.35)
14-day NOEC	2.75	2.75
14-day LOEC	8.01	8.01

[1)] Value not reliable. Pooled control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.

Effects on Shoot Fresh Weight following a 14-day exposure of *Ceratophyllum demersum* to Mesosulfuron-methyl technical

	Growth rate (shoot fresh weight in g) [µg Mesosulfuron-methyl technical/L]	Yield (shoot fresh weight in g) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
14-day EC_{50}	16.5	11.2
95% Conf. Limits	13.1 – 21.3	8.86 – 14.2
14-day EC_{20}	3.38 [1)]	2.38 [1)]
95% Conf. Limits	(2.34 - 4.53)	(1.62 - 3.22)
14-day EC_{10}	1.47 [1)]	1.06 [1)]
95% Conf. Limits	(0.895 - 2.15)	(0.635 - 1.56)
14-day NOEC	3.05	3.05
14-day LOEC	9.77	9.77
Geometric mean measured concentrations		
14-day EC_{50}	16.0	10.6
95% Conf. Limits	12.4 - 21.0	8.22 - 13.6
14-day EC_{20}	2.93 [1)]	2.00 [1)]
95% Conf. Limits	(1.98 - 4.00)	(1.33 - 2.77)
14-day EC_{10}	1.20 [1)]	0.840 [1)]
95% Conf. Limits	(0.709 - 1.80)	(0.487 - 1.27)
14-day NOEC	2.75	2.75
14-day LOEC	8.01	8.01

[1)] Value not reliable. Pooled control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.

Effects on Shoot Dry Weight following a 14-day exposure of *Ceratophyllum demersum* to Mesosulfuron-methyl technical

	Growth rate (shoot dry weight in g) [µg Mesosulfuron-methyl technical/L]	Yield (shoot dry weight in g) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
14-day EC_{50}	21.4	9.34 [2)]
95% Conf. Limits	15.4 – 31.3	7.20 – 12.5
14-day EC_{20}	2.11 [1)]	1.83 [1)]
95% Conf. Limits	(1.16 - 3.24)	(1.15 - 2.56)
14-day EC_{10}	0.627 [1)]	0.779 [1)]
95% Conf. Limits	(0.261 - 1.14)	(0.399 - 1.23)
14-day NOEC	9.77	9.77
14-day LOEC	31.3	31.3
Geometric mean measured concentrations		
14-day EC_{50}	21.3 [2)]	8.63 [2)]
95% Conf. Limits	14.9 - 32.1	6.56 - 11.8
14-day EC_{20}	1.77 [1)]	1.53 [1)]
95% Conf. Limits	(0.934 - 2.80)	(0.936 - 2.19)
14-day EC_{10}	0.482 [1)]	0.622 [1)]
95% Conf. Limits	(0.188 - 0.915)	(0.305 - 1.01)

	14-day NOEC	8.01	8.01
	14-day LOEC	32.6	32.6

[1)] Value not reliable. Pooled control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.
[2)] Value is not considered to be reliable as the pooled control CV slightly exceeded the effect level

Reference: KCP 10.2-06_04

Report: Mesosulfuron-methyl technical: Growth inhibition of *Ceratophyllum demersum* in water/sediment system. Maren D, 2018, S18-00149.

Guideline(s): Yes, OECD 239 (2014)

Deviations: none

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): N/A

Materials and methods

Test item: Mesosulfuron-methyl technical, Batch No.: 20160408; purity (analysed): 96.78 % w/w

Test species: *Ceratophyllum demersum*

Test design: Five replicates per test item concentration and ten replicates for the control and solvent control were used. There was one plant per replicate. The duration of the test was 14 days. The test was performed under static test conditions. On day 14 plants were harvested from each treatment group for assessment of shoot length, shoot fresh weight, shoot dry weight and number and length of side shoots.

Endpoints: Endpoints reported are the $EC_{10,\ 20,\ 50}$ values for yield ($E_yC_{10,\ 20,\ 50}$) and growth rate ($E_rC_{10,\ 20,\ 50}$) based on the increase in total shoot length and shoot fresh weight and shoot dry weight respectively after 14 days of exposure. The NOEC and LOEC values for yield and growth rate were also determined. Endpoints based on total plant fresh weight and dry weight were not reported as *Ceratophyllum demersum* is an unrooted aquatic macrophyte.

Test rates: The nominal concentrations of the test item during the test were 0.954, 3.05, 9.77, 31.3 and 100 µg/L. An untreated control and a solvent control were tested in parallel. The test item was spiked to the water.

Test conditions: Temperature, pH-value and oxygen saturation of the test solutions measured after 0, 7 and 14 days are reported.

Samples analysed: Test item concentrations in the definitive test were verified by analyses of mesosulfuron-methyl at all concentration levels by analysing the overlaying water from samples taken at test start and test end and wet sediment from samples taken at test termination on day 14. The pore water from the highest concentration level was analysed from samples taken at test termination on day 14.

Dates of work: 07 Mar 2018 – 19 Apr 2018

Results and discussions

Findings:

The measured concentration of the test item in the test vessels based on the mesosulfuron-methyl content in the freshly prepared test solution ranged between 84 and 128% of nominal in the overlaying water. The mean measured content for all concentrations in the freshly prepared test solutions was 103% of nominal for mesosulfuron-methyl. In the aged test solutions the measured concentration of the test item based on the mesosulfuron-methyl content in the test vessels ranged between 80 and 101% of nominal in the overlaying water. The mean measured concentration of the test item in aged test solutions based on the mesosulfuron-methyl content in the test vessels was 89% of nominal in the overlaying water. In the sediment, concentrations of mesosulfuron-methyl above the LOQ were detectable at nominal concentrations of 31.3 and 100 µg/L with respective recoveries of 4 and 5% of the applied amount at test end after 14 days. In pore water < 1 % of the applied amount was measured after 14 days at the highest nominal concentration level of 100 µg test item/L.

Since mean measured concentrations of mesosulfuron-methyl were between 80% and 120% of nominal, all toxicological endpoints were evaluated using nominal concentrations of the test item. In addition as one initial measured concentration was above 120% of nominal, endpoints based on geometric mean measured concentrations of fresh and aged test solutions are also shown in the summary tables and conclusion.

Following exposure to Mesosulfuron-methyl technical, inhibition effects on *Ceratophyllum demersum* for total shoot length were found to be slightly more sensitive than for shoot fresh weight and shoot dry weight for the EC_{50} as indicated in the tables below.

Summary of Effects on Total Shoot Length following a 14-day exposure of *Ceratophyllum demersum* to Mesosulfuron-methyl technical

	Growth rate (total shoot length in cm) [µg Mesosulfuron-methyl technical/L]	Yield (total shoot length in cm) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
14-day EC_{50}	8.66	8.20
95% Conf. Limits	7.41 – 10.1	6.98 – 9.61
14-day EC_{20}	3.89 [1)]	3.54 [1)]
95% Conf. Limits	(3.13 - 4.66)	(2.81 - 4.27)
14-day EC_{10}	2.56 [1)]	2.28 [1)]
95% Conf. Limits	(1.95 - 3.18)	(1.71 - 2.86)
14-day NOEC	3.05	3.05
14-day LOEC	9.77	9.77
Geometric mean measured concentrations		
14-day EC_{50}	7.77	7.37
95% Conf. Limits	6.60 - 9.16	6.22 - 8.73
14-day EC_{20}	3.32 [1)]	2.98 [1)]
95% Conf. Limits	(2.65 - 4.00)	(2.35 - 3.63)
14-day EC_{10}	2.12 [1)]	1.86 [1)]
95% Conf. Limits	(1.60 - 2.66)	(1.38 - 2.35)
14-day NOEC	2.75	2.75
14-day LOEC	8.01	8.01

[1)] Value not reliable. Pooled control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.

Summary of Effects on Shoot Fresh Weight following a 14-day exposure of *Ceratophyllum demersum* to Mesosulfuron-methyl technical

	Growth rate (shoot fresh weight in g) [µg Mesosulfuron-methyl technical/L]	Yield (shoot fresh weight in g) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
14-day EC_{50}	16.5	11.2
95% Conf. Limits	13.1 – 21.3	8.86 – 14.2
14-day EC_{20}	3.38 [1)]	2.38 [1)]
95% Conf. Limits	(2.34 - 4.53)	(1.62 - 3.22)
14-day EC_{10}	1.47 [1)]	1.06 [1)]
95% Conf. Limits	(0.895 - 2.15)	(0.635 - 1.56)
14-day NOEC	3.05	3.05
14-day LOEC	9.77	9.77
Geometric mean measured concentrations		
14-day EC_{50}	16.0	10.6
95% Conf. Limits	12.4 - 21.0	8.22 - 13.6
14-day EC_{20}	2.93 [1)]	2.00 [1)]
95% Conf. Limits	(1.98 - 4.00)	(1.33 - 2.77)
14-day EC_{10}	1.20 [1)]	0.840 [1)]
95% Conf. Limits	(0.709 - 1.80)	(0.487 - 1.27)
14-day NOEC	2.75	2.75
14-day LOEC	8.01	8.01

[1)] Value not reliable. Pooled control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.

Summary of Effects on Shoot Dry Weight following a 14-day exposure of *Ceratophyllum demersum* to Mesosulfuron-methyl technical

	Growth rate (shoot dry weight in g) [µg Mesosulfuron-methyl technical/L]	Yield (shoot dry weight in g) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
14-day EC_{50}	21.4	9.34 [2)]
95% Conf. Limits	15.4 – 31.3	7.20 – 12.5
14-day EC_{20}	2.11 [1)]	1.83 [1)]
95% Conf. Limits	(1.16 - 3.24)	(1.15 - 2.56)
14-day EC_{10}	0.627 [1)]	0.779 [1)]
95% Conf. Limits	(0.261 - 1.14)	(0.399 - 1.23)
14-day NOEC	9.77	9.77
14-day LOEC	31.3	31.3
Geometric mean measured concentrations		
14-day EC_{50}	21.3 [2)]	8.63 [2)]
95% Conf. Limits	14.9 - 32.1	6.56 - 11.8
14-day EC_{20}	1.77 [1)]	1.53 [1)]
95% Conf. Limits	(0.934 - 2.80)	(0.936 - 2.19)
14-day EC_{10}	0.482 [1)]	0.622 [1)]
95% Conf. Limits	(0.188 - 0.915)	(0.305 - 1.01)
14-day NOEC	8.01	8.01
14-day LOEC	32.6	32.6

[1)] Value not reliable. Pooled control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.
[2)] Value is not considered to be reliable as the pooled control CV slightly exceeded the effect level

The average pH-value was determined to be 8.69 ± 0.47, the average temperature was measured to be 19.8 ± 0.4 °C and the oxygen saturation was determined to be 123 ± 13 %. The test item had no influence on the pH-value of the test solutions.

Conclusions:

Following exposure of the aquatic unrooted macrophyte *Ceratophyllum demersum* to Mesosulfuron-methyl technical for 14 days the E_rC_{50} and E_yC_{50} values based on total shoot length were 8.66 µg/L and 8.20 µg/L (nominal) respectively. The NOEC based on total shoot length was 3.05 µg/L (nominal) for growth rate and yield. Corresponding E_rC_{50} and E_yC_{50} values based on geometric mean measured concentrations were 7.77 µg/L and 7.37 µg/L (mean measured). The NOEC based total shoot length was 2.75 µg/L (mean measured) for growth rate and yield.

The E_rC_{50} and E_yC_{50} values based on biomass (shoot fresh weight) were 16.5 µg/L and 11.2 µg/L (nominal) respectively. The NOEC for growth rate and yield based on biomass (shoot fresh weight) was 3.05 µg/L (nominal) respectively. Corresponding E_rC_{50} and E_yC_{50} values based on geometric mean measured concentrations were 16.0 µg/L and 10.6 µg/L (mean measured). The NOEC based on fresh weight was 2.75 µg/L (mean measured) for growth rate and yield.

The E_rC_{50} value based on biomass (shoot dry weight) was 21.4 µg/L (nominal). The E_yC_{50} value based on biomass (shoot dry weight) was estimated to be 9.34 µg/L (nominal) but considered to not be reliable as the pooled control CV slightly exceeded the effect level. The NOEC based on biomass (shoot dry weight) was 9.77 µg/L (nominal) for growth rate and yield. Corresponding E_rC_{50} and E_yC_{50} values based on geometric mean measured concentrations were 21.3 µg/L and 8.63 µg/L (mean measured). The NOEC based on dry weight was 8.01 µg/L (mean measured) for growth rate and yield.

A 2.2.3.6 Vallisneria spiralis

Comments of zRMS:	The study is considered valid. All validity criteria were met. • Increase in shoot length of control plants: Mean total shoot length must at least double during the exposure period • Increase in shoot fresh weight of control plants Mean shoot fresh weight must at least double during the exposure period • Coefficient of variation for yield (based on shoot fresh weight of control plants): Must not exceed 35 % between replicates **In the current study:** Increase in shoot length of control plants: The shoot length increased by a factor of 2.1 after 21 days of exposure, Increase in shoot fresh weight of control plants: The shoot fresh weight increased by a factor of 2.7 after 21 days of exposure, validity criterion was therefore met Coefficient of variation for yield (based on shoot fresh weight in Control): 22.0 %, Sublethal effects in Control: Control plants did not show any sign of chlorosis and were visibly free from contamination by other organisms such as algae and/or bacterial film, validity criterion was therefore met

Agreed endpoints:

Effects on Total Shoot Length following a 21-day exposure of *Vallisneria* spiralis to Mesosulfuron-methyl technical

	Growth rate (total shoot length in cm) [µg Mesosulfuron-methyl technical/L]	Yield (total shoot length in cm) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
21-day EC_{50}	43.5	36.8
95% Conf. Limits	37.6 - 50.3	32.0 - 42.3
21-day EC_{20}	22.2 [1)]	19.5 [1)]
95% Conf. Limits	(17.9 - 26.3)	(15.8 - 23.0)
21-day EC_{10}	15.6 [1)]	13.9 [1)]
95% Conf. Limits	(11.8 - 19.2)	(10.7 - 17.0)
21-day NOEC	9.77	9.77
21-day LOEC	31.3	31.3
Geometric mean measured concentrations		
21-day EC_{50}	51.1	43.5
95% Conf. Limits	44.4 - 58.8	37.9 - 49.8
21-day EC_{20}	26.8 [1)]	23.5 [1)]
95% Conf. Limits	(21.6 - 31.6)	(19.1 - 27.7)
21-day EC_{10}	19.1 [1)]	17.1 [1)]
95% Conf. Limits	(14.5 - 23.4)	(13.1 - 20.8)
21-day NOEC	11.5	11.5
21-day LOEC	37.9	37.9

[1)] Value not reliable. Pooled control CV exceeded the effect level (EC_x).
Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.

Effects on Shoot Fresh Weight following a 21-day exposure of *Vallisneria spiralis* to Mesosulfuron-methyl technical

	Growth rate (shoot fresh weight in g) [µg Mesosulfuron-methyl technical/L]	Yield (shoot fresh weight in g) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
21-day EC_{50}	56.1	35.6
95% Conf. Limits	42.9 -77.9	28.1 - 46.7
21-day EC_{20}	13.1	8.656 [1)]
95% Conf. Limits	9.71 - 16.8	(6.43 - 11.1)
21-day EC_{10}	6.10 [1)]	4.13 [1)]
95% Conf. Limits	(4.04 - 8.35)	(2.76 - 5.64)
21-day NOEC	9.77	9.77
21-day LOEC	31.3	31.3
Geometric mean measured concentrations		
21-day EC_{50}	65.1	41.3

	95% Conf. Limits	49.8 - 90.4	32.5 - 54.1
	21-day EC_{20}	15.0	9.89 [1)]
	95% Conf. Limits	11.1 - 19.4	(7.32 - 12.7)
	21-day EC_{10}	6.99 [1)]	4.69 [1)]
	95% Conf. Limits	(4.60 - 9.60)	(3.12 - 6.42)
	21-day NOEC	11.5	11.5
	21-day LOEC	37.9	37.9
	[1)] Value not reliable. Pooled control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.		

Reference: KCP 10.2-06_05

Report: Mesosulfuron-methyl technical: Growth inhibition of *Vallisneria spiralis* in water/sediment system. Maren D, 2018, S18-00150.

Guideline(s): Yes, OECD 239 (2014)

Deviations: In addition to the guideline recommendation which only evaluates the shoot fresh weight and dry weight, the total plant fresh weight and dry weight, comprising roots and shoots was assessed as well. Evaluating total plant fresh weight and dry weight avoids underestimating effects on rooted aquatic macrophytes, especially for test items which may affect root development.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): N/A

Materials and methods

Test item: Mesosulfuron-methyl technical, Batch No.: 20160408; purity (analysed): 96.78 % w/w

Test species: *Vallisneria spiralis*

Test design: Five replicates per test item concentration and ten replicates for the control and solvent control were used. There was one plant per replicate. The duration of the test was 21 days. The test was performed under static test conditions. On day 21 plants were harvested from each treatment group for assessment of shoot length, shoot fresh weight, shoot dry weight, root fresh and dry weight and number of shoots.

Endpoints: Endpoints reported are the $EC_{10,\ 20,\ 50}$ values for yield ($E_yC_{10,\ 20,\ 50}$) and growth rate ($E_rC_{10,\ 20,\ 50}$) based on the increase in total shoot length, shoot fresh weight and shoot dry weight respectively after 21 days of exposure. The NOEC and LOEC values for yield and growth rate were also determined. Endpoints based on total plant fresh weight and dry weight are reported for completeness in the Appendices.

Test rates: The nominal concentrations of the test item during the test were 0.298, 0.954, 3.05, 9.77, 31.3 and 100 µg/L. An untreated control and a solvent control were tested in parallel. The test item was spiked to the water.

Test conditions:	Temperature, pH-value and oxygen saturation of the test solutions measured after 0, 7, 14 and 21 days are reported.
Samples analysed:	Test item concentrations in the definitive test were verified by analyses of mesosulfuron-methyl at all concentration levels by analysing the overlaying water from samples taken at test start and test end and wet sediment from samples taken at test termination on day 21. The pore water from the highest concentration level was analysed from samples taken at test termination on day 21.
Dates of work:	19 Apr 2018 – 23 Jul 2018

Results and discussions

Findings**:**

The measured concentration of the test item in the test vessels based on the mesosulfuron-methyl content in the freshly prepared test solution ranged between 117 and 128% of nominal in the overlaying water. The mean measured content for all concentrations in the freshly prepared test solutions was 124% of nominal for mesosulfuron-methyl. In the aged test solutions the measured concentration of the test item based on the mesosulfuron-methyl content in the test vessels ranged between 87 and 118% of nominal in the overlaying water. The mean measured concentration of the test item in aged test solutions based on the mesosulfuron-methyl content in the test vessels was 107% of nominal in the overlaying water. In the sediment, concentrations of mesosulfuron-methyl above the LOQ were detectable at concentrations of 9.77, 31.3 and 100 µg/L with recoveries between 10 and 11% of the applied amount at test end after 21 days. In pore water < 1 % of the applied amount was measured after 21 days at the highest nominal concentration level of 100 µg test item/L.

All toxicological endpoints were evaluated using nominal concentrations of the test item. Since the mean measured content for all concentrations in the freshly prepared test solutions was above 120% of nominal, endpoints based on geometric mean measured concentrations of fresh and aged test solutions are also reported.

Following exposure to Mesosulfuron-methyl technical, inhibition effects on *Vallisneria spiralis* for total shoot length, shoot fresh and dry weight were found to be equaly sensitive when comparing the E_yC_{50} values as indicated in the tables below.

Summary of Effects on Total Shoot Length following a 21-day exposure of *Vallisneria spiralis* to Mesosulfuron-methyl technical

	Growth rate (total shoot length in cm) [µg Mesosulfuron-methyl technical/L]	Yield (total shoot length in cm) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
21-day EC_{50}	43.5	36.8
95% Conf. Limits	37.6 - 50.3	32.0 - 42.3
21-day EC_{20}	22.2 [1)]	19.5 [1)]
95% Conf. Limits	(17.9 - 26.3)	(15.8 - 23.0)
21-day EC_{10}	15.6 [1)]	13.9 [1)]
95% Conf. Limits	(11.8 - 19.2)	(10.7 - 17.0)
21-day NOEC	9.77	9.77
21-day LOEC	31.3	31.3
Geometric mean measured concentrations		
21-day EC_{50}	51.1	43.5
95% Conf. Limits	44.4 - 58.8	37.9 - 49.8
21-day EC_{20}	26.8 [1)]	23.5 [1)]
95% Conf. Limits	(21.6 - 31.6)	(19.1 - 27.7)
21-day EC_{10}	19.1 [1)]	17.1 [1)]
95% Conf. Limits	(14.5 - 23.4)	(13.1 - 20.8)
21-day NOEC	11.5	11.5
21-day LOEC	37.9	37.9

[1)] Value not reliable. Pooled control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.

Summary of Effects on Shoot Fresh Weight following a 21-day exposure of *Vallisneria spiralis* to Mesosulfuron-methyl technical

	Growth rate (shoot fresh weight in g) [µg Mesosulfuron-methyl technical/L]	Yield (shoot fresh weight in g) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
21-day EC_{50}	56.1	35.6
95% Conf. Limits	42.9 -77.9	28.1 - 46.7
21-day EC_{20}	13.1	8.656 [1)]
95% Conf. Limits	9.71 - 16.8	(6.43 - 11.1)
21-day EC_{10}	6.10 [1)]	4.13 [1)]
95% Conf. Limits	(4.04 - 8.35)	(2.76 - 5.64)
21-day NOEC	9.77	9.77
21-day LOEC	31.3	31.3
Geometric mean measured concentrations		
21-day EC_{50}	65.1	41.3
95% Conf. Limits	49.8 - 90.4	32.5 - 54.1
21-day EC_{20}	15.0	9.89 [1)]
95% Conf. Limits	11.1 - 19.4	(7.32 - 12.7)
21-day EC_{10}	6.99 [1)]	4.69 [1)]
95% Conf. Limits	(4.60 - 9.60)	(3.12 - 6.42)
21-day NOEC	11.5	11.5
21-day LOEC	37.9	37.9

[1)] Value not reliable. Pooled control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.

Summary of Effects on Shoot Dry Weight following a 21-day exposure of *Vallisneria spiralis* to Mesosulfuron-methyl technical

	Growth rate (shoot dry weight in g) [µg Mesosulfuron-methyl technical/L]	Yield (shoot dry weight in g) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
21-day EC_{50}	31.3	18.4
95% Conf. Limits	22.5 - 50.0	14.2 - 25.4
21-day EC_{20}	7.33	4.69
95% Conf. Limits	5.39 - 9.56	3.43 - 6.03
21-day EC_{10}	3.43 [1)]	2.29 [1)]
95% Conf. Limits	(2.17 - 4.75)	(1.46 - 3.17)
21-day NOEC	3.05	3.05
21-day LOEC	9.77	9.77
Geometric mean measured concentrations		
21-day EC_{50}	38.6	22.0
95% Conf. Limits	27.2 - 63.7	16.8 - 31.2
21-day EC_{20}	8.29	5.16
95% Conf. Limits	6.01 - 11.0	3.72 - 6.73
21-day EC_{10}	3.71 [1)]	2.42 [1)]
95% Conf. Limits	(2.29 - 5.21)	(1.50 - 3.40)
21-day NOEC	3.08	3.08
21-day LOEC	11.5	11.5

[1)] Value not reliable. Pooled control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.

The average pH-value was determined to be 7.72 ± 0.43, the average temperature was measured to be 20.2 ± 0.5 °C and the oxygen saturation was determined to be 105 ± 8%. The test item had no influence on the pH-value of the test solutions.

Conclusions:

Following exposure of the aquatic rooted eudicotyledon macrophyte *Vallisneria spiralis* to Mesosulfuron-methyl technical for 21 days the E_rC_{50} and E_yC_{50} values based on total shoot length were 43.5 µg/L and 36.8 µg/L (nominal) respectively. The NOEC based on total shoot length was 9.77 µg/L (nominal) for growth rate and yield. Corresponding E_rC_{50} and E_yC_{50} values based on geometric mean measured concentrations were 51.1 µg/L and 43.5 µg/L (mean measured). The NOEC based total shoot length was 11.5 µg/L (mean measured) for growth rate and yield.

The E_rC_{50} and E_yC_{50} values based on biomass (shoot fresh weight) were 56.1 µg/L and 35.6 µg/L (nominal) respectively. The NOEC for growth rate and yield based on biomass (shoot fresh weight) was 9.77 µg/L (nominal) respectively. Corresponding E_rC_{50} and E_yC_{50} values based on geometric mean measured concentrations were 65.1 µg/L and 41.3 µg/L (mean measured). The NOEC based on fresh weight was 11.5 µg/L (mean measured) for growth rate and yield.

The E_rC_{50} and E_yC_{50} values based on biomass (shoot dry weight) were 31.3 µg/L and 18.4 µg/L (nominal). The NOEC based on biomass (shoot dry weight) was 3.05 µg/L (nominal) for growth rate and yield. Corresponding E_rC_{50} and E_yC_{50} values based on geometric mean measured concentrations were 38.6 µg/L and 22.0 µg/L (mean measured). The NOEC based on shoot dry weight was 3.08 µg/L (mean measured) for growth rate and yield.

A 2.2.3.7 Glyceria maxima

<table>
<tr><td>Comments of zRMS:</td><td>
The study is considered valid. All validity criteria were met.
The study is considered valid. All validity criteria were met.

Increase in shoot length of control plants: Mean total shoot length must at least double during the exposure period
Increase in shoot fresh weight of control plants Mean shoot fresh weight must at least double during the exposure period
Coefficient of variation for yield (based on shoot fresh weight of control plants): Must not exceed 35 % between replicates

In the correct study:
<table>
<tr><td>Increase in shoot length of control plants:</td><td>The shoot length increased by a factor of 2.6 after 14 days of exposure,</td></tr>
<tr><td>Increase in shoot fresh weight of control plants:</td><td>The shoot fresh weight increased by a factor of 2.8 after 14 days of exposure,</td></tr>
<tr><td>Coefficient of variation for yield (based on shoot fresh weight in control):</td><td>16.9 %,</td></tr>
<tr><td>Sublethal effects in control:</td><td>Control plants did not show any sign of chlorosis and were visibly free from contamination by other organisms such as algae and/or bacterial film,</td></tr>
</table>
Agreed endpoints:
Effects on Total Shoot Length following a 14-day exposure of Glyceria maxima to Mesosulfuron-methyl technical
<table>
<tr><th></th><th>Growth rate (total shoot length in cm) [µg Mesosulfuron-methyl technical/L]</th><th>Yield (total shoot length in cm) [µg Mesosulfuron-methyl technical/L]</th></tr>
<tr><td colspan="3">Nominal concentrations</td></tr>
<tr><td>14-day EC50</td><td>160</td><td>97.7</td></tr>
<tr><td>95% Conf. Limits</td><td>125 – 209</td><td>76.4 - 124</td></tr>
<tr><td>14-day EC20</td><td>29.0</td><td>19.1 1)</td></tr>
<tr><td>95% Conf. Limits</td><td>19.3 – 39.8</td><td>(12.5 - 26.5)</td></tr>
<tr><td>14-day EC10</td><td>11.8 1)</td><td>8.15 1)</td></tr>
<tr><td>95% Conf. Limits</td><td>(6.77 - 17.9)</td><td>(4.64 - 12.4)</td></tr>
<tr><td>14-day NOEC</td><td>9.54</td><td>9.54</td></tr>
<tr><td>14-day LOEC</td><td>30.5</td><td>30.5</td></tr>
</table>
1) Values not reliable. Pooled control CV exceeded the effect level (ECx). Please refer to OECD guideline 239, paragraph 5 which specifies that EC10 and EC20 values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.
</td></tr>
</table>

Effects on Shoot Fresh Weight following a 14-day exposure of *Glyceria maxima* to Mesosulfuron-methyl technical

	Growth rate (shoot fresh weight in g) [µg Mesosulfuron-methyl technical/L]	**Yield (shoot fresh weight in g) [µg Mesosulfuron-methyl technical/L]**
Nominal concentrations		
14-day EC_{50}	**65.8**	52.7
95% Conf. Limits	54.5 – 79.0	43.5 – 63.3
14-day EC_{20}	21.9	17.7
95% Conf. Limits	16.4 – 27.7	13.1 – 22.6
14-day EC_{10}	12.3 [1)]	10.0 [1)]
95% Conf. Limits	(8.55 - 16.4)	(6.84 - 13.5)
14-day NOEC	9.54	9.54
14-day LOEC	30.5	30.5

[1)] Values not reliable. Pooled control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.

Effects on Shoot Dry Weight following a 14-day exposure of *Glyceria maxima* to Mesosulfuron-methyl technical

	Growth rate (shoot dry weight in g) [µg Mesosulfuron-methyl technical/L]	**Yield (shoot dry weight in g) [µg Mesosulfuron-methyl technical/L]**
Nominal concentrations		
14-day EC_{50}	490	255
95% Conf. Limits	348 – 763	193 – 351
14-day EC_{20}	64.9	40.1
95% Conf. Limits	43.1 – 89.9	26.6 – 55.3
14-day EC_{10}	22.5	15.3 [1)]
95% Conf. Limits	12.2 – 34.9	(8.51 - 23.4)
14-day NOEC	30.5	30.5
14-day LOEC	97.7	97.7

[1)] Values not reliable. Pooled control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.

Reference:	KCP 10.2-06_06
Report	Mesosulfuron-methyl technical: Growth inhibition of *Glyceria maxima* in water/sediment system. Maren D, 2018, S18-00151.
Guideline(s):	Yes, OECD 239 (2014)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	N/A

Materials and methods

Test item:	Mesosulfuron-methyl technical, Batch No.: 20160408; purity (analysed): 96.78 % w/w
Test species:	*Glyceria maxima* H.
Test design:	Five replicates per test item concentration and ten replicates for the control and solvent control were used. There was one plant per replicate. The duration of the test was 14 days. The test was performed under static test conditions. On day 14 plants were harvested from each treatment group for assessment of shoot length, shoot fresh weight, shoot dry weight and number and length of side shoots.
Endpoints:	Endpoints reported are the $EC_{10,\ 20,\ 50}$ values for yield ($E_yC_{10,\ 20,\ 50}$) and growth rate ($E_rC_{10,\ 20,\ 50}$) based on the increase in total shoot length, shoot fresh weight and shoot dry weight respectively after 14 days of exposure. The NOEC and LOEC values for yield and growth rate were also determined.
Test rates:	The nominal concentrations of the test item during the test were 9.54, 30.5, 97.7, 313 and 1000 µg/L. An untreated control and a solvent control were tested in parallel. The test item was spiked to the water.
Test conditions:	Temperature, pH-value and oxygen saturation of the test solutions measured after 0, 7 and 14 days are reported.
Samples analysed:	Test item concentrations in the definitive test were verified by analyses of mesosulfuron-methyl at all concentration levels by analysing the overlaying water from samples taken at test start and test end and wet sediment from samples taken at test termination on day 14. The pore water from the highest concentration level was analysed from samples taken at test termination on day 14.
Dates of work:	23 Apr 2018 – 28 Jun 2018

Results and discussions

Findings:	The measured concentration of the test item in the test vessels based on the mesosulfuron-methyl content in the freshly prepared test solution ranged between 108 and 117% of nominal in the overlaying water. The mean measured content for all concentrations in the freshly prepared test solutions was 112% of nominal for mesosulfuron-methyl. In the aged test solutions the measured concentration of the test item based on the mesosulfuron-methyl

content in the test vessels ranged between 98 and 109% of nominal in the overlaying water. The mean measured concentration of the test item in aged test solutions based on the mesosulfuron-methyl content in the test vessels was 104% of nominal in the overlaying water. In the sediment, concentrations of mesosulfuron-methyl above the LOQ were detectable at all concentrations with recoveries between 6 and 11% of the applied amount at test end after 14 days. In pore water < 1 % of the applied amount was measured after 14 days at the highest nominal concentration level of 1000 µg test item/L.

Since all measured concentrations of mesosulfuron-methyl were between 80% and 120% of nominal, all toxicological endpoints were evaluated using nominal concentrations of the test item. For completeness, endpoints based on geometric mean measured concentrations of fresh and aged test solutions are also reported in the Appendices.

Following exposure to Mesosulfuron-methyl technical, inhibition effects on *Glyceria maxima* for shoot fresh weight were found to be more sensitive than for shoot dry weight and total shoot length when comparing the E_yC_{50} values as indicated in the tables below.

Summary of Effects on Total Shoot Length following a 14-day exposure of *Glyceria maxima* to Mesosulfuron-methyl technical

	Growth rate (total shoot length in cm) [µg Mesosulfuron-methyl technical/L]	Yield (total shoot length in cm) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
14-day EC_{50}	160	97.7
95% Conf. Limits	125 – 209	76.4 - 124
14-day EC_{20}	29.0	19.1 [1)]
95% Conf. Limits	19.3 – 39.8	(12.5 - 26.5)
14-day EC_{10}	11.8 [1)]	8.15 [1)]
95% Conf. Limits	(6.77 - 17.9)	(4.64 - 12.4)
14-day NOEC	9.54	9.54
14-day LOEC	30.5	30.5

[1)] Values not reliable. Pooled control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.

Summary of Effects on Shoot Fresh Weight following a 14-day exposure of *Glyceria maxima* to Mesosulfuron-methyl technical

	Growth rate (shoot fresh weight in g) [µg Mesosulfuron-methyl technical/L]	Yield (shoot fresh weight in g) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
14-day EC_{50}	65.8	52.7
95% Conf. Limits	54.5 – 79.0	43.5 – 63.3
14-day EC_{20}	21.9	17.7
95% Conf. Limits	16.4 – 27.7	13.1 – 22.6
14-day EC_{10}	12.3 [1)]	10.0 [1)]
95% Conf. Limits	(8.55 - 16.4)	(6.84 - 13.5)
14-day NOEC	9.54	9.54
14-day LOEC	30.5	30.5

[1)] Values not reliable. Pooled control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.

Summary of Effects on Shoot Dry Weight following a 14-day exposure of *Glyceria maxima* to Mesosulfuron-methyl technical

	Growth rate (shoot dry weight in g) [µg Mesosulfuron-methyl technical/L]	Yield (shoot dry weight in g) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
14-day EC_{50}	490	255
95% Conf. Limits	348 – 763	193 – 351
14-day EC_{20}	64.9	40.1
95% Conf. Limits	43.1 – 89.9	26.6 – 55.3
14-day EC_{10}	22.5	15.3 [1)]
95% Conf. Limits	12.2 – 34.9	(8.51 - 23.4)
14-day NOEC	30.5	30.5
14-day LOEC	97.7	97.7

[1)] Values not reliable. Pooled control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.

The average pH-value was determined to be 7.59 ± 0.18, the average temperature was measured to be 19.8 ± 0.4 °C and the oxygen saturation was determined to be 100 ± 4 %. The test item had no influence on the pH-value of the test solutions.

Conclusions:

Following exposure of the aquatic rooted monodicotyledon macrophyte *Glyceria maxima* to Mesosulfuron-methyl technical for 14 days the E_rC_{50} and E_yC_{50} values based on total shoot length were 160 µg/L and 97.7 µg/L (nominal) respectively. The NOEC based on total shoot length was 9.54 µg/L (nominal) for growth rate and yield.

The E_rC_{50} and E_yC_{50} values based on biomass (shoot fresh weight) were 65.8 µg/L and 52.7 µg/L (nominal) respectively. The NOEC for growth rate and yield based on biomass (shoot fresh weight) was 9.54 µg/L (nominal) respectively.

The E_rC_{50} and E_yC_{50} values based on biomass (shoot dry weight) were 490 µg/L and 255 µg/L (nominal). The NOEC based on biomass (shoot dry weight) was 30.5 µg/L (nominal) for growth rate and yield.

A 2.2.3.8 Elodea canadensis

Comments of zRMS:	The study is considered valid. All validity criteria were met. • Increase in shoot length of control plants: Mean total shoot length must at least double during the exposure period • Increase in shoot fresh weight of control plants Mean shoot fresh weight must at least double during the exposure period • Coefficient of variation for yield (based on shoot fresh weight of control plants): Must not exceed 35 % between replicates **In the current study:** Doubling Time of Shoot length in Control: 8.2 days, Doubling Time of Shoot fresh weight in Control: 7.7 days, Coefficient of variation for yield (based on shoot fresh weight in Control): 22.2 %, Sublethal effects in Control: Control plants did not show any sign of chlorosis and were visibly free from contamination by other organisms such as algae and/or bacterial film, **Agreed endpoints:** **Effects on Total Shoot Length following a 14-day exposure of *Elodea canadensis* to Mesosulfuron-methyl technical**.

	Growth rate (total shoot length in cm) [µg Mesosulfuron-methyl technical/L]	**Yield (total shoot length in cm) [µg Mesosulfuron-methyl technical/L]**
Nominal concentrations		
14-day EC_{50}	10.2	4.57
95% Conf. Limits	7.76 – 13.3	3.41 – 5.95
14-day EC_{20}	1.60	0.789 1)
95% Conf. Limits	0.978 – 2.32	(0.455 - 1.19)
14-day EC_{10}	0.607 1)	0.315 1)
95% Conf. Limits	(0.311 - 0.990)	(0.153 - 0.533)
14-day NOEC	0.954	0.954
14-day LOEC	3.05	3.05
Geometric mean measured concentrations		
14-day EC_{50}	9.08	3.92
95% Conf. Limits	6.82 - 12.1	2.88 - 5.16
14-day EC_{20}	1.28	0.616 1)
95% Conf. Limits	0.760 - 1.90	(0.345 - 0.951)
14-day EC_{10}	0.459 1)	0.234 1)
95% Conf. Limits	(0.226 - 0.770)	(0.109 - 0.409)
14-day NOEC	0.792	0.792
14-day LOEC	2.62	2.62

1) Value not reliable. Solvent control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated

Effects on Shoot Fresh Weight following a 14-day exposure of *Elodea canadensis* to Mesosulfuron-methyl technical.

	Growth rate (shoot fresh weight in g) [µg Mesosulfuron-methyl technical/L]	**Yield (shoot fresh weight in g) [µg Mesosulfuron-methyl technical/L]**
Nominal concentrations		
14-day EC_{50}	10.7	6.13
95% Conf. Limits	8.52 – 13.5	4.85 – 7.64
14-day EC_{20}	2.34	1.45 [1)]
95% Conf. Limits	1.61 – 3.15	(0.979 - 1.97)
14-day EC_{10}	1.05 [1)]	0.681 [1)]
95% Conf. Limits	(0.644 - 1.54)	(0.410 - 1.00)
14-day NOEC	0.954	0.954
14-day LOEC	3.05	3.05
Geometric mean measured concentrations		
14-day EC_{50}	9.57	5.31
95% Conf. Limits	7.50 - 12.2	4.16 - 6.70
14-day EC_{20}	1.92	1.17 [1)]
95% Conf. Limits	1.29 - 2.62	(0.773 - 1.61)
14-day EC_{10}	0.826 [1)]	0.528 [1)]
95% Conf. Limits	(0.491 - 1.23)	(0.310 - 0.794)
14-day NOEC	0.792	0.792
14-day LOEC	2.62	2.62

[1)] Value not reliable. Solvent control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.

Effects on Shoot Fresh Weight following a 14-day exposure of *Elodea canadensis* to Mesosulfuron-methyl technical.

	Growth rate (shoot fresh weight in g) [µg Mesosulfuron-methyl technical/L]	**Yield (shoot fresh weight in g) [µg Mesosulfuron-methyl technical/L]**
Nominal concentrations		
14-day EC_{50}	10.7	6.13
95% Conf. Limits	8.52 – 13.5	4.85 – 7.64
14-day EC_{20}	2.34	1.45 [1)]
95% Conf. Limits	1.61 – 3.15	(0.979 - 1.97)
14-day EC_{10}	1.05 [1)]	0.681 [1)]
95% Conf. Limits	(0.644 - 1.54)	(0.410 - 1.00)
14-day NOEC	0.954	0.954
14-day LOEC	3.05	3.05
Geometric mean measured concentrations		
14-day EC_{50}	9.57	5.31
95% Conf. Limits	7.50 - 12.2	4.16 - 6.70
14-day EC_{20}	1.92	1.17 [1)]
95% Conf. Limits	1.29 - 2.62	(0.773 - 1.61)
14-day EC_{10}	0.826 [1)]	0.528 [1)]
95% Conf. Limits	(0.491 - 1.23)	(0.310 - 0.794)
14-day NOEC	0.792	0.792
14-day LOEC	2.62	2.62

[1)] Value not reliable. Solvent control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.

Reference:	KCP 10.2-06_07
Report	Mesosulfuron-methyl technical: Growth inhibition of *Elodea canadensis* in water/sediment system. Maren D, 2018, S18-00152.
Guideline(s):	Yes, OECD 239 (2014)
Deviations:	In addition to the guideline recommendation which only evaluates the shoot fresh weight and dry weight, the total plant fresh weight and dry weight, comprising roots and shoots was assessed as well. Evaluating total plant fresh weight and dry weight avoids underestimating effects on rooted aquatic macrophytes, especially for test items which may affect root development.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	N/A

Materials and methods

Test item:	Mesosulfuron-methyl technical, Batch No.: 20160408; purity (analysed): 96.78 % w/w
Test species:	*Elodea canadensis*
Test design:	Five replicates per test item concentration and ten replicates for the control and solvent control were used. There was one plant per replicate. The duration of the test was 14 days. The test was performed under static test conditions. On day 14 plants were harvested from each treatment group for assessment of shoot length, shoot fresh weight, shoot dry weight, root fresh and dry weight and number and length of side shoots.
Endpoints:	Endpoints reported are the $EC_{10,\ 20,\ 50}$ values for yield ($E_yC_{10,\ 20,\ 50}$) and growth rate ($E_rC_{10,\ 20,\ 50}$) based on the increase in total shoot length and shoot fresh weight and shoot dry weight respectively after 14 days of exposure. The NOEC and LOEC values for yield and growth rate were also determined. Endpoints based on total plant fresh weight and dry weight are reported for completeness in the Appendices.
Test rates:	The nominal concentrations of the test item during the test were 0.954, 3.05, 9.77, 31.3 and 100 µg/L. An untreated control and a solvent control were tested in parallel. The test item was spiked to the water.
Test conditions:	Temperature, pH-value and oxygen saturation of the test solutions measured after 0, 7 and 14 days are reported.
Samples analysed:	Test item concentrations in the definitive test were verified by analyses of mesosulfuron-methyl at all concentration levels by analysing the overlaying water from samples taken at test start and test end and wet sediment from samples taken at test termination on day 14. The pore water from the highest concentration level was analysed from samples taken at test termination on day 14.
Dates of work:	07 Mar 2018 – 19 Apr 2018
Findings:	The measured concentration of the test item in the test vessels based on the mesosulfuron-methyl content in the freshly prepared test solution ranged between 83 and 111% of nominal in the overlaying water. The mean measured

content for all concentrations in the freshly prepared test solutions was 95% of nominal for mesosulfuron-methyl. In the aged test solutions the measured concentration of the test item based on the mesosulfuron-methyl content in the test vessels ranged between 77 and 105% of nominal in the overlaying water. The mean measured concentration of the test item in aged test solutions based on the mesosulfuron-methyl content in the test vessels was 86% of nominal in the overlaying water. In the sediment, concentrations of mesosulfuron-methyl above the LOQ were detectable at nominal concentrations of 31.3 and 100 µg/L with respective recoveries of 6 and 5% of the applied amount at test end after 14 days. In pore water < 1% of the applied amount was measured after 14 days at the highest nominal concentration level of 100 µg test item/L.

Since mean measured concentrations of mesosulfuron-methyl were between 80% and 120% of nominal, all toxicological endpoints were evaluated using nominal concentrations of the test item. In addition as one aged sample was above 120% of nominal, endpoints based on geometric mean measured concentrations of fresh and aged test solutions are also shown in the summary tables and conclusion.

Following exposure to Mesosulfuron-methyl technical, inhibition effects on *Elodea canadensis* for total shoot length and shoot fresh weight were found to be more sensitive than for shoot dry weight for the EC_{50} as indicated in the tables below.

Summary of Effects on Total Shoot Length following a 14-day exposure of *Elodea canadensis* to Mesosulfuron-methyl technical

	Growth rate (total shoot length in cm) [µg Mesosulfuron-methyl technical/L]	Yield (total shoot length in cm) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
14-day EC_{50}	10.2	4.57
95% Conf. Limits	7.76 – 13.3	3.41 – 5.95
14-day EC_{20}	1.60	0.789 [1)]
95% Conf. Limits	0.978 – 2.32	(0.455 - 1.19)
14-day EC_{10}	0.607 [1)]	0.315 [1)]
95% Conf. Limits	(0.311 - 0.990)	(0.153 - 0.533)
14-day NOEC	0.954	0.954
14-day LOEC	3.05	3.05
Geometric mean measured concentrations		
14-day EC_{50}	9.08	3.92
95% Conf. Limits	6.82 - 12.1	2.88 - 5.16
14-day EC_{20}	1.28	0.616 [1)]
95% Conf. Limits	0.760 - 1.90	(0.345 - 0.951)
14-day EC_{10}	0.459 [1)]	0.234 [1)]
95% Conf. Limits	(0.226 - 0.770)	(0.109 - 0.409)
14-day NOEC	0.792	0.792
14-day LOEC	2.62	2.62

[1)] Value not reliable. Solvent control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.

Summary of Effects on Shoot Fresh Weight following a 14-day exposure of *Elodea canadensis* to Mesosulfuron-methyl technical

	Growth rate (shoot fresh weight in g) [µg Mesosulfuron-methyl technical/L]	Yield (shoot fresh weight in g) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
14-day EC_{50}	10.7	6.13
95% Conf. Limits	8.52 – 13.5	4.85 – 7.64
14-day EC_{20}	2.34	1.45 [1)]
95% Conf. Limits	1.61 – 3.15	(0.979 - 1.97)
14-day EC_{10}	1.05 [1)]	0.681 [1)]
95% Conf. Limits	(0.644 - 1.54)	(0.410 - 1.00)
14-day NOEC	0.954	0.954
14-day LOEC	3.05	3.05
Geometric mean measured concentrations		
14-day EC_{50}	9.57	5.31
95% Conf. Limits	7.50 - 12.2	4.16 - 6.70
14-day EC_{20}	1.92	1.17 [1)]
95% Conf. Limits	1.29 - 2.62	(0.773 - 1.61)
14-day EC_{10}	0.826 [1)]	0.528 [1)]
95% Conf. Limits	(0.491 - 1.23)	(0.310 - 0.794)
14-day NOEC	0.792	0.792
14-day LOEC	2.62	2.62

[1)] Value not reliable. Solvent control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.

Summary of Effects on Shoot Dry Weight following a 14-day exposure of *Elodea canadensis* to Mesosulfuron-methyl technical

	Growth rate (shoot dry weight in g) [µg Mesosulfuron-methyl technical/L]	Yield (shoot dry weight in g) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
14-day EC_{50}	> 100	> 100
95% Conf. Limits	n.d.	n.d.
14-day EC_{20}	56.4	34.4 [1)]
95% Conf. Limits	40.9 - 81.8	(25.0 - 44.4)
14-day EC_{10}	26.7 [1)]	17.8 [1)]
95% Conf. Limits	(15.5 - 37.3)	(10.5 - 24.6)
14-day NOEC	31.3	100
14-day LOEC	100	n.d.
Geometric mean measured concentrations		
14-day EC_{50}	> 108	> 108
95% Conf. Limits	n.d.	n.d.
14-day EC_{20}	56.5	32.2 [1)]
95% Conf. Limits	39.2 - 86.3	(22.5 - 43.1)
14-day EC_{10}	24.2 [1)]	15.2 [1)]
95% Conf. Limits	(13.0 - 35.2)	(8.36 - 21.9)
14-day NOEC	28.5	108
14-day LOEC	108	n.d.

[1)] Value not reliable. Solvent control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.
n.d. = not determined

The average pH-value was determined to be 7.74 ± 0.43, the average temperature was measured to be 18.7 ± 0.5 °C and the oxygen saturation was determined to be 112 ± 8 %. The test item had no influence on the pH-value of the test solutions.

Conclusions:

Following exposure of the aquatic rooted macrophyte *Elodea canadensis* to Mesosulfuron-methyl technical for 14 days the E_rC_{50} and E_yC_{50} values based on total shoot length were 10.2 µg/L and 4.57 µg/L (nominal) respectively. The NOEC based on total shoot length was 0.954 µg/L (nominal) for growth rate and yield. Corresponding E_rC_{50} and E_yC_{50} values based on geometric mean measured concentrations were 9.08 µg/L and 3.92 µg/L (mean measured). The NOEC based total shoot length was 0.792 µg/L (mean measured) for growth rate and yield.

The E_rC_{50} and E_yC_{50} values based on biomass (shoot fresh weight) were 10.7 µg/L and 6.13 µg/L (nominal) respectively. The NOEC for growth rate and yield based on biomass (shoot fresh weight) was 0.954 µg/L (nominal) respectively. Corresponding E_rC_{50} and E_yC_{50} values based on geometric mean measured concentrations were 9.57 µg/L and 5.31 µg/L (mean measured). The NOEC based on fresh weight was 0.792 µg/L (mean measured) for growth rate and yield.

The E_rC_{50} and E_yC_{50} values based on biomass (shoot dry weight) were estimated to be > 100 µg/L (nominal). The NOEC based on biomass (shoot dry weight) was 31.3 µg/L (nominal) for growth rate and 100 µg/L (nominal) yield. Corresponding E_rC_{50} and E_yC_{50} values based on geometric mean measured concentrations were estimated to be > 108 µg/L (mean measured).

The NOEC based on biomass (shoot dry weight) was 28.5 µg/L (mean measured) for growth rate and 108 µg/L (mean measured) yield.

A 2.2.3.9 Wolffia arrhiza

Comments of zRMS:

The study is considered valid. All validity criteria were met.

- Increase in shoot length of control plants: Mean total shoot length must at least double during the exposure period
- Increase in shoot fresh weight of control plants: Mean shoot fresh weight must at least double during the exposure period
- Coefficient of variation for yield (based on shoot fresh weight of control plants): Must not exceed 35 % between replicates

In the current study:

Doubling Time of Shoot length in Control:	8.2 days
Doubling Time of Shoot fresh weight in Control:	7.7 days
Coefficient of variation for yield (based on shoot fresh weight in Control):	22.2 %
Sublethal effects in Control:	Control plants did not show any sign of chlorosis and were visibly free from contamination by other organisms such as algae and/or bacterial film

Agreed endpoints:

Effects on Frond Number following a 7-day exposure of *Wolffia arrhiza* to Mesosulfuron-methyl technical

	Growth rate (frond number) [µg Mesosulfuron-methyl technical/L]	**Yield (frond number) [µg Mesosulfuron-methyl technical/L]**
Nominal concentrations		
7-day EC_{50}	5.64	2.10
95% Conf. Limits	2.98 - 13.4	0.970 - 4.65
7-day EC_{20}	1.49	0.627
95% Conf. Limits	0.473 - 2.83	0.145 - 1.28
7-day EC_{10}	0.742	0.333
95% Conf. Limits	0.145 - 1.57	0.0461 - 0.766
7-day NOEC	0.610	0.191
7-day LOEC	1.95	0.610
Geometric mean of measured concentrations		
7-day EC_{50}	7.45	2.79
95% Conf. Limits	3.86 - 18.5	1.29 - 6.17
7-day EC_{20}	1.98	0.840
95% Conf. Limits	0.592 - 3.82	0.194 - 1.72
7-day EC_{10}	0.988	0.449
95% Conf. Limits	0.176 - 2.12	0.0619 - 1.03
7-day NOEC	0.793	0.264
7-day LOEC	2.67	0.793

Effects on Fresh Weight following a 7-day exposure of *Wolffia arrhiza* to Mesosulfuron-methyl technical.

	Growth rate (fresh weight) [µg Mesosulfuron-methyl technical/L]	Yield (fresh weight) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
7-day EC_{50}	4.23	1.56
95% Conf. Limits	2.33 - 8.75	0.774 - 3.07
7-day EC_{20}	1.11	0.444
95% Conf. Limits	0.389 - 2.05	0.120 - 0.874
7-day EC_{10}	0.554	0.230[1)]
95% Conf. Limits	0.129 - 1.14	(0.0397 - 0.514)
7-day NOEC	0.610	0.191
7-day LOEC	1.95	0.610
Geometric mean of measured concentrations		
7-day EC_{50}	5.59	2.08
95% Conf. Limits	3.03 - 11.9	1.04 - 4.05
7-day EC_{20}	1.48	0.598
95% Conf. Limits	0.498 - 2.76	0.165 - 1.17
7-day EC_{10}	0.741	0.312[1)]
95% Conf. Limits	0.162 - 1.54	(0.0555 - 0.688)
7-day NOEC	0.793	0.264
7-day LOEC	2.67	0.793

[1)] Value not reliable. Pooled control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.

Effects on Dry Weight following a 7-day exposure of *Wolffia arrhiza* to Mesosulfuron-methyl technical.

	Growth rate (dry weight) [µg Mesosulfuron-methyl technical/L]	Yield (dry weight) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
7-day EC_{50}	17.7[2)]	3.95
95% Conf. Limits	(12.0 - 29.9)	3.09 - 5.17
7-day EC_{20}	2.65	0.764
95% Conf. Limits	1.86 - 3.61	0.531 - 1.02
7-day EC_{10}	0.982	0.324[1)]
95% Conf. Limits	0.581 - 1.44	(0.197 - 0.472)
7-day NOEC	0.610	0.610
7-day LOEC	1.95	1.95
Geometric mean of measured concentrations		
7-day EC_{50}	23.2[2)]	5.23
95% Conf. Limits	(15.8 - 39.2)	4.09 - 6.83
7-day EC_{20}	3.51	1.02
95% Conf. Limits	2.47 - 4.77	0.714 - 1.37
7-day EC_{10}	1.31	0.436[1)]
95% Conf. Limits	0.777 - 1.91	(0.267 - 0.634)
7-day NOEC	0.793	0.793
7-day LOEC	2.67	2.67

[1)] Value not reliable. Pooled control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 ich specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control nts fall below the effect level being estimated.

[2)] Value not reliable. No inhibition > 50% was observed.

Reference:	KCP 10.2-06_08
Report	Mesosulfuron-methyl technical: Growth inhibition of *Wolffia arrhiza* in water/sediment system. Maren D, 2018, S18-00153.
Guideline(s):	Yes, OECD 239 (2014)
Deviations:	In deviation to the guideline recommendation which adjusts the pH of STEINBERG medium to 5.5 +/- 0.2, the pH was adjusted to 7.5 +/-. This mostly avoids degradation of the test item within seven days.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	N/A

Materials and methods

Test item:	Mesosulfuron-methyl technical, Batch No.: 20160408; purity (analysed): 96.78 % w/w
Test species:	*Wolffia arrhiza.*
Test design:	In a static dose-response test vegetative growth was observed for a test period of 7 days by measuring the increase in frond number, fresh weight and dry weight.
Endpoints:	Inhibition of growth was assessed by the determination of NOEC/LOEC values and $EC_{10,\ 20,\ 50}$ values for the following response variables: - Growth rates of frond numbers, fresh weight and dry weight - Yield of frond numbers, fresh weight and dry weight Additionally the doubling time of frond numbers was determined.
Test rates:	A static main test with nominal concentrations of 20.0, 6.25, 1.95, 0.610 and 0.191 µg/L, control and solvent control was performed.
Test conditions:	Six replicates were employed for the control, solvent control and three for each test item concentration. The test was performed in 250 mL glass beakers each containing 200 mL test solution. The pH was recorded after 0 and 7 days in all treatments. Temperature was recorded at days 0, 2, 4 and 7. Light intensity of the continuous illumination was measured at test start.
Samples analysed:	Analytical samples were taken from the controls and all test item concentrations from samples taken at test start (fresh) and after seven days from aged solutions. Samples of all test item concentrations and controls were analysed from samples taken at t= 0 days fresh and 7 days aged.
Statistics:	NOEC and LOEC values were determined by using a multiple comparison method, $EC_{10,\ 20,\ 50}$ values were determined by probit analysis, where possible.
Dates of work:	19 Feb 2018 – 27 Apr 2018
Findings:	
Test conditions:	The pH-value of fresh test solutions ranged from 7.27 to 7.30, the pH-value of aged solutions at day 7 ranged from 7.43 to 7.75. The temperature was

	measured to be 22.5 – 23.8 °C during the test and the mean light intensity at the beginning of the test was 6998 lux.
Analytical Results:	The measured initial concentrations of mesosulfuron-methyl ranged from 112% to 122% of nominal with a mean initial concentration of 117% of nominal. At test end the measured content of mesosulfuron-methyl ranged from 141% to 158% of nominal with a mean initial concentration of 151% of nominal. All toxicological endpoints were evaluated using nominal concentrations of the test item (Mesosulfuron-methyl technical). In addition, as the recoveries of the freshly prepared solution of concentration level 1.95 µg/L and aged solutions of all concentrations levels where above 120%, endpoints based on the geometric mean measured concentration of fresh and aged test solutions are also shown in the summary tables and conclusion.
Statistical Results:	Following exposure to Mesosulfuron-methyl technical, inhibition effects on *Wolffia arrhiza* for frond number and fresh weight were found to be slightly more sensitive than for dry weight when comparing the EC_{50} values as indicated in the tables below.

Summary of Effects on Frond Number following a 7-day exposure of *Wolffia arrhiza* to Mesosulfuron-methyl technical

	Growth rate (frond number) [µg Mesosulfuron-methyl technical/L]	Yield (frond number) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
7-day EC_{50}	5.64	2.10
95% Conf. Limits	2.98 - 13.4	0.970 - 4.65
7-day EC_{20}	1.49	0.627
95% Conf. Limits	0.473 - 2.83	0.145 - 1.28
7-day EC_{10}	0.742	0.333
95% Conf. Limits	0.145 - 1.57	0.0461 - 0.766
7-day NOEC	0.610	0.191
7-day LOEC	1.95	0.610
Geometric mean of measured concentrations		
7-day EC_{50}	7.45	2.79
95% Conf. Limits	3.86 - 18.5	1.29 - 6.17
7-day EC_{20}	1.98	0.840
95% Conf. Limits	0.592 - 3.82	0.194 - 1.72
7-day EC_{10}	0.988	0.449
95% Conf. Limits	0.176 - 2.12	0.0619 - 1.03
7-day NOEC	0.793	0.264
7-day LOEC	2.67	0.793

Summary of Effects on Fresh Weight following a 7-day exposure of *Wolffia arrhiza* to Mesosulfuron-methyl technical

	Growth rate (fresh weight) [µg Mesosulfuron-methyl technical/L]	Yield (fresh weight) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
7-day EC_{50}	4.23	1.56
95% Conf. Limits	2.33 - 8.75	0.774 - 3.07
7-day EC_{20}	1.11	0.444
95% Conf. Limits	0.389 - 2.05	0.120 - 0.874
7-day EC_{10}	0.554	0.230[1)]
95% Conf. Limits	0.129 - 1.14	(0.0397 - 0.514)
7-day NOEC	0.610	0.191
7-day LOEC	1.95	0.610
Geometric mean of measured concentrations		
7-day EC_{50}	5.59	2.08
95% Conf. Limits	3.03 - 11.9	1.04 - 4.05
7-day EC_{20}	1.48	0.598
95% Conf. Limits	0.498 - 2.76	0.165 - 1.17
7-day EC_{10}	0.741	0.312[1)]
95% Conf. Limits	0.162 - 1.54	(0.0555 - 0.688)
7-day NOEC	0.793	0.264
7-day LOEC	2.67	0.793

[1)] Value not reliable. Pooled control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.

Summary of Effects on Dry Weight following a 7-day exposure of *Wolffia arrhiza* to Mesosulfuron-methyl technical

	Growth rate (dry weight) [µg Mesosulfuron-methyl technical/L]	Yield (dry weight) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
7-day EC_{50}	17.7[2)]	3.95
95% Conf. Limits	(12.0 - 29.9)	3.09 - 5.17
7-day EC_{20}	2.65	0.764
95% Conf. Limits	1.86 - 3.61	0.531 - 1.02
7-day EC_{10}	0.982	0.324[1)]
95% Conf. Limits	0.581 - 1.44	(0.197 - 0.472)
7-day NOEC	0.610	0.610
7-day LOEC	1.95	1.95
Geometric mean of measured concentrations		
7-day EC_{50}	23.2[2)]	5.23
95% Conf. Limits	(15.8 - 39.2)	4.09 - 6.83
7-day EC_{20}	3.51	1.02
95% Conf. Limits	2.47 - 4.77	0.714 - 1.37
7-day EC_{10}	1.31	0.436[1)]
95% Conf. Limits	0.777 - 1.91	(0.267 - 0.634)
7-day NOEC	0.793	0.793
7-day LOEC	2.67	2.67

[1)] Value not reliable. Pooled control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.
[2)] Value not reliable. No inhibition > 50% was observed.

Conclusions:

Following exposure of the aquatic macrophyte *Wolffia arrhiza* to Mesosulfuron-methyl technical for 7 days the E_rC_{50} and E_yC_{50} values based on frond number were 5.64 µg/L and 2.10 µg/L (nominal) respectively. The NOEC based on frond number was 0.610 µg/L (nominal) for growth rate and 0.191 µg/L for yield. Corresponding E_rC_{50} and E_yC_{50} values based on geometric mean measured concentrations were 7.45 µg/L and 2.79 µg/L (mean measured). The NOEC based on frond number was 0.793 µg/L (mean measured) for growth rate and 0.264 µg/L for yield.

The E_rC_{50} and E_yC_{50} values based on biomass (fresh weight) were 4.23 µg/L and 1.56 µg/L (nominal) respectively. The NOEC for growth rate and yield based on biomass (fresh weight) were 0.610 µg/L and 0.191 µg/L (nominal) respectively. Corresponding E_rC_{50} and E_yC_{50} values based on geometric mean measured concentrations were 5.59 µg/L and 2.08 µg/L (mean measured). The NOEC based on fresh weight for growth rate and yield were 0.793 µg/L and 0.264 µg/L (mean measured) respectively.

The E_rC_{50} and E_yC_{50} values based on biomass (dry weight) were 17.7 µg/L and 3.95 µg/L (nominal). The NOEC based on biomass (dry weight) was 0.610 µg/L (nominal) for growth rate and yield. Corresponding E_rC_{50} and E_yC_{50} values based on geometric mean measured concentrations were 23.2 µg/L and 5.23 µg/L (mean measured). The NOEC based on dry weight was 0.793 µg/L (mean measured) for growth rate and yield.

A 2.2.3.10 Spirodela polyrhiza

Comments of zRMS:

The study is considered valid. All validity criteria were met.

- The doubling time of frond numbers in the control should be less than 2.5 days (< 60 hours).

In the current study:

The doubling time of frond numbers in the control was 1.722 days (corresponding to 41.3 hours).

Agreed endpoints:

Effects on Frond Number following a 7-day exposure of *Spirodela polyrhiza* to Mesosulfuron-methyl technical

	Growth rate (frond number) [µg Mesosulfuron-methyl technical/L]	**Yield (frond number) [µg Mesosulfuron-methyl technical/L]**
Nominal concentrations		
7-day EC_{50}	3.35	1.48
95% Conf. Limits	2.04 - 5.60	0.690 - 3.03
7-day EC_{20}	1.20	0.639
95% Conf. Limits	0.521 - 1.97	0.155 - 1.20
7-day EC_{10}	0.698	0.412[1)]
95% Conf. Limits	0.232 - 1.25	(0.0631 - 0.834)
7-day NOEC	0.610	0.610
7-day LOEC	1.95	1.95
Geometric mean of measured concentrations		
7-day EC_{50}	3.56	1.63
95% Conf. Limits	1.84 - 7.42	0.545 - 4.93
7-day EC_{20}	1.31	0.740
95% Conf. Limits	0.386 - 2.42	0.0546 - 1.56
7-day EC_{10}	0.774	0.489[1)]
95% Conf. Limits	0.147 - 1.56	(0.0129 - 1.09)
7-day NOEC	0.787	0.787
7-day LOEC	1.87	1.87

1) Value not reliable. Pooled control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.

Effects on Fresh Weight following a 7-day exposure of *Spirodela polyrhiza* to Mesosulfuron-methyl technical

	Growth rate (fresh weight) [µg Mesosulfuron-methyl technical/L]	**Yield (fresh weight) [µg Mesosulfuron-methyl technical/L]**
Nominal concentrations		
7-day EC_{50}	2.49	1.39
95% Conf. Limits	1.34 - 4.74	0.677 - 2.90
7-day EC_{20}	1.10	0.600
95% Conf. Limits	0.362 - 1.92	0.158 - 1.11
7-day EC_{10}	0.720	0.387[1)]
95% Conf. Limits	0.164 - 1.34	(0.0652 - 0.768)
7-day NOEC	0.610	0.610
7-day LOEC	1.95	1.95
Geometric mean of measured concentrations		
7-day EC_{50}	2.64	1.54
95% Conf. Limits	1.54 - 4.79	0.731 - 3.55
7-day EC_{20}	1.22	0.712
95% Conf. Limits	0.487 - 2.01	0.153 - 1.32
7-day EC_{10}	0.814	0.476[1)]
95% Conf. Limits	0.239 - 1.42	(0.0569 - 0.930)
7-day NOEC	0.787	0.787
7-day LOEC	1.87	1.87

1) Value not reliable. Pooled control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.
2)

Effects on Dry Weight following a 7-day exposure of *Spirodela polyrhiza* to Mesosulfuron-methyl technical

	Growth rate (dry weight) [µg Mesosulfuron-methyl technical/L]	**Yield (dry weight) [µg Mesosulfuron-methyl technical/L]**
Nominal concentrations		
7-day EC_{50}	12.7	4.06
95% Conf. Limits	9.48 - 18.3	2.15 - 8.74
7-day EC_{20}	2.72	1.07
95% Conf. Limits	2.00 - 3.53	0.331 - 2.03
7-day EC_{10}	1.21	0.532
95% Conf. Limits	0.785 - 1.68	0.104 - 1.14
7-day NOEC	0.610	0.610
7-day LOEC	1.95	1.95
Geometric mean of measured concentrations		
7-day EC_{50}	13.1	4.33
95% Conf. Limits	9.85 - 18.7	2.08 - 11.2
7-day EC_{20}	2.92	1.20
95% Conf. Limits	2.18 - 3.77	0.279 - 2.44
7-day EC_{10}	1.34	0.613

	95% Conf. Limits	0.885 - 1.83	0.0769 -1.40	
	7-day NOEC	0.787	0.787	
	7-day LOEC	1.87	1.87	

Reference: KCP 10.2-06_09

Report: Mesosulfuron-methyl technical: Growth inhibition of *Spirodela polyrhiza* in water/sediment system. Maren D, 2018, S18-00154.

Guideline(s): Yes, OECD 221 (2006)

Deviations: In deviation to the guideline recommendation which adjusts the pH of STEINBERG medium to 5.5 +/- 0.2, the pH was adjusted to 7.5 +/-. This mostly avoids degradation of the test item within seven days.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): N/A

Materials and methods

Test item: Mesosulfuron-methyl technical, Batch No.: 20160408; purity (analysed): 96.78 % w/w

Test species: *Spirodela polyrhiza.*

Test design: In a static dose-response test vegetative growth was observed for a test period of 7 days by measuring the increase in frond number, fresh weight and dry weight.

Endpoints: Inhibition of growth was assessed by the determination of NOEC/LOEC values and $EC_{10,\ 20,\ 50}$ values for the following response variables:

- Growth rates of frond numbers, fresh weight and dry weight
- Yield of frond numbers, fresh weight and dry weight

Additionally the doubling time of frond numbers was determined.

Test rates: A static main test with nominal concentrations of 20.0, 6.25, 1.95, 0.610 and 0.191 µg/L, control and solvent control was performed.

Test conditions: Six replicates were employed for the control, solvent control and three for each test item concentration. The test was performed in 500 mL glass beakers each containing 200 mL test solution. The pH was recorded after 0 and 7 days in all treatments. Temperature was recorded at days 0, 2, 4 and 7. Light intensity of the continuous illumination was measured at test start.

Samples analysed: Analytical samples were taken from the controls and all test item concentrations from samples taken at test start (fresh) and after seven days from aged solutions. Samples of all test item concentrations and controls were analysed from samples taken at t= 0 days fresh and 7 days aged.

Statistics: NOEC and LOEC values were determined by using a multiple comparison method, $EC_{10, 20, 50}$ values were determined by probit analysis, where possible.

Dates of work: 19 Feb 2018 – 12 Apr 2018

Findings:

Test conditions: The pH-value of fresh test solutions ranged from 7.64 to 7.66, the pH-value of aged solutions at day 7 ranged from 8.85 to 9.08. The temperature was measured to be 23.3 – 24.6 °C during the test and the mean light intensity at the beginning of the test was 7692 lux.

Analytical Results: The measured initial concentrations of mesosulfuron-methyl ranged from 83% to 122% of nominal with a mean initial concentration of 102% of nominal. At test end the measured content of mesosulfuron-methyl ranged from 110% to 152% of nominal with a mean initial concentration of 128% of nominal.

All toxicological endpoints were evaluated using nominal concentrations of the test item (Mesosulfuron-methyl technical). In addition, as the recoveries of the freshly prepared solution of concentration level 0.191 µg/L and aged solutions of concentrations levels 0.191, 0.610 and 20.0 µg/L where above 120%, endpoints based on geometric mean measured concentrations of fresh and aged test solutions are also shown in the summary tables and conclusion.

Statistical Results: Following exposure to Mesosulfuron-methyl technical, inhibition effects on *Spirodela polyrhiza* for frond number and fresh weight were found to be more sensitive than for dry weight when comparing the E_yC_{50} values as indicated in the tables below.

Summary of Effects on Frond Number following a 7-day exposure of *Spirodela polyrhiza* to Mesosulfuron-methyl technical

	Growth rate (frond number) [µg Mesosulfuron-methyl technical/L]	Yield (frond number) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
7-day EC_{50}	3.35	1.48
95% Conf. Limits	2.04 - 5.60	0.690 - 3.03
7-day EC_{20}	1.20	0.639
95% Conf. Limits	0.521 - 1.97	0.155 - 1.20
7-day EC_{10}	0.698	0.412[1)]
95% Conf. Limits	0.232 - 1.25	(0.0631 - 0.834)
7-day NOEC	0.610	0.610
7-day LOEC	1.95	1.95
Geometric mean of measured concentrations		
7-day EC_{50}	3.56	1.63
95% Conf. Limits	1.84 - 7.42	0.545 - 4.93
7-day EC_{20}	1.31	0.740
95% Conf. Limits	0.386 - 2.42	0.0546 - 1.56
7-day EC_{10}	0.774	0.489[1)]
95% Conf. Limits	0.147 - 1.56	(0.0129 - 1.09)
7-day NOEC	0.787	0.787
7-day LOEC	1.87	1.87

[1)] Value not reliable. Pooled control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.

Summary of Effects on Fresh Weight following a 7-day exposure of *Spirodela polyrhiza* to Mesosulfuron-methyl technical

	Growth rate (fresh weight) [µg Mesosulfuron-methyl technical/L]	Yield (fresh weight) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
7-day EC_{50}	2.49	1.39
95% Conf. Limits	1.34 - 4.74	0.677 - 2.90
7-day EC_{20}	1.10	0.600
95% Conf. Limits	0.362 - 1.92	0.158 - 1.11
7-day EC_{10}	0.720	0.387[1)]
95% Conf. Limits	0.164 - 1.34	(0.0652 - 0.768)
7-day NOEC	0.610	0.610
7-day LOEC	1.95	1.95
Geometric mean of measured concentrations		
7-day EC_{50}	2.64	1.54
95% Conf. Limits	1.54 - 4.79	0.731 - 3.55
7-day EC_{20}	1.22	0.712
95% Conf. Limits	0.487 - 2.01	0.153 - 1.32
7-day EC_{10}	0.814	0.476[1)]
95% Conf. Limits	0.239 - 1.42	(0.0569 - 0.930)
7-day NOEC	0.787	0.787
7-day LOEC	1.87	1.87

[1)] Value not reliable. Pooled control CV exceeded the effect level (EC_x). Please refer to OECD guideline 239, paragraph 5 which specifies that EC_{10} and EC_{20} values are only reliable and appropriate in tests where coefficients of variation in control plants fall below the effect level being estimated.

Summary of Effects on Dry Weight following a 7-day exposure of *Spirodela polyrhiza* to Mesosulfuron-methyl technical

	Growth rate (dry weight) [µg Mesosulfuron-methyl technical/L]	Yield (dry weight) [µg Mesosulfuron-methyl technical/L]
Nominal concentrations		
7-day EC_{50}	12.7	4.06
95% Conf. Limits	9.48 - 18.3	2.15 - 8.74
7-day EC_{20}	2.72	1.07
95% Conf. Limits	2.00 - 3.53	0.331 - 2.03
7-day EC_{10}	1.21	0.532
95% Conf. Limits	0.785 - 1.68	0.104 - 1.14
7-day NOEC	0.610	0.610
7-day LOEC	1.95	1.95
Geometric mean of measured concentrations		
7-day EC_{50}	13.1	4.33
95% Conf. Limits	9.85 - 18.7	2.08 - 11.2
7-day EC_{20}	2.92	1.20
95% Conf. Limits	2.18 - 3.77	0.279 - 2.44
7-day EC_{10}	1.34	0.613
95% Conf. Limits	0.885 - 1.83	0.0769 -1.40
7-day NOEC	0.787	0.787
7-day LOEC	1.87	1.87

Conclusions:

Following exposure of the aquatic macrophyte *Spirodela polyrhiza* to Mesosulfuron-methyl technical for 7 days the E_rC_{50} and E_yC_{50} values based on frond number were 3.35 µg/L and 1.48 µg/L (nominal) respectively. The NOEC based on frond number was 0.610 µg/L (nominal) for growth rate and yield. Corresponding E_rC_{50} and E_yC_{50} values based on geometric mean measured concentrations were 3.56 µg/L and 1.63 µg/L (mean measured). The NOEC based on frond number was 0.787 µg/L (mean measured) for growth rate and yield.

The E_rC_{50} and E_yC_{50} values based on biomass (fresh weight) were 2.49 µg/L and 1.39 µg/L (nominal) respectively. The NOEC for growth rate and yield based on biomass (fresh weight) was 0.610 µg/L (nominal). Corresponding E_rC_{50} and E_yC_{50} values based on geometric mean measured concentrations were 2.64 µg/L and 1.54 µg/L (mean measured). The NOEC based on fresh weight was 0.787 µg/L (mean measured) for growth rate and yield.

The E_rC_{50} and E_yC_{50} values based on biomass (dry weight) were 12.7 µg/L and 4.06 µg/L (nominal). The NOEC based on biomass (dry weight) was 0.610 µg/L (nominal) for growth rate and yield. Corresponding E_rC_{50} and E_yC_{50} values based on geometric mean measured concentrations were 13.1 µg/L and 4.33 µg/L (mean measured). The NOEC based on dry weight was 0.787 µg/L (mean measured) for growth rate and yield.

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

Comments of zRMS:	The study is considered valid. All validity criteria were met. • The mortality ≤10% in the control groups in both of the oral and contact tests. • In the test with the reference item the LD_{50} =0.13 µg a.i./bee $_{oral}$ and the $LD_{50\ contact}$ = 0.11 µg/bee **Agreed endpoints:** **48 h $LD_{50\ oral}$: > 194.95 µg formulated product/bee** **48 h $LD_{50\ contact}$: > 200 µg formulated product/bee**

Reference: KCP 10.3.1.1-01

Report: Acute contact and oral toxicity of Iodosulfuron + Mesosulfuron (0.6% + 3%) WG on honey bees (*Apis mellifera*), Sipos K., 2018, 17/173-116MT

Guideline(s): Yes (OECD 213 & 214; Guideline 2001/59/EC, Annex Part C, No. C. 16 & C. 17)

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

Test item: Iodosulfuron + Mesosulfuron (0.6 + 3) WG, batch no.: R-BAA
content of a.i.:
Iodosulfuron: 6 g/kg (nominal)
5.6 g/kg (analyzed)
Mesosulfuron: 30 g/kg (nominal)
29.2 g/kg (analyzed)

Reference item: BAS 152 11 I (BASF SE) Batch no.: FRE-001578 (Dimethoate 400 g/L nominal; 429.0 g/L analysed)

Test species: *Apis mellifera* L. subspecies *Carniolan* (honey bee); derived from healthy and queen-right colonies; source: Györgyi Purger Pordánné, Szentgál, Hive No.: 22

Test system: A limit test was performed in both the oral and contact main test with one concentration of 200 µg formulated product/bee.

Oral test: the bees were starved for up to approximately 2 hours before treatment. Subsequently, 200 µL sucrose solution mixed with the test item was injected in each feeding tube. Each tube was

	weighed after feeding to allow an exact calculation of consumed amount. The control groups received 200 µL sucrose solution. **Contact test**: the bees were anesthetized with CO_2 before the 1 µl of the test item solution, deionised water, acetone or 50 % v/v acetone solution was dropped onto the dorsal side of the thorax of each bee using a micropipette. The bees were returned to the cage, allowed to recover and supplied with 50 % w/v sucrose solution, *ad libitum*. **Observations:** Mortality: The numbers of dead bees were recorded at 4, 24 and 48 hours after the start of the experiment. Toxic symptoms: toxic symptoms were recorded at 4, 24 and 48 hours after the start of the experiment.
Endpoints:	Mortality, behavioural impairments
Treatments:	Control oral test: 50% (w/v) aqueous sucrose solution Control contact test: 50% (v/v) aceton solution and deionised water Test item: 200 µg formulated product/bee. Toxic standard: 0.07, 0.12, 0.19 and 0.30 µg dimethoate/bee
Test conditions:	Temperature: 25.0 – 25.6 °C Relative humidity: 51 – 57% Photoperiod: 24h darkness (except during assessments)
Exposure time:	48 hours
Statistics:	The 24h and 48h oral and contact LD_{50} of the test item could not be calculated (because it was a limit test). The 24h oral and contact LD_{50} of the reference item with their 95% confidence limits were calculated using Probit-analysis by TOXSTAT 3.5 software.

Results and discussions

No behavioural impairments were observed in the test item treatments or in the controls, in either the oral or contact test. In the oral test the treated groups consumed the test solution during 4-6 hours of exposure. No repellent effect was observed. The mortality was at the accepted level (≤10%) in the control groups in both of the oral and contact tests. In the test with the reference item the LD_{50} was 0.13 µg a.i./bee and the contact LD_{50} was 0.11 µg/bee, which confirms that the bees were reacting normally under test conditions.

Conclusion

ORAL (24 h) LD501:	> 194.95 µg formulated product/bee
ORAL (48 h) LD501:	> 194.95 µg formulated product/bee
CONTACT (24 h) LD50:	> 200 µg formulated product/bee
CONTACT (48 h) LD50:	> 200 µg formulated product/bee

The acute contact and acute oral toxicity of Iodosulfuron + Mesosulfuron (0.6+3) WG was tested on honey bees (Apis mellifera L.) under laboratory conditions. Based on the results obtained the LD50 (48 h) in the contact toxicity test was determined to be higher than 200 µg formulated product/bee. No adverse effects were noticed on behaviour. In the oral toxicity test the LD50 (48 h) was determined to be higher than 194.95 µg formulated product/bee. No adverse effects were noticed on behaviour.

[1] *Remark: Based on test solution consumption*

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. • Control mortality (48h): ≤ 10 % (being 0%) • LD_{50} – value of the reference (24 h): 0.10 – 0.35 µg a.s./bee (24 h=0.125 µg a.s./bee) **Agreed endpoints:** **48 h $_{oral}$ LD_{50} > 198.9 µg product/bee** **48 h $_{oral}$ LD_{50} >7.1 µg total a.s./bee**

Reference:	KCP 10.3.1.1-04
Report	Acute oral toxicity of GLOB289H + Actirob (adjuvant) to the honeybee *Apis mellifera* L., under laboratory conditions. Franke M., 2019, 19 48 BAA 0089
Guideline(s):	Yes (OECD 214; 1998)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item: GLOB289H + Actirob
GLOB289H; Batch No.: R-BAA

Content of active substance (a.s.):	nominal	analysed
Iodosulfuron-methyl-sodium:	0.6% w/w	0.560% w/w
Mesosulfuron-methyl:	3% w/w	3.001% w/w
Mefenpyr-diethyl (safener):	9% w/w	8.804% w/w

Actirob (adjuvant): Batch no. LC20370511;
Nominal content of active ingredient: Rapeseed oil methyl ester 842 g/L

Reference item: Dimethoate 400 g/L nominal; 429.0 g/L analysed)

Test species: Honeybee – *Apis mellifera* L. Buckfast. (Hymenoptera, Apoidea): worker bees of a healthy and queen-right colony; young adult worker bees were collected in the morning before use; apiary: BioChem agrar GmbH, Kupferstr. 6, 04828 Machern OT Gerichshain, Germany

Test design:	Test item: 48-h; 5 dose rates of test item (GLOB289H + Actirob), 1 dose rate of the adjuvant (solo), comprising 3 replicates each of 10 bees; The mortality and the behaviour were assessed 4, 24, 48 hours after application for the oral test Controls and reference: 50 % w/v sucrose solution and 50 % w/v sucrose solution containing 3.3 % v/v Pottok (adjuvant).
Endpoints:	Mortality, behavioural impairments
Dose rates:	Test item: mixing ratio of GLOB289H:Actirob was 1:3.33 200.0, 120.0, 72.0, 43.2, 25.9 µg product/bee Control (Actirob): 3.33% v/v adjuvant solution
Test conditions:	Temperature: 23.7 – 25.2 °C Relative humidity: 45 - 67% Photoperiod: 24h darkness (except during assessments) Food: 50% (w/v) sucrose solution (after application *ad libitum*)
Statistics:	Statistical program used: ToxRat Professional 3.3.0 (2018) Calculation of LD_{50} values: Test item: no LD50-calculation (due to low mortality) Reference item: Probit analysis (maximum likelihood regression) Statistical significance of mortality values: Test item: Fisher's Exact Binominal Test after Bonferroni-Holm Correction ($p \leq 0.05$) Reference item: Fisher's Exact Binominal Test after Bonferroni-Holm Correction ($p \leq 0.05$)
Validity criteria:	Control mortality (48h): ≤ 10 % LD_{50} – value of the reference (24 h): 0.10 – 0.35 µg a.s./bee
Experimental phase:	22-24 October 2019

Results and discussions

After 48 hours, no mortality occurred in the control group fed with sucrose solution as well as the adjuvant control (sucrose solution containing Actirob). In the test item treatment group, no mortality occurred after oral consumption of ≤ 198.9 µg GLOB289H (+ Actirob)/bee within the 48 hours testing period.

LD_{50}-values of the contact toxicity test

LD_{50} values	Contact toxicity test	
	24 h	48 h
LD_{50} [µg product/bee]	**> 198.9**	**> 198.9**
LD_{50} [µg total a.s./bee]*	**> 7.1**	**> 7.1**

the given dose rates based on the formulated product GLOB289H; * based on sum of analysed content of a.s. of GLOB289H

The oral LD_{50} (24 h) of the reference item was calculated to be 0.125 µg a.s./bee. All validity criteria (control and reference) were achieved.

Conclusion

The acute oral toxicity of GLOB289H + Actirob (adjuvant) was tested on honeybees under laboratory conditions over 48 hours. The oral LD_{50} after 48 hours was > 198.9 µg GLOB289H (+ Actirob)/bee.

<table>
<tr><td>Comments of zRMS:</td><td>The study is considered valid. All validity criteria were met.

• After 48 hours, no mortality occurred in the control group fed with sucrose solution as well as the adjuvant control (sucrose solution containing Pottok)
• LD_{50} – value of the reference (24 h): 0.10 – 0.35 µg a.s./bee

Agreed endpoints:

48 h $_{oral}$ LD_{50} > 196.1 µg product/bee
48 h $_{oral}$ LD_{50} >7.0 µg total a.s./bee</td></tr>
</table>

Reference:	KCP 10.3.1.1-05
Report	Acute oral toxicity of GLOB289H + Pottok (adjuvant) to the honeybee *Apis mellifera* L., under laboratory conditions. Franke M., 2019, 19 48 BAA 0088
Guideline(s):	Yes (OECD 214; 1998)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item: <u>GLOB289H + Pottok</u>

GLOB289H; Batch No.: R-BAA

Content of active substance (a.s.):	<u>nominal</u>	<u>analysed</u>
Iodosulfuron-methyl-sodium:	0.6% w/w	0.560% w/w
Mesosulfuron-methyl:	3% w/w	3.001% w/w
Mefenpyr-diethyl (safener):	9% w/w	8.804% w/w

Pottok (adjuvant): Batch no. BH-MAA

Reference item: Dimethoate 400 g/L nominal; 429.0 g/L analysed)

Test species:	Honeybee – *Apis mellifera* L. Buckfast. (Hymenoptera, Apoidea): worker bees of a healthy and queen-right colony; young adult worker bees were collected in the morning before use; apiary: BioChem agrar GmbH, Kupferstr. 6, 04828 Machern OT Gerichshain, Germany
Test design:	Test item: 48-h; 5 dose rates of test item (GLOB289H + Pottok), 1 dose rate of the adjuvant (solo), comprising 3 replicates each of 10 bees; The mortality and the behaviour were assessed 4, 24, 48 hours after application for the oral test Controls and reference: 50 % w/v sucrose solution and 50 % w/v sucrose solution containing 0.1 % v/v Pottok (adjuvant).
Endpoints:	Mortality, behavioural impairments
Dose rates:	Test item: Pottok was applied at a concentration 0.1% v/v 200.0, 120.0, 72.0, 43.2, 25.9 µg product/bee Control (Pottok): 0.1% v/v adjuvant solution
Test conditions:	Temperature: 23.7 – 25.2 °C Relative humidity: 45 - 67% Photoperiod: 24h darkness (except during assessments) Food: 50% (w/v) sucrose solution (after application *ad libitum*)
Statistics:	Statistical program used: ToxRat Professional 3.3.0 (2018) Calculation of LD_{50} values: Test item: no LD50-calculation (due to low mortality) Reference item: Probit analysis (maximum likelihood regression) Statistical significance of mortality values: Test item: Fisher's Exact Binominal Test after Bonferroni-Holm Correction ($p \leq 0.05$) Reference item: Fisher's Exact Binominal Test after Bonferroni-Holm Correction ($p \leq 0.05$)

Results and discussions

After 48 hours, no mortality occurred in the control group fed with sucrose solution as well as the adjuvant control (sucrose solution containing Pottok). In the test item treatment group, no statistically significant mortality occurred after oral consumption of ≤ 196.1 µg GLOB289H + Pottok/bee within the 48 hours testing period.

LD_{50}-values of the oral toxicity test

LD_{50} values	Oral toxicity test	
	24 h	48 h
LD_{50} [µg product/bee]	**> 196.1**	**> 196.1**
LD_{50} [µg total a.s./bee]*	**> 7.0**	**> 7.0**

* based on sum of analysed content of a.s. the given dose rates based on the formulated product GLOB289H

The oral LD_{50} (24 h) of the reference item was calculated to be 0.125 µg a.s./bee. All validity criteria (control and reference) were achieved.

Conclusion
The acute oral toxicity of GLOB289H + Pottok (adjuvant) was tested on honeybees under laboratory conditions over 48 hours. The oral LD_{50} after 48 hours was > 196.1 µg GLOB289H + Pottok/bee.

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. • Control mortality (48h): ≤ 10 % (being 0%) • LD_{50} of the reference (24 h): 0.10 – 0.30 µg a.s./bee (24 h=0.179 µg a.s./bee and LD50 (48h) =0.157 µg a.s./bee) **Agreed endpoints:** **48 h $_{contact}$ LD_{50} > 200 µg product/bee** **48 h $_{contact}$ LD_{50} >7.1 µg total a.s./bee**

Reference: KCP 10.3.1.1-02

Report: Acute contact toxicity of GLOB289H + Actirob (adjuvant) to the honeybee *Apis mellifera* L. under laboratory conditions. Franke M., 2019, 19 48 BAA 0019

Guideline(s): Yes (OECD 214; 1998)

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

Test item: GLOB289H + Actirob
GLOB289H; Batch No.: R-BAA

Content of active substance (a.s.):	nominal	analysed
Iodosulfuron-methyl-sodium:	0.6% w/w	0.560% w/w
Mesosulfuron-methyl:	3% w/w	3.001% w/w
Mefenpyr-diethyl (safener):	9% w/w	8.804% w/w

Actirob (adjuvant): Batch no. LC20370511;
Nominal content of active ingredient: Rapeseed oil methyl ester 842 g/L

Reference item: Dimethoate 400 g/L nominal; 429.0 g/L analysed)

Test species: Honeybee – *Apis mellifera* L. iberiensis E. (Hymenoptera, Apoidea): worker bees of a healthy and queen-right colony; young adult worker bees were collected in the morning before use; apiary: Joaquin Cordero, Paseo de Moro No. 19, 41370 Cazalla (Seville), Spain

Test design: Test item:
48-h; 5 dose rates of test item (GLOB289H + Actirob), 1 dose rate of the adjuvant (solo), comprising 3 replicates each of 10 bees;
The mortality and the behaviour were assessed 4, 24, 48 hours after application for the contact test
Controls and reference:

	deionised water, tween solution (deionised water with 1 % (v/v) Tween®80 as wetting agent) and an adjuvant control (Actirob)
Endpoints:	Mortality, behavioural impairments
Treatments:	Test item (GLOB289 + Actirob): mixing ratio of GLOB289H : Actirob was 1 :3.333 200.0, 120.0, 72.0, 43.2, 25.9 µg product/bee Control (Actirob) 0.67 µL adjuvant/bee
Test conditions:	Temperature: 23.3 – 26.8 °C Relative humidity: 51.2 – 69.4% Photoperiod: 24h darkness (except during assessments)
Statistics:	Statistical program used: ToxRat Professional 3.2.1 (2015) Calculation of LD_{50} values: Test item: no LD50-calculation (due to low mortality) Reference item: Spearman-Karber computation Statistical significance of mortality values: Test item: Fisher's Exact Binominal Test after Bonferroni-Holm Correction ($p \leq 0.05$) Reference item: Fisher's Exact Binominal Test after Bonferroni-Holm Correction ($p \leq 0.05$)
Validity criteria:	Control mortality (48h): ≤ 10% LD_{50} – value of the reference (24h): 0.10 – 0.30 µg a.s./bee
Experimental phase:	05 – 07 March 2019

Results and discussions

After 48 hours, no mortality occurred in both control groups either treated with deionised water or tween solution. The adjuvant solo tested revealed a mortality of 6.7 % that is not statistically significant in comparison with the tween control group. In the test item treatment group, mortality of 16.7 and 3.3 % without any statistical significance occurred after thoracic application of 200.0 and 120.0 µg GLOB289H + Actirob/bee, respectively, after 48 hours.

LD_{50}-values of the contact toxicity test

LD_{50} values	Contact toxicity test	
	24 h	48 h
LD_{50} [µg product/bee]	> 200.0	> 200.0
LD_{50} [µg total a.s./bee]*	> 7.1	> 7.1

; * based on sum of analysed content of a.s. the given dose rates based on the formulated product GLOB289H

The contact LD50 (24 h) of the reference item was calculated to be 0.179 µg a.s./bee. All validity criteria (control and reference) were achieved.

Conclusion

The acute contact toxicity of GLOB289H + Actirob (adjuvant) was tested on honeybees under laboratory conditions over 48 hours. The contact LD50 after 48 hours was > 200.0 µg GLOB289H + Actirob/bee that is corresponding to > 7.1 µg total a.s./bee.

Comments of zRMS:	The study is considered valid. All validity criteria were met. • Control mortality (48 h): ≤ 10 % • LD_{50} of the reference (24 h): 0.10 – 0.35 µg a.s./bee **Agreed enpoint:** **48 h $LD_{50\ contact}$> 200 µg product/bee**

Reference: KCP 10.3.1.1-03

Report: Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (6 + 30 + 90) g/kg (SAP63H) + adjuvant (Pottok) – Acute contact toxicity to the honey bee, *Apis mellifera* L., under laboratory conditions. Martin M., 2019, S19-00809

Guideline(s): Yes (OECD 214; 1998)

Deviations:
- Behavioural abnormalities in the reference item treatment were not recorded since the reference item is known to be toxic to honey bees and therefore effects are expected. Moreover, the dose range covers the expected LD_{50} values.
- A 2µL droplet was used for contact application instead of a 1 µL droplet, because a higher volume ensures a more reliable dispersion of the application solution. Test facility experience has proven that this volume is suitable and no adverse effects on the outcome of the study are to be expected.

GLP: Yes

Acceptability: Yes

Materials and methods

Test item: Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (6 + 30 + 90) g/kg WG (SAP63H) + adjuvant (POTTOK)

<u>SAP63H</u>
Batch code: F-DBA
Expiry date: April 2020
Iodosulfuron-methyl-sodium: 4.6 g/kg (analysed)
Mesosulfuron-methyl: 27.2 g/kg (analysed)
Mefenpyr-diethyl (safener): 89.9 g/kg analysed

<u>Adjuvant: POTTOK</u>
Batch code: BH-MAA
Ingredient: Non-ionic surfactant
Density: 1.013 g/mL
Expiry date: December 2019

Mixing Ratio: For the application, a volume of Adjuvant POTTOK equivalent to 0.1% of the application solution volume is mixed with SAP63H.

Reference item: BAS 152 11 I.
Batch code: FRE-001578.

	Active ingredient: Dimethoate. Content of a.i. analysed: 429.0 g/L.
Test species:	Adult worker Honey bees (Apis mellifera L.) Commercial beehives from the in-house test facility stock, adequately fed, healthy and as far as possible disease-free and queen-right. The hives from which the bees were obtained were not previously exposed to any chemical treatments within one month of test initiation. Honey bees were picked up from the outer combs of the hive and distributed into test cages one day before start of exposure. The collected honey bees were kept under test conditions until test start. During the acclimatisation period they were fed ad libitum with untreated 50 % (w/v) aqueous sucrose solution.
Test design:	Dose-response test with a duration of 48 hours (up to 96 hours). Five different concentrations of the Test Item were applied to the bees of the Test Item groups, and four different concentrations of the Reference Item were applied to the bees of the Reference Item groups. One control group and one adjuvant control group were included and exposed for the same period of time under identical climatic conditions to the treated groups. Test item groups consisted of 5 replicates per dose, containing 10 bees each. Reference item groups consisted of 4 replicates per dose, containing 10 bees each. Control groups consisted of 5 replicates each, containing 10 bees per replicate. Mortality was assessed at 4, 24 and 48 hours after exposure start for the control, Test Item and Reference Item groups. Behavioural abnormalities were recorded at each observation interval, except for the Reference Item groups, as it can be assumed that moribund and affected bees of these groups will die by the end of the test.
Treatments:	Control groups: - Control group (deionized water) C1 - Adjuvant control group (Pottok) C2 (0.1% of the water volume of POTTOK/bee) Test item groups: - Test item (SAP63H + POTTOK) T1 – T5 Target doses: 25.90, 43.17, 71.97, 119.98 and 200.00 µg SAP63H/bee + 0.1% of the application solution of POTTOK/bee* *in the following, e.g. 25.90, 43.17, 71.97, 119.98 and 200.00 µg SAP63H/bee + 0.1% of the application solution of POTTOK/bee, will be referred to as 25.90, 43.17, 71.97, 119.98 and 200.00 µg SAP63H/bee Reference Item groups: - Reference item (Dimethoate) R1 – R4 Target doses: 0.080, 0.120, 0.180 and 0.270 µg dimethoate/bee.
Test conditions:	Temperature: 24.9 – 25.2 °C Relative humidity: 56.9 – 59.5% Photoperiod: 24h darkness (except during assessments)
Statistics:	Statistical calculations were made with the statistical software SPSS 19.0.0. All tests were performed with an alpha level of 0.05. No mortality was recorded in any of the control groups, so no comparison for statistically significant differences between controls was performed.

The 24h LD_{50} contact values with 95% confidence limits of the reference item was calculated by Probit analysis using linear maximum likelihood regression. There was no observed mortality above 50%, so it was not possible to calculate the LD_{50} values with 95% confidence limits of the contact exposure to the test item.

Results and discussions

the control group (deionized water), 0.00 % mortality was observed during the 24 hour test period. At the end of the test, 48 hours after exposure, also 0.00 % mortality was observed.
In the adjuvant control group (deionized water), 0.00 % mortality was observed during the 24 hour test period. At the end of the test, 48 hours after exposure, also 0.00 % mortality was observed.
In the Reference Item group (dimethoate), the obtained value for the 24 hour period LD50 was 0.165 µg dimethoate/bee. Therefore, all validity criteria were met.
In the Test Item doses of 25.90, 43.17, 71.97, 119.98 and 200.00 µg SAP63H/bee, the cumulative mean mortality was 2.00, 2.00, 2.00, 2.00 and 0.00 %, respectively, both 24 and 48 hours after exposure. As no mortality was recorded in the control groups, mortality in the Test item and Reference item groups was not corrected. According to these results, for the control and treated groups in the contact toxicity test, it was not possible to determine a LD50 value for 24h or 48 h, so it was estimated to be higher than 200.00 µg SAP63H/bee for both periods. At the end of the test (48 hours after contact exposure) no symptoms of affected bees were observed.

LD_{50} values in the contact toxicity tests with Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (6 + 30 + 90) g/kg WG (SAP63H) + 0.1% of the application volume of POTTOK:

Test	Period	LD_{50} [µg SAP63H/bee]	95 % c.l.
Contact toxicity test	24 h	> 200.00	n.d.
	48 h	> 200.00	n.d.

n.d.: not determined; c.l.: confidence limits.

Conclusion

All validity criteria were met and sensitivity of the test organisms could be confirmed. Accordingly, the study was deemed valid.
In an Acute Contact toxicity test with Iodosulfuron-methyl-sodium + Mesosulfuron-methyl
+ Mefenpyr-diethyl (6 + 30 + 90) g/kg WG (SAP63H) + adjuvant (POTTOK), the estimated LD50 values were > 200 µg SAP63H/bee.

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

A 2.3.1.2.1 Honey bee larvae

Comments of zRMS:	The study is considered valid. All validity criteria were met. • Control mortality was ≤ 15% on D8, • Corrected cumulated mortality in the reference item dose of 7.4 µg a.i./larva was ≥ 50% on D8. • Adult emergence in the control was ≥ 70% on D22, **Agreed endpoints:** **ED_{50} (successful adult emergence up to D22) =28.4 µg product/larva**, which is equivalent to an EC50 of 180 mg product/kg food. **ED_{10}** and **ED_{20} (D22)** = **151.9 and 55.4 µg product/larva**, respectively, which is equivalent to an EC_{10} and EC20 (D22) of 960 and 350 mg product/kg food, respectively. **NOED = 32.5 µg product/larva** and the corresponding NOEC = 206 mg product/kg food.

Reference: KCP 10.3.1.2-01

Report: GLOB289H – Repeated exposure of honey bee (*Apis mellifera L.*) larvae under laboratory conditions (*in vitro*), Kleebaum K., 2018, 17 48 BLC 0089

Guideline(s): Yes; OECD 237 Guideline for testing chemicals: Honey bee (*Apis mellifera*) larval toxicity test, single exposure (2013) & Guidance Document on Honey Bee Larval Toxicity Test following Repeated Exposure, Series on Testing and Assessment, No. 239. OECD (2016)

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

In a test under laboratory conditions, honeybee larvae (*Apis mellifera* L.) were repeatedly exposed to GLOB289H. The toxicity of the test item was determined at total doses of 833.6, 370.5, 164.6, 73.2, 32.5 and 14.4 µg product/larva (corresponding to 30.0, 13.3, 5.9, 2.6, 1.2 and 0.5 µg a.i./larva). The concentrations of test item in the diet were 5270, 2342, 1041, 463, 206 and 91 mg product/kg food (corresponding to 190, 84, 37, 17, 7.4 and 3.3 mg total a.i./kg food).
Additionally, honeybee larvae were treated with Dimethoate tech. as reference item at a total concentration of 7.4 µg dimethoate/larva or with an untreated diet as control.

Test item: GLOB289H, Batch No.: R-BAA
Content of a.i.: Iodosulfuron: 6 g/kg (nominal), 5.6 g/kg (analysed); Mesosulfuron: 30 g/kg (nominal), 29.2 g/kg (analysed)

Reference item: Dimethoate tech. (analysed purity: 98.8% w/w)

Test species: *Apis mellifera iberiensis Engel*, first instar larvae; derived from three healthy and queen-right colonies; source: Beekeeper Joaquin Cordero, Paseo de Colón

	No. 19, 41370 Cazalla (Sevilla), Spain
Test design:	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 GLOB289H diluted in the larval food (aqueous sugar solution mixed with royal jelly). After the applications no additional feedings of the larvae took place. In total, 3 treatment groups were set up: 6 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 cumulated larval mortality were done after 24, 48, 72, 96 and 120 hours (respectively D4, D5, D6, D7 and D8). Additionally other observations of 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.
Endpoints :	Successful adult emergence (dose-effect relationship), moratlity, qualitative observations: e.g. body size, remaining food.

Test concentrations:	Controls:	AC	untreated diet B/C (50% aqueous sugar solution with 50% royal jelly)
	Test item:	AT	treated diet B/C at a concentration of 5270 mg product/kg food
		BT	treated diet B/C at a concentration of 2342 mg product/kg food
		CT	treated diet B/C at a concentration of 1041 mg product/kg food
		DT	treated diet B/C at a concentration of 463 mg product/kg food
		ET	treated diet B/C at a concentration of 206 mg product/kg food
		FT	treated diet B/C at a concentration of 91 mg product/kg food
	Reference item:	AR	treated diet B/C at a concentration of 47 mg a.i./kg food

Test conditions:	Temperature:	34.0 °C – 34.9 °C	
	Relative humidity:	D1-D8:	90 – 100%
		D8-D15:	78 – 85%
		D15-D22:	57 – 65%
	Photoperiod:	Darkness (except during assessments)	
	Food:	50% aqueous sugar solution and 50% royal jelly	

Statistics:	Descriptive statistics; Step-down Cochran-Armitage Test for mortality data (one sided greater, alpha = 0.05) and NOED/NOEC. LD/LC_{50} values were determined by Weibull analysis using linear max. likelihood regression.

Results and discussions

On D8, a larval mortality of 0.0% was observed in the control (AC). Pupal mortality (between D8 and D22) was 13.9% in the control. The control group showed a total mortality of 13.9% on D22. In the test

item groups, larval mortalities on D8 ranged between 5.6 and 94.4%. Pupal mortalities ranged between 17.6 and 100.0% in the test item treatment groups. Total mortalities on D22 ranged between 22.2 and 100.0%. Mortality in the reference (AR) was above 50% across all replicates on D8, being 86.1%.
On D8, one individual in one replicate (treated with 370.5 µg product/larva) showed an irregularity, in this case remaining food.
In the final assessment at D22, an adult emergence rate of 86.1% was determined for the honey bees in the control group (AC). In the test item groups the adult honey bees emerged at rates ranging between 0.0% and 77.8% following an application of 833.6, 370.5, 164.6, 73.2, 32.5 and 14.4 µg product/larva, respectively, during the larval stages. On D22, larvae treated with 833.6, 370.5, 164.6 and 73.2 µg product/larva, respectively, showed mortality, which was statistically significantly increased if compared to the control.
The concentrations of active substances in the test item stock solutions A and F ranged between 88% and 107% of the respective nominal concentration. No test item was detected in the control specimen. Because control mortality was □ 15% on D8, corrected cumulated mortality in the reference item dose of 7.4 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 GLOB289H to larvae of *Apis mellifera* L.

Treatment group	Test solution ID	Dose	Concentration	On D8 Larval mortality D3 to D8 [%]		Mean OO [%]	On D22 Pupal mortality D8-D22 [%]		Total mortality D3-D22 [%]		Adult emergence rate [%]
		[µg prod/ larva]	[mg prod/ kg food]	abs.	corr.		abs.	corr.	abs.	corr.	abs.
Control	AC	-	-	0.0	-	0.0	13.9	-	13.9	-	86.1
Test Item	AT	833.6	5270	94.4	-	0.0	100.0	100.0	100.0	100.0	0.0*
	BT	370.5	2342	33.3	-	3.7	83.3	80.6	88.9	87.1	11.1*
	CT	164.6	1041	11.1	-	0.0	46.9	38.3	52.8	45.2	47.2*
	DT	73.2	463	8.3	-	0.0	27.3	15.5	33.3	22.6	66.7*
	ET	32.5	206	5.6	-	0.0	20.6	7.8	25.0	12.9	75.0
	FT	14.4	91	5.6	-	0.0	17.6	4.4	22.2	9.7	77.8
Reference Item	AR	[µg a.i./ larva] 7.4	[mg a.i./ kg food] 47	86.1	-	0.0	80.0	76.8	97.2	96.8	2.8

Treatment	Endpoint: Successful adult emergence	Up to D22
Test item doses	ED_{50} [µg product/larva] [2] (95% CL)	28.4 (17.4 – 46.4)
	ED_{20} [µg product/larva] [2] (95% CL)	55.4 (38.6 – 79.6)
	ED_{10} [µg product/larva] [2] (95% CL)	151.9 (121.1 – 190.5)
	NOED [µg product/larva] [1]	32.5
Test item concentrations	EC_{50} [mg product/kg food] [2] (95% CL)	180 (110 – 294)
	EC_{20} [mg product/kg food] [2] (95% CL)	350 (244 – 503)
	EC_{10} [mg product/kg food] [2] (95% CL)	960 (765 – 1204)
	NOEC [mg product/kg food] [1]	206

Conclusion

In a repeated exposure larval toxicity study with GLOB289H, the ED50 (successful adult emergence up to D22) was calculated to be 28.4 µg product/larva, which is equivalent to an EC50 of 180 mg product/kg food.
The ED10 and ED20 (D22) was determined to be 151.9 and 55.4 µg product/larva, respectively, which is equivalent to an EC10 and EC20 (D22) of 960 and 350 mg product/kg food, respectively. The respective NOED was 32.5 µg product/larva and the corresponding NOEC was 206 mg product/kg food.

A 2.3.1.2.2 Adult honey bees

Comments of zRMS:	The study is considered valid. All validity criteria were met. • Control mortality < 15 % and mortality (observed: 3.3% for aqueous sucrose solution and 6.7% for 0.1% xanthan) • Mortality in the reference group was > 50 % after 10 days (observed: 96.7%) **Agreed endpoints:** **LDD_{50}=115 µg consumed product/bee/day (**equivalent to 4.14 µg consumed a.i./bee/day) **LC_{50} = 4.531 g product/kg food** (equivalent to 0.163 g a.i./kg food) **NOEDD=45.7 µg consumed product/bee/day** (equivalent to 1.65 µg consumed a.i./bee/day) **NOEC = 1.326 g product/kg food** (equivalent to 0.048 g a.i./kg food)

Reference:	KCP 10.3.1.2-02
Report	Chronic toxicity of Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (0.6+3+9)% WG to the honey bee *Apis mellifera* L. under laboratory conditions. Ruhland S., 2018, 17 48 BAC 0055
Guideline(s):	Yes; OECD 245 (2017)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item:	Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (0.6+3+9) % WG, Batch: R-BAA Content of a.i.: Iodosulfuron-methyl-sodium: 6 g/kg (nom), 5.6 g/kg (analysed) Mesosulfuron-methyl: 30 g/kg (nom), 29.2 g/kg (analysed) Mefenpyr-diethyl: 90 g/kg (nom), N/A (analysed)
Reference item:	Dimethoate tech. (analysed purity: 98.8% w/w)
Test species:	*Apis mellifera* L. subspecies *iberiensis* (honey bee), max. 2 days old bees; derived from healthy and queen-right colonies; source: Beekeeper Joaquin Cordero, Paseo del Moro No. 19, 41370 Cazalla (Seville), Spain

Test design:	In a 10 day chronic test, young adults of *Apis mellifera* L. were daily exposed to 5 doses of Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (0.6+3+9) % WG in treated food (50% (w/v) aqueous sucrose solution + 0.1% (w/v) xanthan). The following treatment groups were set up: 5 doses of the test item, 2 untreated control groups (50% (w/v) aqueous sucrose solution and 50% (w/v) aqueous sucrose solution + 0.1% (w/v) xanthan) and 1 dose of the toxic standard with 3 replicates per dose and 10 bees per replicate. Assessments of bee mortality and behavioural effects were done daily during the study. In the analytical phase of the study the concentrations of the active substances in the highest and lowest test item feeding concentration on D0 were determined.
Endpoints :	Mortality, behavioural impairments

Test concentrations:	Controls:	AC	50% (w/v) aqueous sucrose solution
		BC	50% (w/v) aqueous sucrose solution + 0.1% (w/v) xanthan
	Test item:	treated diet at nominal doses of 833, 416, 208, 104 and 52.1 µg product/bee/day (30.0, 15.0, 7.50, 3.75 and 1.87 µg a.i./bee/day*) corresponding to concentrations of 21.210, 10.605, 5.303, 2.651 and 1.326 g product/kg food (0.764, 0.382, 0.191, 0.095 and 0.048 g a.i./kg food*) * sum of nominal content of both active substances	
	Reference:	treated diet at a nominal dose of 27.3 ng Dimethoate/bee/day (corresponding to a concentration of 0.696 mg a.i./kg food).	

Test conditions:	Temperature:	31.9 °C – 34.1 °C
	Relative humidity:	59.6 – 69.8 %
	Photoperiod:	Darkness (except during assessments)
	Food:	50% aqueous sugar solution

Statistics:	Descriptive statistics; Step-down Cochran-Armitage Test for mortality data (one sided greater, alpha = 0.05) and NOED/NOEC. LD/LC_{50} values were determined with Probit analysis using linear maximum likelihood regression along with the 95% confidence limits. Statistical program: ToxRat Professional 3.2.1 (2015).

Results and discussions

After 10 days, a mortality of 3.3 % in control group AC was observed. In the viscosifier control group BC a mortality of 6.7 % was recorded. Taking into account the actual food uptake and the evaporated amount of feeding solution the bees effectively consumed doses of 325, 155, 127, 92.1 and 45.7 µg product/bee/day which caused mortalities of 100.0, 90.0, 73.3, 20.0 and 3.3 %, respectively after 10 days. Mortalities in the 325, 155, 127 and 92.1 µg consumed product/bee/day treatment groups were statistically significantly increased compared to the viscosifier control.

Based on these data the LDD_{50} was determined to be 115 µg consumed product/bee/day (equivalent to 4.14 µg consumed a.i./bee/day) and the LC_{50} to be 4.531 g product/kg food (equivalent to 0.163 g a.i./kg food), respectively.
The NOEDD was determined to be 45.7 µg consumed product/bee/day (equivalent to 1.65 µg consumed a.i./bee/day) and the NOEC to be 1.326 g product/kg food (equivalent to 0.048 g a.i./kg food), respectively.

On the last day of the test, treatment related behavioural abnormalities could be observed in both the middle and second lowest test item group. Bees were described as being affected in terms of uncoordinated movements.

The recovery rates of iodosulfuron-methyl-sodium were 107 % in the highest and 105 % in the lowest test item dose and of mesosulfuron-methyl 106 % in the highest and 108 % in the lowest test item dose (samples taken on the first day of application). No test item has been detected in the control sample.

The effective reference dosage in the study was 16.0 ng a.i./bee/day, which caused a mean mortality of 96.7 %.

In the test item group the food consumption ranged between 14.6 and 34.7 mg solution per bee per day which is 37.2 % to 88.4 % of the expected amount (control AC: on average 41.3 mg/bee/day = 105.1 %, viscosifier control BC: on average 35.6 mg/bee/day = 90.8 %) with a tendency of higher food uptake in the lower test item dosages. The food consumption per cage was corrected by subtracting the mean evaporation figure of each day of application.

Because control mortality was < 15 % and mortality in the reference group was > 50 % after 10 days the study can be regarded as valid.

The results are summarised in Table I.

Table I **Mean mortality, behaviour of bees and toxicity of Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (0.6+3+9) % WG after 10 days in a chronic toxicity feeding test**

Treatment group	Treatment group ID	Daily dose nominal	Daily dose consumed	Concentration	After 10 days: Mean mortality absolute	Mean mortality corrected	Number of bees with behavioural abnormalities**
		[µg product/bee/day]		[g product/ kg food]	[%]	[%]	
Control	AC	-	-	-	3.3	-	0 out of 29
Viscosifier control	BC	-	-	-	6.7	-	0 out of 28
Test item	AT	833	325	21.210	100.0*	100.0	-
	BT	416	155	10.605	90.0*	89.3	0 out of 3
	CT	208	127	5.303	73.3*	71.4	1 out of 8
	DT	104	92.1	2.651	20.0*	14.3	4 out of 24
	ET	52.1	45.7	1.326	3.3	0.0	0 out of 29
		[ng a.i./bee/day]		**[mg a.i./ kg food]**			
Reference item	AR	27.3	16.0	0.696	96.7	96.6	1 out of 1

	Endpoints	10 d
Test item doses	LDD_{50} [µg consumed product/bee/day][1]	**115** (106 – 123)
	LDD_{50} [µg consumed a.i./bee/day][1]	**4.14** (3.82 – 4.43)
	NOEDD [µg consumed product/bee/day][2]	**45.7**
	NOEDD [µg consumed a.i./bee/day][2]	**1.65**
Test item concentrations	LC_{50} [g product/kg food][1]	**4.531** (3.823 – 5.367)
	LC_{50} [g a.i./kg food][1]	**0.163** (0.137 – 0.193)
	NOEC [g product/kg food][2]	**1.326**
	NOEC [g a.i./kg food][2]	**0.048**

Results are averages based on 3 replicates, containing 10 bees each; Calculations are performed with non-rounded values and corrected for evaporation
corrected: test item treatment group was corrected for mortality of untreated solvent control BC, reference item group was corrected for mortality of untreated control AC (according to SCHNEIDER-ORELLI 1947), negative values are treated as "0"
* Statistically significant difference in pairwise comparison between treatment and and untreated viscosifier control (BC) (Step-down Cochran-Armitage Test Procedure; $\alpha = 0.05$; one-sided greater)
** Number of bees with behavioural abnormalities referring to number of remaining bees
1 Median lethal dietary dose/concentration (95 % cl lower-upper) was calculated using Probit analysis (linear max. likelihood regression)
2 No observed effect dietary dose/concentration was calculated using Step-down Cochran-Armitage Test Procedure ($\alpha = 0.05$; one sided greater)

Conclusion

The chronic oral toxicity of Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (0.6+3+9)% WG on young adult bees (*Apis mellifera* L.) was investigated in a 10 day chronic, dose-response feeding study under laboratory conditons.
The LDD_{50} was determined to be 115 µg consumed product/bee/day (equivalent to 4.14 µg consumed a.i./bee/day) and the LC_{50} to be 4.531 g product/kg food (equivalent to 0.163 g a.i./kg food), respectively.
The NOEDD was determined to be 45.7 µg consumed product/bee/day (equivalent to 1.65 µg consumed a.i./bee/day) and the NOEC to be 1.326 g product/kg food (equivalent to 0.048 g a.i./kg food), respectively.

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

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 Tests on arthropods other than bees

A 2.3.2.1 KCP 10.3.2.1 Using artificial substrates

Comments of zRMS:	The study is considered valid. All validity criteria were met. • Mortality in the control group: ≤ 20 % (dead and escaped mites) on day 7 (observed 2.0%) • Corrected mortality in the reference group: 50 – 100 % on day 7 (observed: 71.4%) • Reproduction in the control group: ≥ 4 eggs per female (only, when a fecundity test was performed with surviving mites of the test item group), • (observed 6.38 eggs per female) **Agreed endpoints:** **LR_{50} > 1 kg product/ha** **$NOER_{mortality}$ ≥ 1 kg product/ha** **ER_{50} > 1 kg product/ha** **$NOER_{reproduction}$ ≥ 1 kg product/ha**

Reference:	KCP10.3.2-01
Report	Effects of Iodosulfuron + Mesosulfuron (0.6+3)% WG on the predatory mite *Typhlodromus pyri* scheuten in a laboratory test, Röhlig U., 2017, 17 48 NTL 0008.
Guideline(s):	IOBC (BLÜMEL et al. 2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item: Iodosulfuron + Mesosulfuron (0.6+3) % WG (GLOB289H)
batch No.: R-BAA
analysed content of a.s.:
iodosulfuron 5.6 g/kg (nominal: 6 g/kg)
mesosulfuron 29.2 g/kg (nominal: 30 g/kg)

Reference Item:	Dimethoate EC 400 (Dimethoate 405.2 g/L, nominal: 400 g/L)
Test species:	Predatory mite Typhlodromus pyri SCHEUTEN, protonymphs (< 24 hours old); source (in the stage of eggs): "Katz Biotech AG", An der Birkenpfuhlheide 10, 15837 Baruth,Germany
Test design:	Protonymphs were exposed to dried spray residues of different application rates of the test item applied on glass plates. 7 treatment groups (5 test item rates, water treated control, reference item) were set up with 5 replicates (consisting of 20 protonymphs) per treatment. Exposure lasted until 14 days after application. Mortality assessments were carried out 3 and 7 days after exposure of the mites and additionally after 9, 11 and 14 days. In addition, for the control and all rates up to and including 1 kg product/ha, the reproduction, i.e. number of eggs per female, was determined (3 assessments, 9, 11 and 14 days after application).
Endpoints :	Mortality after exposure over 7 days, including determination of a LR50 (Lethal Rate 50 %, rate resulting in 50 % mortality) Reproductive capacity of the surviving mites from day 7-14 including determination of a ER50 (Effect Rate 50 %, rate resulting in 50 % effect on reproduction)
Test concentrations:	Control (deionised water) Test item (Iodosulfuron + Mesosulfuron (0.6+3) % WG): 0.0625 – 0.125 – 0.25 – 0.5 – 1 kg product/ha in 200 L /ha of deionised water Reference item (Dimethoate EC 400): 15 mL/ha in 200 L /ha of deionised water
Test conditions:	Temperature: 23 °C – 27 °C Relative humidity: 68 – 72 % Photoperiod: 16 hours light : 8 hours dark Light intensity: 2060 lux Food: pollen (pine and birch)
Statistics:	Multiple Sequentially-rejective Chi2-2x2 Table test after BONFERRONI-HOLM ($\alpha = 0.05$) for mortality (test item) Chi2 2x2 Table test ($\alpha = 0.05$) for mortality (reference item) WILLIAMS-t-test for reproductive capacity ($\alpha = 0.05$)

Results and discussion

After 7 days, in the water-treated control a mortality of 2.0 % was observed. In the test item treatments mortality ranged between 1.0 % and 3.0 %. This resulted in corrected mortality rates between -1.0 % and 1.0 %. No statistically significant effects on mortality were determined at rates up to and including 1 kg product/ha compared to the control. The LR50 was estimated to be > 1 kg product/ha. The NOER for mortality was considered to be ≥ 1 kg product/ha.

The reproductive capacity of the mites was assessed in the control group and at all rates up to and including 1 kg product/ha. The reproduction rate amounted to 6.38 eggs/female in the control treatment. The reproduction rate in the different test item treated groups ranged from 6.42 eggs/female to 6.94 eggs/female. Thus an effect on reproduction between -0.6 % and -8.8 % was calculated for the test item treated groups compared to the control. No statistically significant effects on reproduction were determined at rates up to and including 1 kg product/ha. The ER50 was estimated to be > 1 kg product/ha. The NOER for reproduction was considered to be ≥ 1 kg product/ha.

The results are summarized below

Effects on predatory mite *Typhlodromus pyri* exposed to fresh dry residues of Iodosulfuron + Mesosulfuron (0.6+3) % WG in a worst-case laboratory test

Treatment	Rate [1] [kg product/ha]	Mortality [2] [%]	Corrected mortality [3] [%]	Mean number of eggs per female [4] [7-14 Day]	Effect on Reproduction [5] [%]
Control	-	2.0	-	6.38	-
Test item	0.0625	2.0 (n.s.)	0	6.66 (n.s.)	-4.4
Test item	0.125	2.0 (n.s.)	0	6.94 (n.s.)	-8.8
Test item	0.25	1.0 (n.s.)	-1.0	6.42 (n.s.)	-0.6
Test item	0.5	1.0 (n.s.)	-1.0	6.98 (n.s.)	-9.4
Test item	1	3.0 (n.s.)	1.0	6.48 (n.s.)	-1.6
	Endpoint [kg product/ha]				
LR_{50}	> 1				
ER_{50}				> 1	

[1] Application rate in 200 L water/ha
[2] Mortality after 7 days of exposure to residues on treated glass plates. The results for mortality in individual test item treatments were compared to that in the control using Multiple Sequentially-rejective Chi^2-2x2 Table test after BONFERRONI-HOLM (α = 0.05).
[3] Corrected mortality according to ABBOTT (1925)
[4] Results for reproduction compared by WILLIAMS-t-test (α = 0.05)
[5] Change in mean number of eggs per female, relative to control. A negative value indicates an increase relative to the control.
n.s. not statistically significant different compared to the control

No unusual observations regarding behaviour were noted in the control and the test item treatment groups at any observation point during the test. The reference item caused a mortality of 72.0 % of exposed mites, resulting in a corrected mortality of 71.4 %.

Conclusion
In a worst-case laboratory study with Iodosulfuron + Mesosulfuron (0.6+3) % WG the LR50 for Typhlodromus pyri was estimated to be > 1 kg product/ha. The NOER for mortality was considered to be ≥ 1 kg product/ha. The ER50 was estimated to be > 1 kg product/ha. The NOER for reproduction was considered to be ≥ 1 kg product/ha.

Comments of zRMS:	The study is considered valid. All validity criteria were met. • Mortality in the control group: ≤ 13 % (48 h), (observed: 2.5%) • Reproduction in the control group: ≥ 5 mummies per female*), (observed: 20.9) (only, when a reproduction test was performed with surviving wasps of the test item group) • Corrected mortality in the reference item group: > 50 % and preferably < 100 % (48 h), (observed: 97.4%) *) there should be no more than 2 control replicates (with surviving wasps) with zero values **Agreed endpoints:** **LR_{50} > 1 kg product/ha.** **NOER $_{mortality}$ ≥ 1 kg product/ha.** **ER50 $_{reproduction}$ > 1 kg product/ha.** **NOER $_{reproduction}$ ≥ 1 kg product/ha.**

Reference:	KCP10.3.2-02
Report	Effects of Iodosulfuron + Mesosulfuron (0.6+3)% WG on the parasitic wasp *Aphidius rhopalosiphi* (Destefani-perez) in a laboratory test, Röhlig U., 2017, 17 48 NAL 0009.
Guideline(s):	IOBC (MEAD-BRIGGS et al. 2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item:	Iodosulfuron + Mesosulfuron (0.6+3) % WG (GLOB289H) batch No.: R-BAA analysed content of a.s.: iodosulfuron 5.6 g/kg (nominal: 6 g/kg) mesosulfuron 29.2 g/kg (nominal: 30 g/kg)
Reference Item:	Dimethoate EC 400 (Dimethoate 405.2 g/L, nominal: 400 g/L)
Test species:	Parasitic wasp Aphidius rhopalosiphi (DESTEFANI-PEREZ), adults (< 48 hours old); source (in the stage of mummies): “Katz Biotech AG”, An der Birkenpfuhlheide 10, 15837 Baruth,Germany
Test design:	Exposure of the wasps was achieved via dried spray residues on glass plates. Seven treatment groups (5 test item rates, water treated control, reference item) were set up with 4 replicates (consisting of 7 females and 3 males, each) per treatment. Mortality assessments were carried out 2, 24 and 48 hours after test initiation. At 48 hours, surviving wasps (15 females per treatment) were removed and their reproductive capacity was assessed by confining them individually over untreated wheat plants infested with adult and nymphal aphids (Rhopalosiphum padi). Assessment of reproduction capacity i.e. number of mummies per female, was made for the control and all test item groups up to and including 1 kg product/ha (1

assessment, 14 days afterapplication).

Endpoints :	Mortality after exposure over 48 hours, including determination of a LR50 (Lethal Rate 50 %, rate resulting in 50 % mortality) Reproductive capacity: number of mummies per female including the determination of an ER_{50} (if possible)
Test concentrations:	Control (deionised water) Test item (Iodosulfuron + Mesosulfuron (0.6+3) % WG): 0.0625 – 0.125 – 0.25 – 0.5 – 1 kg product/ha in 200 L /ha of deionised water Reference item (Dimethoate EC 400): 0.3 mL/ha in 200 L /ha of deionised water
Test conditions:	Temperature: 19 °C – 22 °C Relative humidity: 63 – 74 % Photoperiod: 16 hours light : 8 hours dark Light intensity: 1420 lux (exposure phase) 2610 lux (parasitisation phase) 7130 lux (reproduction phase) Food: pollen (pine and birch)
Statistics:	Multiple Sequentially-rejective Chi2-2x2 Table test after BONFERRONI-HOLM ($\alpha = 0.05$) for mortality (test item) FISHER's Exact Binomial Test ($\alpha = 0.05$) for mortality (reference item) WILLIAMS-t-test for reproductive capacity ($\alpha = 0.05$)

Results and discussion

After 48 hours, in the water-treated control a mortality of 2.5 % was observed. In the test item treatments, mortality ranged between 2.5 % and 12.5 %. This resulted in corrected mortality rates of 0 % and 10.3 %. No statistically significant effects on mortality were determined at any of the tested rates. The LR50 was estimated to be > 1 kg product/ha. The NOER for mortality was considered to be ≥ 1 kg product/ha.

The mean number of mummies per female per day in the test item treatment groups ranged between 18.9 and 22.1, compared to the control with 20.9 mummies/female. No statistically significant effects on reproductive capacity were determined in any of the test item treatment groups. The ER50 was estimated to be > 1 kg product/ha. The NOER for reproduction was considered to be ≥ 1 kg product/ha.
The results are summarised below.

Effects on the parasitic wasp *Aphidius rhopalosiphi* exposed to Iodosulfuron + Mesosulfuron (0.6+3) % WG in a worst-case laboratory test

Treatment	Rate [1] [kg product/ha]	Mortality [2] [%]	Corrected Mortality [3] [%]	Reproduction [4] [mean number of mummies/female]	Effects on reproduction [5] [%]
Control	-	2.5	-	20.9	-
Test item	0.0625	2.5 (n.s.)	0	22.1 (n.s.)	-5.7
Test item	0.125	2.5 (n.s.)	0	20.9 (n.s.)	0
Test item	0.25	5.0 (n.s.)	2.6	19.4 (n.s.)	7.2
Test item	0.5	7.5 (n.s.)	5.1	19.4 (n.s.)	7.2
Test item	1	12.5 (n.s.)	10.3	18.9 (n.s.)	9.6
	Endpoint [kg product/ha]				
LR_{50}	**> 1**			**-**	
ER_{50}	**-**			**> 1**	

[1] Application rate in 200 L water/ha
[2] Mortality after 48 hours of exposure to the test item on treated glass plates. The results for mortality in individual treatments were compared to that in the control using Multiple Sequentially-rejective FISHER Test after BONFERRONI-HOLM (α = 0.05).
[3] Corrected mortality according to ABBOTT (1925).
[4] Reproduction: mean number of parasitised aphids (mummies)/surviving female. The results for the test item treatments and control were compared by WILLIAMS-t-test (α = 0.05).
[5] Change in mean number of mummies per female, relative to control. A negative value indicates an increase and a positive value indicates a decrease, relative to the control.
n.s. not statistically significant different compared to the control

No unusual observations regarding behavior were noted in the control and the test item treatment groups at any observation point during the test.
The reference item cause a mortality of 97.5% of exposed wasps, resulting in a corrected mortality of 97.5%.

Conclusion
In a worst-case laboratory study with Iodosulfuron + Mesosulfuron (0.6+3) % WG, the LR50 for Aphidius rhopalosiphi was estimated to be > 1 kg product/ha. The NOER for mortality was considered to be ≥ 1 kg product/ha. The ER50 for reproduction was estimated to be > 1 kg product/ha. The NOER for reproduction was considered to be ≥ 1 kg product/ha.

<table>
<tr><td>Comments of zRMS:</td><td>The study is considered valid. All validity criteria were met.
Mortality in the control group: ≤ 20 % (dead and escaped mites) on day 7, (observed: 0% in the water control and 2.0% in the adjuvant)
Corrected mortality in the reference group: 50 – 100 % on day 7, (observed: 76%)
Reproduction in the control group: ≥ 4 eggs per female (only, when a fecundity test was performed with surviving mites of the test item group), (observed: 6.45 eggs per female in th water control and 6.56 eggs per female in the adjuvant)
Agreed endpoints:

NOER (no observed effect rate) $_{mortality}$ ≥ 1 kg GLOB289H/ha + 2 L Actirob B/ha
NOER (no observed effect rate) $_{reproduction}$ ≥ 1 kg GLOB289H/ha + 2 L Actirob B/ha</td></tr>
</table>

Reference:	KCP10.3.2-03
Report	Effects of GLOB289H + Actirob B on the predatory mite *Typhlodromus pyri* SCHEUTEN in a laboratory test, Röhlig U., 2019, 19 48 NTL 0002.
Guideline(s):	IOBC (BLÜMEL et al. 2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item: GLOB289H + Actirob B

GLOB289H
batch No.: R-BAA
analysed content of a.s.:
iodosulfuron-methyl-sodium 0.560 % w/w (nominal: 0.6 % w/w)
mesosulfuron-methyl 3.0001 % w/w (nominal: 3 % w/w)
mefenpyr-diethyl (safener) 8.804 % w/w (nominal: 9% w/w)

Actirob B
batch No.: LC2037051
nominal content of a.s.: 842 g/L rapeseed oil methyl ester

Reference Item: Dimethoate EC 400 (Dimethoate 429.0 g/L, nominal: 400 g/L)

Test species: Predatory mite Typhlodromus pyri SCHEUTEN, protonymphs (< 24 hours old); source (in the stage of eggs): “Katz Biotech AG”, An der Birkenpfuhlheide 10, 15837 Baruth, Germany

Test design: Protonymphs were exposed to dried spray residues of different application rates of the test item applied on glass plates. 5 treatment groups (2 test item rates, water treated control, adjuvant control, reference item) were set up with 5 replicates (consisting of 20 protonymphs) per treatment.
Exposure lasted until 14 days after application. Mortality assessments were carried out 3 and 7 days after exposure of the mites and additionally after 9, 11 and 14 days. In addition, for the control, the adjuvant control and the both test item rates, the reproduction, i.e. number of eggs per female, was determined (3 assessments, 9, 11 and 14 days after application).

Endpoints : Mortality after exposure over 7 days
Reproductive capacity of the surviving mites from day 7-14

Test concentrations: Control (deionised water)
Adjuvant control (2L Actirob B/ha)
Test item (GLOB289H + Actirob B):
- 0.5 kg GLOB289H/ha + 1 L Actriob B/ha
- 1 kg GLOB289H/ha + 2 L Actirob B/ha

Reference item (Dimethoate EC 400): 15 ml/ha
All substances were applied in 200 L/ha, sprayed on glass plates, via laboratory spraying equipment and air dried afterwards.

Test conditions:	Temperature:	23 °C – 25 °C
	Relative humidity:	66 – 74 %
	Photoperiod:	16 hours light : 8 hours dark
	Light intensity:	2030 lux
	Food:	pollen (pine and birch)

Statistics: Multiple Sequentially-rejective Chi2-2x2 Table test after BONFERRONI-HOLM (α = 0.05) for mortality (test item)
Chi^2 2x2 Table test (α = 0.05) for mortality (reference item)
DUNNETT'S Multiple t-test (α = 0.05) for reproductive capacity

Results and discussion

After 7 days, in the water-treated control a mortality of 0 % and in the adjuvant control a mortality of 2.0 % were observed. In the test item treatments mortality was 2.0 % and 1.0 %. This resulted in corrected mortality rates between 0 % and -1.0 %, compared to the adjuvant control. No statistically significant effects on mortality were determined at both rates compared to the control (Multiple Sequentially-rejective Chi2-2x2 Table test after BONFERRONI-HOLM, α = 0.05). The NOER (no observed effect rate) for mortality was considered to be ≥ 1 kg GLOB289H/ha + 2 L Actirob B/ha. The reproductive capacity of the mites was assessed in both control groups and in both test item rates. The reproduction rate amounted to 6.45 eggs/female in the water treated control and 6.56 eggs/female in the adjuvant control treatment. The reproduction rate in both test item treated groups ranged from 6.86 eggs/female to 6.51 eggs/female. Thus an effect on reproduction between -4.6 % and 0.8 % was calculated for the test item treated groups compared to the adjuvant control. No statistically significant effects on reproduction were determined at rates up to and including 1 kg GLOB289H/ha + 2 L Actirob B/ha (DUNNETT'S Multiple Sequential t-test, α = 0.05). The NOER (no observed effect rate) for reproduction was considered to be ≥ 1 kg GLOB289H/ha + 2 L Actirob B/ha.

The results are summarized below.

Effects on predatory mite *Typhlodromus pyri* exposed to fresh dry residues of GLOB289H + Actirob B in a worst-case laboratory test

Treatment	Rate [1] [product/ha]	Mortality [2] [%]	Corrected mortality [3] [%]	Mean number of eggs per female [4] [7-14 Day]	Effect on Reproduction [5] [%]
Control	-	0.0	-	6.45	-
Adjuvant Control	2 L	2.0	-	6.56	
Test item	0.5 kg + 1 L	2.0 (n.s.)	0	6.86 (n.s.)	-4.6
Test item	1 kg + 2 L	1.0 (n.s.)	-1.0	6.51 (n.s.)	0.8

Test item: GLOB289H + Actirob B
1 Application rate in 200 L/ha
2 Mortality after 7 days of exposure to residues on treated glass plates. The results for mortality in individual test item treatments were compared to that in the control using Multiple Sequentially-rejective Chi2-2x2 Table test after BONFERRONI-HOLM (α = 0.05).
3 Corrected mortality according to ABBOTT (1925) compared to the adjuvant control
4 Results for reproduction compared by DUNNETT'S Multiple Sequential t-test (α = 0.05)
5 Change in mean number of eggs per female, relative to the adjuvant control. A negative value indicates an increase and a positive value indicates a decrease, relative to the control.
n.s. not statistically significant different compared to the control

No unusual observations regarding behavior were noted in the control and the test item treatment groups at any observation point during the test.

The reference item caused a mortality of 76.0 % of exposed mites, resulting in a corrected mortality of 76.0 % compared to the water treated control.

Conclusion
In a worst-case laboratory study with GLOB289H + Actirob B on Typhlodromus pyri the NOER (no observed effect rate) for mortality was considered to be ≥ 1 kg GLOB289H/ha + 2 L Actirob B/ha.
The NOER (no observed effect rate) for reproduction was considered to be ≥ 1 kg GLOB289H/ha + 2 L Actirob B/ha.

Comments of zRMS:	The study is considered valid. All validity criteria were met. • Control mortality The mean mortality in control should be ≤ 10 % after 48 hour of exposure , (observed: 0.0 %). • Reference item mortality The reference item should cause mean corrected 48-hour mortality between 50 % and 100 %. (observed: 73.33%) • The mean number of mummies per female in the control should be 5.0 mummies/female (observed: 67.93) • No more than 2 females should fail to produce mummies (observed : 0 female) **Agreed endpoints:** **LR_{50} =0.833 kg test item/ha + 0.2 L adjuvant/ha.** **ER_{50} ≥0.833 kg test item/ha + 0.2 L adjuvant/ha,** **(NOER (48h mortality) and repellency≥0.833 kg test item/ha+ 0.2 L adjuvant/ha.** **NOER (fecundity)≥ 0.500 kg test item/ha + 0.2 L adjuvant/ha.**

Reference:	KCP10.3.2-07
Report	Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (6 + 30 + 90) g/kg WG (SAP63H) + adjuvant (Pottok) – Toxicity to the aphid parasitoid *Aphidius rhopalosiphi* De Stafani Perez (Hymenoptera, Braconidae) under Extended Laboratory Conditions, Luna R., 2019, S19-02623.
Guideline(s):	IOBC (MEAD-BRIGGS et al. 2010)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item:	Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyrdiethyl (6 + 30 + 90) g/kg WG (SAP63H), Batch No. F-DBA, Content of a.i. (nominal/analysed): Iodosulfuron-methyl-sodium: 6.0 / 4.6 g/kg Mesosulfuron-methyl: 30.0 / 27.2 g/kg Mefenpyr-diethyl 90.0 / 89.9 g/kg + adjuvant (POTTOK), Batch No. BH-MAA
Reference Item:	Dimethoate 40% w/v EC; BAS 152 11 I, Batch No. FRE-001578,

Content of a.i. (nominal/analysed): 400 / 429.0 g/L

Test species: Aphidius rhopalosiphi De Stefani Perez, life stage at start of exposure: adult wasps (less than 48 hours old)

Test design: Adjuvant, test item + adjuvant and reference item were diluted in deionised water and applied with a laboratory track sprayer to barley seedlings. A control group applied with deionised water was included in the study. All applications were performed with a spray volume of 400 L/ha. After assembling of test units five adult female wasps were introduced into each test unit (6 replicates per treatment). The settling behaviour of the wasps was assessed during the initial three hours after their release. Direct treatment effects and any change in behaviour with respect to the control were assessed approximately 2, 24 and 48 hours. Reproduction (mummies/female) was assessed 11 days following a 24-hour parasitation period. Reproduction was assessed for the control group and each test item group, where the corrected mortality was below 50%, including the adjuvant treatment.

Endpoints : To determine the effects of Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (6 + 30 + 90) g/kg WG (SAP63H) + adjuvant (POTTOK) on the mortality and reproduction (parasitisation) of the aphid parasitoid *Aphidius rhopalosiphi* under extended laboratory conditions.

Test concentrations: Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (6 + 30 + 90) g/kg WG (SAP63H) + adjuvant (POTTOK):

0.108 kg of formualted product (FP)/ha + 0.072 L (adjuvant)/ha
0.180 kg FP/ha + 0.120 L/ha
0.300 kg FP/ha + 0.2 L/ha
0.500 kg FP/ha + 0.2 L/ha
0.833 kg FP/ha + 0.2 L/ha

Treatment with adjuvant alone in water, at 0.2 L/ha
Reference item: 0.0100 L FP/ha

Test conditions:

Temperature:	19.9 – 20.4° C
Relative humidity:	76.7 – 83.3 %
Light regime:	16 h light / 8 h darkness
Light intensity:	434 – 503 lux during mortality 3012 – 4674 lux during parasitation 5920 – 11502 lux during development of mummies

Statistics: Because no changes in mortality were observed among any treatment, no statistical analyses were performed to detect significant differences between mortality data of the test item groups and the control group. It was not possible to determine the LR50 by probit analysis since mortality data with the tested rates of the test item were less than 50%.
Repellency data met normality (Shapiro-Wilk’s Test) and homoscedasticity (Levene´s Test). The Dunnett’s multiple t-test was performed with repellency during 3.0 h in order to study possible significant differences compared to control (one-sided smaller, α=0.05, analysing the percentage of adults settling on the plants).
Reproduction data met normality (Shapiro-Wilk’s Test) and homoscedasticity (Levene´s Test). The Dunnett`s Multiple t-test was performed with number of offspring at 14 days (one-sided smaller, α=0.05) in order to study possible significant differences compared to control.

The adjuvant data (treatment "A" with adjuvant but without test item) was compared to the control water group in order to study any significant difference in reproduction between them. The parametric two-sample test (Student-t for homogeneous variances, one-sided smaller, α=0.05) was used since normality (Shapiro-Wilk's Test) and homoscedasticity (Levene´s Test) were met.
Reductions of reproduction with the tested rates of the test item + adjuvant were less than 50% and therefore, the ER50 was estimated according to the percentage of reduction relative to the control.

Dates of work: 26th March – 19 April 2019

Results

Treatment group(1)	Rates Test item [kg/ha] + Adjuvant [L/ha]	Mean mortality [%]	Corrected mortality [%]	Reproduction (2) [mummies/female]	Reduction in reproduction rate [%]
Control	(Deionised water)	0.00	---	67.93	---
Adjuvant (POTTOK)	0 + 0.200	0.00	0.00	64.50	5.05
SAP63H+ adjuvant (POTTOK)	0.108 + 0.072	0.00	0.00	61.80	9.03
	0.180 + 0.120	0.00	0.00	47.36 *	30.29
	0.300 + 0.200	0.00	0.00	67.27	0.98
	0.500 + 0.200	0.00	0.00	60.29	11.26
	0.833 + 0.200	0.00	0.00	45.33 *	33.27

(1): Test item (SAP63H): Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (6 + 30 + 90) g/kg WG
(2): *: Statistically significant decreased compared to the control (Dunnett's Multiple t-Test, one-sided smaller, α=0.05)

The reference item caused a corrected mortality of 73.33 %.

	Endpoints, [kg test item/ha + L adjuvant/ha]
LR_{50}	n.d.; [> 0.833 + 0.200]
ER_{50}	n.d.; [> 0.833 + 0.200]
NOER (mortality)	≥ 0.833 + 0.200
NOER (reproduction)	= 0.500 + 0.200
NOER (repellency)	≥ 0.833 + 0.200

n.d.: not determined as corrected mortality and reduction on reproduction were below 50% up to 0.833 g formulated product /ha (relative to the control)

Conclusion
Mortality less than 10 % (0.0 %) and acceptable reproductive capacity (67.93 mummies per female) were observed during the 48-hour exposure period and subsequent fecundity assessment in the control group, applied with deionised water. The toxic reference item caused 73.33 % corrected mortality and confirmed the sensitivity of the test species and the test conditions. The treatment with only adjuvant did not cause mortality (0.0 %) and did not show significant differences in reproduction when compared to the control group (Student-t test, one side smaller, α=0.05); 5.05 % reduction relative to the control.
Under these extended laboratory test conditions, LR50 was estimated to be greater than the maximum tested rate of 0.833 kg test item/ha + 0.2 L adjuvant/ha. ER50 based on the reproductive capacity (esti-

mated application rate at which the fecundity is reduced by 50 % as compared to the control) was estimated to be greater than the tested rate of 0.833 kg test item/ha + 0.2 L adjuvant/ha.
The NOER values for lethal effects (48h mortality) and repellency were estimated to be equal to or higher than 0.833 kg test item/ha+ 0.2 L adjuvant/ha. The NOER for sublethal effects (fecundity) was determined to be equal to the tested rate of 0.500 kg test item/ha + 0.2 L adjuvant/ha.

A 2.3.2.2 KCP 10.3.2.1 Extended laboratory tests

Comments of zRMS:	The study is considered valid. All validity criteria were met. • Mortality in the control group: ≤ 10 % (48 hours), (observed: 3.3.% in water and Actirob B control) • Reproduction in the control group: ≥ 5 mummies per female*) (only, when a reproduction test was performed with surviving wasps of the test item group), (observed: 20.6 in the water control, and 21.3 in ActirobB control)) • Corrected mortality in the reference item group: > 50 % (48 hours) , (observed: 96.6%) **LR_{50} > 0.833 kg GLOB289H/ha + 1 L Actirob B/ha.** **$NOER_{mortality}$ = 0.833 kg GLOB289H/ha + 1 L Actirob B/ha.** **ER_{50} > 0.833 kg GLOB289H/ha + 1 L Actirob B/ha.** **$NOER_{reproduction}$ = 0.833 kg GLOB289H/ha + 1 L Actirob B/ha.**

Reference:	KCP10.3.2-04
Report	Effects of GLOB289H + Actirob B on the parasitic wasp *Aphidius rhopalosiphi* (DESTEFANI-PEREZ) in an extended laboratory test, Röhlig U., 2019, 19 48 NAE 0009
Guideline(s):	IOBC (MEAD-BRIGGS et al. 2009)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item: GLOB289H + Actirob B

GLOB289H
batch No.: R-BAA
analysed content of a.s.:
iodosulfuron-methyl-sodium 0.560 % w/w (nominal: 0.6 % w/w)
mesosulfuron-methyl 3.0001 % w/w (nominal: 3 % w/w)
mefenpyr-diethyl (safener) 8.804 % w/w (nominal: 9% w/w)

Actirob B
batch No.: LC20370511
nominal content of a.s.: 842 g/L rapeseed oil methyl ester

Reference Item: Dimethoate EC 400 (Dimethoate 429.0 g/L, nominal: 400 g/L)

Test species: Parasitic wasp Aphidius rhopalosiphi (DESTEFANI-PEREZ), adults (< 48 hours

old); source (in the stage of mummies):
"Katz Biotech AG", An der Birkenpfuhlheide 10, 15837 Baruth, Germany

Test design: Exposure of the adults was achieved via air-dried spray residues on treated, potted barley plants. Seven treatment groups (5 test item rates, water treated control, reference item) were set up with 6 replicates (consisting of 5 females) per treatment.
Mortality assessments were carried out 2, 24 and 48 hours after start of exposure of the wasps. At 48 hours, surviving wasps (15 females per treatment) were removed and their reproductive capacity was assessed by confining them individually over untreated wheat plants infested with adult and nymphal aphids (*Rhopalosiphum padi*). Assessment of reproduction capacity, i.e. number of mummies per female, was made for the water-treated control, the adjuvant-treated control and all test item treated groups (1 assessment, 14 days after application).

Endpoints : Mortality: number of dead wasps, including the determination of the LR_{50}.
Reproductive capacity: number of mummies per female, including the determination of the ER_{50}.

Test concentrations: Control (deionised water)
Adjuvant control (1L Actirob B/ha)
Test item (GLOB289H + Actirob B):
- 0.108 kg GLOB289H/ha + 1 L Actriob B/ha
- 0.180 kg GLOB289H/ha + 1 L Actirob B/ha
- 0.300 kg GLOB289H/ha + 1 L Actriob B/ha
- 0.500 kg GLOB289H/ha + 1 L Actirob B/ha
- 0.833 kg GLOB289H/ha + 1 L Actriob B/ha

Reference item (Dimethoate EC 400): 10 ml/ha
All substances were applied in 400 L/ha, sprayed on potted barley plants via laboratory spraying equipment and air dried afterwards.

Test conditions:

Temperature:	19 °C – 22 °C
Relative humidity:	66 – 74 %
Photoperiod:	16 hours light : 8 hours dark
Light intensity:	1150 lux (mortality phase) 5630 lux (parasitisation phase) 7480 lux (reproduction phase)
Food:	10 % w/w aqueous fructose solution

Statistics: Multiple sequentially-rejective FISHER test after BONFERRONI-HOLM ($\alpha = 0.05$) for mortality (test item)
FISHER'S exact binominal test ($\alpha = 0.05$) for mortality (adjuvant control and reference item)
DUNNETT'S t-test ($\alpha = 0.05$) for repellence and reproductive capacity (test item)
STUDENT-t-test ($\alpha = 0.05$) for repellence and reproductive capacity (adjuvant control)

Results and discussion

In the water-treated control as well as in the Actirob B adjuvant control a mortality of 3.3 % was observed. In the test item treatments mortality ranged between 0 % and 6.7 %. This resulted in corrected mortality rates between -3.4 % and 3.4 %. No statistically significant effects on mortality were determined in test item treatments up to and including 0.833 kg GLOB289H/ha + 1 L Actirob B/ha (Multiple Sequentially rejective FISHER test after BONFERRONI-HOLM, α = 0.05). The LR_{50} for GLOB289H + Actirob B was estimated to be > 0.833 kg GLOB289H/ha + 1 L Actirob B/ha. The NOER (no observed effect rate) for mortality was = 0.833 kg GLOB289H/ha + 1 L Actirob B/ha. No statistically significant effects on mortality were determined in the Actirob B adjuvant control compared to the water treated control. The mean number of mummies per female in the test item treatments was between 20.9 and 21.9, and 21.3 mummies per female in the Actirob B adjuvant control and 20.6 mummies per female in the water treated control. No statistically significant effects on reproductive capacity were determined in all test item treatments, up to and including 0.833 kg GLOB289H/ha + 1 L Actirob B/ha (DUNNETT'S -t-test, α = 0.05), as well as in the Actirob B adjuvant control compared to the water-treated control. The ER_{50} was estimated to be > 0.833 kg GLOB289H/ha + 1 L Actirob B/ha.
The NOER (no observed effect rate) for reproduction was = 0.833 kg GLOB289H/ha + 1 L Actirob B/ha.

The results are summarised below.

Effects on the parasitic wasp (*Aphidius rhopalosiphi*) exposed to GLOB289H + Actirob B in an extended laboratory test

Treatment	Rate [1] [product/ha]	Mortality [2] [%]	Corrected Mortality [3] [%]	Reproduction [4] [mean number of mummies/female]	Effects on reproduction [5] [%]
Water Control	-	3.3	-	20.6	-
Adjuvant Control	1 L	3.3	0	21.3	3.3
Test item	0.108 kg + 1 L	0 (n.s.)	-3.4	21.2 (n.s.)	-2.9
Test item	0.180 kg + 1 L	6.7 (n.s.)	3.4	20.9 (n.s.)	-1.5
Test item	0.300 kg + 1 L	3.3 (n.s.)	0	21.9 (n.s.)	-6.3
Test item	0.500 kg + 1 L	6.7 (n.s.)	3.4	21.0 (n.s.)	-1.9
Test item	0.833 kg + 1 L	6.7 (n.s.)	3.4	20.9 (n.s.)	-1.5
	Endpoint [kg GLOB289 H/ha + L Actirob B/ha]				
LR_{50}	> 0.833 kg GLOB289H/ha + 1 L Actirob B/ha.				
ER_{50}	> 0.833 kg GLOB289H/ha + 1 L Actirob B/ha.				

Test item: GLOB289H + Actirob B
[1] Application rate in 400 L/ha
[2] Mortality after 48 hours of exposure to the test item on treated barley plants. The results for mortality in individual treatments were compared to that in the water-treated control using Multiple Sequentially-rejective FISHER test after BONFERRONI-HOLM (α = 0.05) for test item and FISHER-t-Test (α = 0.05) for adjuvant control
[3] Corrected mortality according to ABOTT (1925)
[4] Reproduction: mean number of parasitised aphids (mummies)/surviving female. The results were compared to the water-treated control by DUNNETT'S-t-test (α = 0.05) for test item and STUDENT-t-Test for adjuvant control. Results for reproduction compared by DUNNETT'S Multiple Sequential t-test (α = 0.05)
[5] Change in mean number of eggs per female, relative to the water-treated control. A negative value indicates an increase and a positive value indicates a decrease, relative to the control.
n.s. not statistically significant different compared to the control

No unusual observations were noted in the control and all test item groups at any observation point during the test. There were no statistically significant differences in the behaviour (wasps settled on the plants as

a criterion for repellence) in all test item groups compared to the water-treated control (DUNNETT'S -t-test, α = 0.05). as well as between the water-treated control and the adjuvant-treated control. (STUDENT -t-test, α = 0.05).
The reference item caused a mortality of 96.7 % of exposed wasps, resulting in a corrected mortality of 96.6 %.

Conclusion
In an extended laboratory study with GLOB289H + Actirob B the LR50 for *Aphidius rhopalosiphi* was estimated to be > 0.833 kg GLOB289H/ha + 1 L Actirob B/ha. The NOER (no observed effect rate) for mortality was = 0.833 kg GLOB289H/ha + 1 L Actirob B/ha.
The ER_{50} was estimated to be > 0.833 kg GLOB289H/ha + 1 L Actirob B/ha. The NOER (no observed effect rate) for reproduction was = 0.833 kg GLOB289H/ha + 1 L Actirob B/ha.

Comments of zRMS**:**	The study is considered valid. All validity criteria were met according to the IOBC Guideline by VOGT et al. (2000) for conducting the laboratory test with Chrysoperla carnea. • Mortality in the control: ≤ 20 % (observed: 7.55) • Mortality in the reference item group: ≥ 50 % (observed: 65%) • Fecundity in the control (mean number of eggs/female and day): ≥ 15 (observed:19.9) • Fertility in the control (mean hatching rate): ≥ 70 % (observed: 74.5%) **Agreed endpoints:** **LR_{50} > 0.833 kg GLOB289H/ha + 1 L Actirob B/ha** **$NOER_{mortality}$= 0.833 kg GLOB289H/ha + 1 L Actirob B/ha** **$NOER_{reproduction}$≥0.833 kg GLOB289H/ha + 1 L Actirob B/ha**

Reference:	KCP10.3.2-05
Report	Effects of GLOB289H + Actirob B on the green lacewing *Chrysoperla carnea* STEPH. in an extended laboratory test, Röhlig U., 2019, 19 48 NCE 0010
Guideline(s):	IOBC (VOGT et al. 2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item: GLOB289H + Actirob B

<u>GLOB289H</u>
batch No.: R-BAA
analysed content of a.s.:
iodosulfuron-methyl-sodium 0.560 % w/w (nominal: 0.6 % w/w)
mesosulfuron-methyl 3.0001 % w/w (nominal: 3 % w/w)
mefenpyr-diethyl (safener) 8.804 % w/w (nominal: 9% w/w)

<u>Actirob B</u>
batch No.: LC20370511
nominal content of a.s.: 842 g/L rapeseed oil methyl ester

Reference Item: Dimethoate EC 400 (Dimethoate 429.0 g/L, nominal: 400 g/L)

Test species: Green lacewing *Chrysoperla carnea* STEPH., larvae (2-3 days old)
source: reared in the laboratory of the test facility

Test design: Exposure of the larvae was achieved via air-dried spray residues on treated bean leaves. Seven treatment groups (5 test item rates, water treated control, reference item) were set up with 40 replicates (consisting of one larva per replicate) per treatment. Exposure lasted until pupae were transferred to oviposition units for development of adults. Mortality assessments were carried out regularly until hatching of the adult lacewings. In addition, for the water-treated control, the adjuvant-treated control and all test item the reproductive performance, i.e. egg deposition and hatching rate, was determined (2 assessments/week, 24 h period each).

Endpoints : Mortality including the estimation of a LR_{50} (Lethal Rate 50 %, rate resulting in 50 % mortality),
Reproductive performance: number of produced eggs per female per day and hatching rate

Test concentrations: Control (deionised water)
Adjuvant control (1L Actirob B/ha)
Test item (GLOB289H + Actirob B):
- 0.108 kg GLOB289H/ha + 1 L Actriob B/ha
- 0.180 kg GLOB289H/ha + 1 L Actirob B/ha
- 0.300 kg GLOB289H/ha + 1 L Actriob B/ha
- 0.500 kg GLOB289H/ha + 1 L Actirob B/ha
- 0.833 kg GLOB289H/ha + 1 L Actriob B/ha

Reference item (Dimethoate EC 400): 40 ml/ha
All substances were applied in 200 L/ha, sprayed on bean leaves via laboratory spraying equipment and air dried afterwards.

Test conditions:

Temperature:	23 °C – 27 °C
Relative humidity:	65 – 72 %
Photoperiod:	16 hours light : 8 hours dark
Light intensity:	1100 lx
Food:	larvae: *Sitotroga cerealella* eggs (UV-sterilised); adults: artificial diet

Statistics: Multiple sequentially-rejective FISHER test after BONFERRONI-HOLM ($\alpha = 0.05$) for mortality (test item)
FISHER'S exact binominal test ($\alpha = 0.05$) for mortality (adjuvant control and reference item)

Results and discussion

In the water-treated control a mortality of 7.5 % was observed. In the test item treatments mortality ranged between 2.5 % and 5.0 %. This resulted in corrected mortality rates between -2.7 % and -5.4 %. No statistically significant effects on mortality were determined in all test item treatment groups (Multiple Sequentially-rejective FISHER Test after BONFERRONI-HOLM, $\alpha = 0.05$), compared to the water-treated control. The LR_{50} for GLOB289H + Actirob B was estimated to be > 0.833 kg GLOB289H/ha + 1

L Actirob B/ha. The NOER (no observed effect rate) for mortality was = 0.833 kg GLOB289H/ha + 1 L Actirob B/ha.
No statistically significant effects on mortality were determined in the Actirob B adjuvant control compared to the water treated control.
No effects on reproduction of *Chrysoperla carnea* occurred, when the test item was applied at rates up to and including 0.833 kg GLOB289H/ha + 1 L Actirob B/ha. In the water-treated control, as well as in the adjuvant-treated control and all test item treatments the number of eggs per female per day was > 15 and the hatching rate was > 70 %.

The results are summarised below.

Effects on the green lacewing *Chrysoperla carnea* exposed to GLOB289H + Actirob B in an extended laboratory test

Treatment	Rate [1] [product/ha]	Mortality [2] [%]	Corrected mortality [3] [%]	Reproduction [eggs/female/ day]	Hatching rate [%]
Water Control	-	7.5	-	19.9	74.5
Adjuvant Control	1 L	2.5 (n.s.)	-5.4	19.8	74.4
Test item	0.108 kg + 1 L	5.0 (n.s.)	-2.7	18.8	74.3
Test item	0.180 kg + 1 L	5.0 (n.s.)	-2.7	19.4	74.8
Test item	0.300 kg + 1 L	2.5 (n.s.)	-5.4	18.7	74.4
Test item	0.500 kg + 1 L	2.5 (n.s.)	-5.4	19.4	74.7
Test item	0.833 kg + 1 L	5.0 (n.s.)	-2.7	19.3	74.8
	Endpoint [kg GLOB289 H/ha + L Actirob B/ha]				
LR_{50}	> 0.833 kg GLOB289H/ha + 1 L Actirob B/ha				

Test item: GLOB289H + Actirob B
1 Application rate in 200 L/ha
2 Mortality: percentage of individuals, which did not reach maturity
3 Corrected mortality according to ABOTT (1925)
n.s. not statistically significant different compared to the control; Multiple Sequentially-rejective FISHER Test after BONFERRONI-HOLM (α = 0.05) for test item or FISHER`s Exact Binomial test (α = 0.05) for adjuvant-treated control and reference item

No unusual observations regarding behaviour were noted in the control and the test item treatment groups at any observation point during the test.
The reference item caused a mortality of 65.0 % of exposed lacewings, resulting in a corrected mortality of 62.2 %.

Conclusion
In an extended laboratory study with GLOB289H + Actirob B the LR50 for *Chrysoperla carnea* was estimated to be > 0.833 kg GLOB289H/ha + 1 L Actirob B/ha. The NOER (no observed effect rate) for mortality was = 0.833 kg GLOB289H/ha + 1 L Actirob B/ha.
No unacceptable effects on reproduction of *Chrysoperla carnea* were observed, when the test item was applied at rates up to and including 0.833 kg GLOB289H/ha + 1 L Actirob B/ha.

Comments of zRMS:	The study is considered valid. All validity criteria were met. • Average number of hatched beetles per replicate of the F1-generation in

	the control: > 400 (observed: 518 for water control and for Actirob B adjuvant 525) • Reduction of the reproductive capacity in the reference item treatment relative to control: ≥ 50 % (observed: reduction of reproductive performance was 87.1%) **Agreed endpoints:** **ER_{50} > 0.833 kg GLOB289H/ha + 1 L Actirob B in 400 L/ha.** **NOER $_{reproduction}$ = 0.500 kg GLOB289H/ha + 1 L Actirob B/ha.**

Reference: KCP10.3.2-06

Report: Effects of GLOB289H + Actirob B on the rove beetle *Aleochara bilineata* GYLL. in an extended laboratory test, Röhlig U., 2019, 19 48 NKE 0006

Guideline(s): IOBC (GRIMM et al. 2000)

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

Test item: GLOB289H + Actirob B

<u>GLOB289H</u>
batch No.: R-BAA
analysed content of a.s.:
iodosulfuron-methyl-sodium 0.560 % w/w (nominal: 0.6 % w/w)
mesosulfuron-methyl 3.0001 % w/w (nominal: 3 % w/w)
mefenpyr-diethyl (safener) 8.804 % w/w (nominal: 9% w/w)

<u>Actirob B</u>
batch No.: LC20370511
nominal content of a.s.: 842 g/L rapeseed oil methyl ester

Reference Item: Dimethoate EC 400 (Dimethoate 429.0 g/L, nominal: 400 g/L)

Test species: Rove beetle *Aleochara bilineata* GYLL., adults (1-7 days old); source: reared in the laboratory of the test facility

Test design: The test item rates, controls and reference item were sprayed via a laboratory spray applicator (tracksprayer) on the soil surface. Exposure of the beetles was reached via air-dried residues on
treated sandy soil (LUFA 2.1).
Eight treatment groups (5 test item rates, water-treated control, adjuvant control and reference item) were set up with 4 replicates (consisting of 10 females and 10 males (10 pairs) per treatment. On day 7, 14 and 21 approx. 500 pupae of Delia antiqua were buried in the sandy soil (LUFA 2.1) of each replicate to be parasitised by the larvae of the beetles. On day 28 the adults were separated from the soil and the sandy soil with the pupae was allowed to dry for seven days. On day 35 the pupae were removed from the soil by a sieve and transferred into a hatching unit. After hatching, the test endpoint reproductive capacity (average number

of hatched beetles of the F_1 generation) was determined (daily assessments during 5 weeks).

Endpoints : Reproductive capacity (average number of hatched beetles of the F_1 generation)

Test concentrations: Control (deionised water)
Adjuvant control (1L Actirob B/ha)
Test item (GLOB289H + Actirob B):

- 0.108 kg GLOB289H/ha + 1 L Actriob B/ha
- 0.180 kg GLOB289H/ha + 1 L Actirob B/ha
- 0.300 kg GLOB289H/ha + 1 L Actriob B/ha
- 0.500 kg GLOB289H/ha + 1 L Actirob B/ha
- 0.833 kg GLOB289H/ha + 1 L Actriob B/ha

Reference item (Dimethoate EC 400): 1.5 L/ha
All substances were applied in 400 L/ha, sprayed onto sandy soil via laboratory spraying equipment and air dried afterwards.

Test conditions:	Temperature:	19 °C – 22 °C
	Relative humidity:	66 – 74 %
	Photoperiod:	16 hours light : 8 hours dark
	Light intensity:	1830 lx
	Food:	*Chironimus* spp. larvae (thawed)

Statistics: WILLIAMS-t-test (α = 0.05) for reproductive capacity (test item)
STUDENT-t-test (α = 0.05) for reproductive capacity (adjuvant and reference item)

Results and discussion

In the water-treated control the average number of hatched beetles of the F1 generation was 518 and in the Actirob B adjuvant control the average number of hatched beetles of the F1 generation was 525. In the test item treatments reproductive capacity ranged between 496 and 520 hatched beetles. This resulted in effects on reproduction between 4.2 % and -0.3 % inhibition. Therefore, the ER_{50} for was estimated to be > 0.833 kg GLOB289H/ha + 1 L Actirob B. No statistically significant differences compared to the control were observed at rates up to and including 0.500 kg GLOB289H + Actirob B (WILLIAMS-t-test, α = 0.05).
The result of the 0.833 kg GLOB289H/ha + 1 L Actirob B/ha treatment group was statistically significant compared to the water treated control, but the result of 496 average number of hatched beetles of the F_1 generation, was above the validity criterion, given for the control at the IOBC guideline GRIMM et al 2000.

The results are summarised below.

Effects on reproductive capacity of the rove beetle (*Aleochara bilineata* GYLL.) exposed to GLOB289H + Actirob B in an extended laboratory test

Treatment	Rate[1] [product/ha]	Reproduction [mean number of emerged beetles per replicate]	Reproduction [absolute number of emerged beetles per treatment group]	Effect on Reproduction[2] [%]
Control	-	518	2072	-
Adjuvant Control	1 L	525 (n.s.)	2099	-1.3
Test item	0.108 kg + 1 L	500 (n.s.)	1998	3.6
Test item	0.180 kg + 1 L	520 (n.s.)	2079	-0.3
Test item	0.300 kg + 1 L	515 (n.s.)	2058	0.7
Test item	0.500 kg + 1 L	520 (n.s.)	2078	-0.3
Test item	0.833 kg + 1 L	496*	1984	4.2
Reference item	1.5 L product/ha	67*	267	87.1

Test item: GLOB289H + Actirob B
[1] Application rate in 400 L/ha
[2] Effect on reproduction according to the following formula: (1-Pt/Pc)*100% calculated on the absolute number of emerged beetles (positive values represent a decreased and negative values indicates an increased reproduction compared to the control).
n.s. not statistically significant different compared to the control; WILLIAMS-t-test ($\alpha = 0.05$)
* significantly difference compared to the control: WILLIAMS-t-test ($\alpha = 0.05$) (test item)
* statistically significantly different compared to the control: STUDENT-t-test ($\alpha = 0.05$) (adjuvant control and reference item)

No unusual observations regarding behaviour were noted in the control and the test item treatment groups at any observation point during the test.

Conclusion
In an extended laboratory study with GLOB289H + Actirob B the ER_{50} for Aleochara bilineata was estimated to be > 0.833 kg GLOB289H/ha + 1 L Actirob B in 400 L/ha. The NOER (no observed effect rate) for reproductive capacity was = 0.500 kg GLOB289H/ha + 1 L Actirob B/ha.

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. • Adult mortality: ≤ 10 % (observed: 1.3 % after 4 weeks) • Number of juveniles per replicate: ≥ 30 (observed: 192, 208, 183, 223, 237, 161, 134 and 147) for replicate 1, 2, 3, 4, 5, 6, 7 and 8, respectively) • Coefficient of variation of reproduction: ≤ 30 % (observed:19.7 %)

	Agreed endpoints: **$NOEC_{mortality, biomass and reproduction}$ =325 mg test item/kg soil dry weight, i LC50 and EC10, EC20 and EC50 > 325 mg test item/kg soil dry weight.**

Reference: KCP 10.4-01

Report: Effects of Iodosulfuron + Mesosulfuron (0.6+3)% WG on the reproduction of the earthworm *Eisenia andrei* in artificial soil, Friedrich S, 2018, 17 48 TEC 0043.

Guideline(s): Yes; OECD 222 (2016)

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

Test item: Iodosulfuron + Mesosulfuron (0.6+3) % WG (GLOB289H)
Batch: R-BAA
iodosulfuron 6 g/kg (nominal), 5.6 g/kg (analysed)
mesosulfuron 30 g/kg (nominal), 29.2 g/kg (analysed)

Reference item: Maypon Flow (Carbendazim, SC 500)

Test species: *Eisenia andrei* (Bouché, 1972)

Test design: Sublethal toxicity earthworm: 56 days;
8 test item treatment groups and an untreated control group,
8 replicates in the control group and 4 replicates in the test item treatment groups, 10 worms per replicate;
Exposure of worms to different concentrations of the test item mixed into the substrate (artificial soil with 10% peat);
assessments of adult mortality, behavioural effects and biomass development after 28 days, and reproduction rate after an additional 28 days (assessed 56 days after application)

Endpoints : Mortality and biomass after 28 days, reproduction after 56 days

Treatments: Control (untreated), test item (Iodosulfuron + Mesosulfuron (0.6+3)% WG)

Test concentrations: 5.3, 9.6, 17.2, 31.0, 55.7, 100, 181, 325 mg test item/kg soil dry weight (spacing factor: 1.8)

Test conditions:

Temperature:	19.0°C – 20.7°C
Light intensity:	650 lux
Photoperiod:	light : dark = 16h : 8h

Statistics: Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm for mortality (a=0.05, one-sided greater), Willems-t-test for biomass change and reproduction (a=0.05, one-sided smaller)
Statistical program: ToxRat Professional 3.2.1 (Ratte 2015)

Results and discussions

No statistically significant effects on mortality were observed at any of the concentrations tested compared to the control. The test item caused no statistically significant change in biomass (change in fresh weight after 4 weeks relative to initial fresh weight) and no statistically significant effects on reproduction compared to the control group at any concentration tested.

Sublethal effects of Iodosulfuron + Mesosulfuron (0.6+3) % WG on *Eisenia andrei* in a 56-day reproduction study

Endpoint	Treatment group (mg test item/kg soil d.w.)								
	Control	5.3	9.6	17.2	31.0	55.7	100	181	325
Mortality of adult worms after 4 weeks (%)	1.3	0.0	2.5	0.0	0.0	2.5	0.0	0.0	2.5
Mean biomass change after 4 weeks (%)	28.2	28.5	26.9	27.7	24.8	30.5	32.1	29.0	25.8
Mean number of juveniles after 8 weeks	185.6	174.5	191.8	206.3	201.3	176.5	176.0	182.5	172.3
Reduction of reproduction compared to control (%)	-	6.0	-3.3	-11.1	-8.4	4.9	5.2	1.7	7.2
	Endpoint (mg test item/kg soil d.w.)								
NOEC (mortality)	325								
NOEC (biomass)	325								
NOEC (reproduction)	325								
LC_{50} (mortality) [1]	> 325								
EC_{10} (reproduction) [1]	> 325								
EC_{20} (reproduction) [1]	> 325								
EC_{50} (reproduction) [1]	> 325								

Not statistically significantly different compared to the control for mortality (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, α = 0.05, one-sided greater) and for biomass and reproduction (Williams-t-test, α = 0.05, one-sided smaller)

Negative values = increase, relative to control

[1] based on estimation of the data

The validity criteria for the control group were met:

- Adult mortality: ≤ 10 % (being 1.3 % after 4 weeks)
- Number of juveniles per replicate: ≥ 30 (being 192, 208, 183, 223, 237, 161, 134 and 147) for replicate 1, 2, 3, 4, 5, 6, 7 and 8, respectively)
- Coefficient of variation of reproduction: ≤ 30 % (being 19.7 %)

Conclusion

In a 56-day earthworm reproduction study with Iodosulfuron + Mesosulfuron (0.6+3) % WG, no statistically significant effects on mortality, biomass and reproduction of the earthworm Eisenia andrei in artificial soil were determined up to and including 325 mg test item/kg soil dry weight.

The NOEC for mortality, biomass and reproduction was determined to be 325 mg test item/kg soil dry weight, i.e. the highest concentration tested. The LC50 and the EC10, EC20 and EC50 values for reproduction were estimated to be > 325 mg test item/kg soil dry weight.

Comments of zRMS:	The study is considered valid. All validity criteria were met. • Adult mortality: ≤ 10 % (observed: 0 % after 4 weeks) • Number of juveniles per replicate: ≥ 30 (observed:248 to 326) • Coefficient of variation of reproduction: ≤ 30 % (observed: 9.6 %) **Agreed endpoints:** **NOEC $_{mortality}$ and change of biomass > 325mg GLOB289H/kg soil dry weight.** **NOEC $_{reproduction}$ =100 mg GLOB289H/kg soil dry weight.** **EC_{10}, EC_{20} and $EC_{50\ reproduction}$ > 325 mg GLOB289H/kg soil dry weight**

Reference: KCP 10.4-02

Report: Effects of GLOB289H + Actirob B on the reproduction of the earthworm *Eisenia andrei* in artificial soil, Friedrich S, 2019, 19 48 TEC 0063.

Guideline(s): Yes; OECD 222 (2016)

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

Test item: GLOB289H + Actirob B
The test item is a mixture of GLOB289H and the adjuvant Actirob B (mixing ratio 500 g GLOB289H : 1L Actirob B)

GLOB289H
Batch no.: R-BAA

Active ingredients/content:	nominal	analysed
Iodosulfuron-methyl-sodium	0.6% w/w	0.5604% w/w
Mesosulfuron-methyl	3% w/w	3.001% w/w
Mefenpyr-diethyl (safener)	9% w/w	8.804% w/w

Test concentrations: 55.7, 100, 181, 325 mg/kg soil dry weight

Actirob B
Batch no.: LC20370511

Active ingredient/content:	nominal
Rapeseed oil methyl ester	842 g/L

Test concentrations: 98.1, 177, 318, 572 mg/kg soil dry weight

Reference item: Maypon Flow (Carbendazim, SC 500)

Test species: *Eisenia andrei* (Bouché, 1972)

Test design: Sublethal toxicity earthworm: 56 days;
4 test item treatment groups and an untreated control group,
8 replicates in the control group and 4 replicates in the test item treatment groups, 10 worms per replicate;
Exposure of worms to different concentrations of the test item mixed into the substrate (artificial soil with 10% peat);
assessments of adult mortality, behavioural effects and biomass development

after 28 days, and reproduction rate after an additional 28 days (assessed 56 days after application)

Treatments: Control (untreated), test item (GLOB289H + Actirob B)

Test conditions: Temperature: 19.9°C – 21.8°C
Light intensity: 640 lux
Photoperiod: light : dark = 16h : 8h

Dates of work: 30 Aug – 25 Oct 2019

Statistics: Williams-t-test for biomass change and reproduction (α = 0.05, one-sided smaller), 3-parametric normal cumulative distribution function (CDF) for calculation of ECx; Statistical program: ToxRat Professional 3.2.1 (Ratte 2015)

Results and discussions

The test item caused no adverse effect on survival of the adult earthworms and no statistically significant change in biomass (change in fresh weight after 4 weeks relative to initial fresh weight) compared to the control group at any concentration tested (Williams-t-test, α = 0.05, one-sided smaller). Statistically significant effects (Williams-t-test, α = 0.05, one-sided smaller) on the number of juveniles compared to the control group were recorded at a concentration of 34.3 mg test item/kg soil d.w.

Effects of GLOB289H + Actirob B on *Eisenia andrei* in a 56-day reproduction study

Endpoint	Treatment group (mg test item/kg soil d.w.)#				
	Control	55.7	100	181	325
Mortality of adult worms after 4 weeks (%)	0.0	0.0	0.0	0.0	0.0
Mean biomass change after 4 weeks (%)	30.2	31.0	29.5	32.1	27.6
Mean number of juveniles after 8 weeks	291.1	295.8	273.3	229.5*	217.3*
Change of reproduction compared to control (%)	-	-1.6	6.1	21.2	25.4
	Endpoint (mg test item/kg soil d.w.)#				
NOEC (mortality)	325				
NOEC (biomass)	325				
NOEC (reproduction)	100				
LC_{50} (mortality) [1]	> 325				
EC_{10} (reproduction) [2]	115 (95 % confidence limits 65.8– 165)				
EC_{20} (reproduction) [2]	217 (95 % confidence limits 166– 273)				
EC_{50} (reproduction) [2]	> 325				

Not statistically significantly different compared to the control for biomass (Williams-t-test, α = 0.05, one-sided smaller)
* statistically significantly different compared to control regarding reproduction (Williams-t-test, α = 0.05, one-sided smaller)
Negative values = increase, relative to control
[1] based on estimation of the data
[2] GLOB289H: based on 3-parametric normal CDF
Concentrations related to GLOB289H only (mixing ratio 500 g GLOB289H : 1 L Actirob B)

The validity criteria for the control group were met:
- Adult mortality: ≤ 10 % (being 0 % after 4 weeks)
- Number of juveniles per replicate: ≥ 30 (being 248 to 326)

- Coefficient of variation of reproduction: ≤ 30 % (being 9.6 %)

Conclusion

In a 56-day earthworm reproduction study with GLOB289H + Actirob B, no adverse effect on survival of the adult earthworms and no statistically significant effects on biomass of the earthworm Eisenia andrei in artificial soil were determined up to and including 325mg GLOB289H/kg soil dry weight, i.e. the highest concentration tested.

The NOEC for mortality and change of biomass was determined to be > 325mg GLOB289H/kg soil dry weight. The NOEC for reproduction was determined to be 100 mg GLOB289H/kg soil dry weight. The EC10, EC20 and EC50 values for reproduction were calculated to be 115, 217 and > 325 mg GLOB289H/kg soil dry weight, respectively.

Comments of zRMS:	The study is considered valid. All validity criteria were met. • Adult mortality: ≤ 10 % (observed: 0 % after 4 weeks) • Number of juveniles per replicate: ≥ 30 (observed:248 to 326) • Coefficient of variation of reproduction: ≤ 30 % (observed: 9.6 %) **Agreed endpoints:** **NOEC $_{mortality ,change of biomass}$ > 325 mg test item/kg soil dry weight.** **NOEC $_{reproduction}$ =100 mg test item/kg soil dry weight.** **EC_{10}, EC_{20} and EC_{50} $_{reproduction}$ =142, 225 and > 325 mg test item/kg soil dry weight, respectively.**

Reference: KCP 10.4-03

Report: Effects of GLOB289H + Pottok on the reproduction of the earthworm *Eisenia andrei* in artificial soil, Friedrich S, 2019, 19 48 TEC 0064.

Guideline(s): Yes; OECD 222 (2016)

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

Test item: GLOB289H + Pottok

The test item is a mixture of GLOB289H and the adjuvant Pottok (mixing ratio 500 g GLOB289H : 0.2 L Actirob B)

<u>GLOB289H</u>

Batch no.: R-BAA

Active ingredients/content:	<u>nominal</u>	<u>analysed</u>
Iodosulfuron-methyl-sodium	0.6% w/w	0.5604% w/w
Mesosulfuron-methyl	3% w/w	3.001% w/w
Mefenpyr-diethyl (safener)	9% w/w	8.804% w/w

Test concentrations: 55.7, 100, 181, 325 mg/kg soil dry weight

<u>Pottok</u>

Batch no.: BH-MAA

Test concentrations: 22.6, 40.6, 73.2, 132 mg/kg soil dry weight

Reference item:	Maypon Flow (Carbendazim, SC 500). The effects of the reference item were investigated in a separate study.
Test species:	*Eisenia andrei* (Bouché, 1972)
Test design:	Sublethal toxicity earthworm: 56 days; 4 test item treatment groups and an untreated control group, 8 replicates in the control group and 4 replicates in the test item treatment groups, 10 worms per replicate; Exposure of worms to different concentrations of the test item mixed into the substrate (artificial soil with 10% peat); assessments of adult mortality, behavioural effects and biomass development after 28 days, and reproduction rate after an additional 28 days (assessed 56 days after application)
Treatments:	Control (untreated), test item (GLOB289H + Pottok)
Test conditions:	Temperature: 19.9°C – 21.8°C Light intensity: 640 lux Photoperiod: light : dark = 16h : 8h
Dates of work:	30 Aug – 25 Oct 2019
Statistics:	Williams-t-test for biomass change and reproduction (α = 0.05, one-sided smaller), 3-parametric normal cumulative distribution function (CDF) for calculation of ECx; Statistical program: ToxRat Professional 3.2.1 (Ratte 2015)

Results and discussions

The test item caused no adverse effect on survival of the adult earthworms and no statistically significant change in biomass (change in fresh weight after 4 weeks relative to initial fresh weight) compared to the control group at any concentration tested (Williams-t-test, α = 0.05, one-sided smaller). Statistically significant effects (Williams-t-test, α = 0.05, one-sided smaller) on the number of juveniles compared to the control group were recorded at a concentration of 34.3 mg test item/kg soil d.w.

Effects of GLOB289H + Pottok on *Eisenia andrei* in a 56-day reproduction study

Endpoint	Treatment group (mg test item/kg soil d.w.)#				
	Control	55.7	100	181	325
Mortality of adult worms after 4 weeks (%)	0.0	0.0	0.0	0.0	0.0
Mean biomass change after 4 weeks (%)	30.2	31.8	30.7	29.3	32.1
Mean number of juveniles after 8 weeks	291.1	324.0	300.3	233.0*	218.0*
Change of reproduction compared to control (%)	-	-11.3	-3.1	20.0	25.1
	Endpoint (mg test item/kg soil d.w.)				
NOEC (mortality) [1]	325				
NOEC (biomass)	325				
NOEC (reproduction)	100				
LC_{50} (mortality) [1]	> 325				
EC_{10} (reproduction) [2]	142 (95 % confidence limits 94.6– 189)				
EC_{20} (reproduction) [2]	225 (95 % confidence limits 182– 267)				
EC_{50} (reproduction) [2]	> 325				

Not statistically significantly different compared to the control for biomass (Williams-t-test, α = 0.05, one-sided smaller)
* statistically significantly different compared to control regarding reproduction (Williams-t-test, α = 0.05, one-sided smaller)
Negative values = increase, relative to control
[1] based on estimation of the data
[2] GLOB289H: based on 3-parametric normal CDF
\# Concentrations related to GLOB289H only (mixing ratio 500 g GLOB289H : 0.2 L Pottok)

The validity criteria for the control group were met:
- Adult mortality: ≤ 10 % (being 0 % after 4 weeks)
- Number of juveniles per replicate: ≥ 30 (being 248 to 326)
- Coefficient of variation of reproduction: ≤ 30 % (being 9.6 %)

Conclusion

In a 56-day earthworm reproduction study with GLOB289H + Pottok, no adverse effect on survival of the adult earthworms and no statistically significant effects on biomass of the earthworm Eisenia andrei in artificial soil were determined up to and including 325 mg test item/kg soil dry weight, i.e. the highest concentration tested.

The NOEC for mortality and change of biomass was determined to be > 325 mg test item/kg soil dry weight. The NOEC for reproduction was determined to be 100 mg test item/kg soil dry weight. The EC10, EC20 and EC50 values for reproduction were calculated to be 142, 225 and > 325 mg test item/kg soil dry weight, respectively.

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

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

Comments of zRMS:	The study is considered valid. All validity criteria were met • Mean adult mortality: ≤ 20 %(observed: 1.3 %) • Mean number of juveniles per test vessel:≥ 100(observed: average of 1125/vessel) • Coefficient of variation for the mean number of juveniles:< 30 % (observed: 10.9 %) **Agreed endpoints:** **LC_{50} >325 mg test item/kg soil dry weight,** **$NOEC_{mortality}$ =100 mg test item/kg soil dry weight.** **$NOEC_{reproduction}$ =100 mg test item/kg soil dry weight.** **EC10, EC20 and EC50 reproduction = 129, 225 and > 325 mg test item/kg soil dry weight, respectively.**

Reference:	KCP 10.4-04
Report	Effects of Iodosulfuron + Mesosulfuron (0.6+3)% WG on the reproduction of the collembolan *Folsomia candida*, Friedrich S., 2018, 17 48 TCC 0043.
Guideline(s):	Yes; OECD 232 (2016)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item:	Iodosulfuron + Mesosulfuron (0.6+3) % WG; Batch: R-BAA Content of a.i.: iodosulfuron 6 g/kg (nominal), 5.6 g/kg (analysed) mesosulfuron 30 g/kg (nominal), 29.2 g/kg (analysed)
Reference item:	Boric acid (100% analysed)
Test species:	Collembola (*Folsomia candida*), age: 9 - 12 days; source: in-house culture
Test design:	Chronic toxicity *Folsomia candida*: 28 days; 8 test item treatment groups and an untreated control group, 8 replicates in the control group and 4 replicates in the test item treatment groups, each containing 10 collembolans (9-12 days old); Exposure of collembolans to different concentrations of the test item mixed into the substrate (artificial soil with 5 % peat); assessments of adult mortality and reproduction 28 days after application
Endpoints :	Mortality and reproduction after 28 days
Treatments:	Control (untreated), test item (Iodosulfuron + Mesosulfuron (0.6+3) % WG)

Test concentrations: 5.3, 9.6, 17.2, 31.0, 55.7, 100, 181, 325 mg test item/kg soil dry weight (spacing factor: 1.8)

Test conditions: Temperature: 19.7 °C – 20.9 °C
Light intensity: 620 lux
Photoperiod: light : dark = 16h : 8h

Statistics: Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm for mortality,
Williams-t-test for reprodduction (α = 0.05, one-sided),
Logit analysis for reproduction
Statistical program: ToxRat Professional 3.2.1 (2015)

Results and discussion

Statistically significant effects on parental mortality were recorded at concentrations of 181 and 325 mg test item/kg soil dry weight. Statistically significant effects on the number of juveniles compared to the control group were recorded at concentrations of 181 and 325 mg test item/kg soil dry weight. Results are summarised in Table 1.

Table 15: Chronic effects of Iodosulfuron + Mesosulfuron (0.6+3) % WG on *Folsomia candida* in a 28-day reproduction study

Endpoint	Treatment group [mg test item/kg soil dry weight]								
	Control	5.3	9.6	17.2	31.0	55.7	100	181	325
Parental mortality [%]	1.3	2.5	2.5	0.0	0.0	0.0	2.5	17.5*	22.5*
Mean No. of juveniles	1125	1140	1176	1090	1163	1168	1099	872*	817*
Reduction of reproduction [%] compared to control	-	-1	-4	3	-3	-4	2	23	27
	Endpoints [mg test item/kg soil dry weight]								
NOEC (mortality)	100								
NOEC (reproduction)	100								
LC_{50} (mortality) [1]	> 325								
EC_{10} (reproduction) [2]	129 (95 % confidence limits 78.6 to 211)								
EC_{20} (reproduction) [2]	225 (95 % confidence limits 172 to 296)								
EC_{50} (reproduction) [2]	> 325								

* statistically significant different compared to the control (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm for mortality, α = 0.05, one-sided greater; Williams-t-test for reproduction, α = 0.05, one-sided smaller)
Negative values = increase, relative to control
[1] based on estimation of the data, [2] based on Logit analysis

In a separate study (BioChem project No. 17 48 TCC 0042, dated 14 August 2017), the EC_{50} (reproduction) of the reference item boric acid was calculated to be 107 mg/kg soil dry weight. The results of the reference test demonstrate the sensitivity of the test system.

The validity criteria for the control group were met:
- Mean adult mortality: ≤ 20 % (observed: 1.3 %)
- Mean number of juveniles per test vessel: ≥ 100 (observed: average of 1125/vessel)

- Coefficient of variation for the mean number of juveniles:< 30 % (observed: 10.9 %)

Conclusion

In a 28-day Folsomia candida reproduction study, in which collembolans were exposed to Iodosulfuron + Mesosulfuron (0.6+3) % WG, the LC50 was estimated to be higher than 325 mg test item/kg soil dry weight, the highest tested concentration. The NOEC for mortality was determined to be 100 mg test item/kg soil dry weight.
The NOEC for reproduction was determined to be 100 mg test item/kg soil dry weight. The EC10, EC20 and EC50 values for reproduction were calculated to be 129, 225 and > 325 mg test item/kg soil dry weight, respectively.

Comments of zRMS:	The study is considered valid. All validity criteria were met. • Mean mortality of adult females: ≤ 20 % (observed: 2.5%) • Mean number of juveniles per replicate: ≥ 50 (observed: 245.6) • Coefficient of variation (mean number of juveniles per replicate): ≤ 30 % (observed: 17.2%) **Agreed endpoints:** **LC_{50} and the EC10, EC20 and EC50 >325 mg test item/kg soil dry weight** **NOEC $_{mortality, reproduction}$ =325 mg test item/kg soil dry weight**

Reference: KCP 10.4-05

Report: Effects of Iodosulfuron + Mesosulfuron (0.6+3)% WG on the reproduction of the predatory mite *Hypoaspis aculeifer*, Schulz L., 2018, report No 17 48 THC 0038.

Guideline(s): Yes, OECD 226

Deviations: No

GLP: Yes

Acceptability: Yes

Material and methods

Test item: Iodosulfuron + Mesosulfuron (0.6+3) % WG; Batch: R-BAA
Content of a.i.:
iodosulfuron 6 g/kg (nominal), 5.6 g/kg (analysed)
mesosulfuron 30 g/kg (nominal), 29.2 g/kg (analysed)

Reference item: Dimethoate (EC 400 g/L, nominal)

Test species: *Hypoaspis aculeifer* (CANESTRINI)
age: adult female mites with an age difference of 2 days
source: in-house culture

Test system: Exposure of female mites to different concentrations of the test item mixed into artificial soil substrate

Test design: The effects of the test item on mortality and reproduction of the soil mite spe-

	cies *Hypoaspis aculeifer* (CANESTRINI) were investigated in a chronic laboratory experiment over a time period of 14 days according to OECD 226. Each of the eight different test item concentrations were homogeneously mixed into artificial soil and filled into glass vessels. Subsequently, the soil mites were introduced on top of the soil and the vessels were covered. Four replicates were performed for the test item groups and eight replicates for the control group; each replicate consisted of ten female soil mites. The mites were fed with *Tyrophagus putrescentiae* (SCHRANK) at the beginning and every two to three days during the whole test period. For the main measured variable, the number of juveniles per test vessel and additionally the mortality of the adult female mites were determined. The reproductive output of the mites exposed to the test item was compared to that of the control in order to determine the no observed effect concentration (NOEC). Assessment of adult mortality and reproduction effects was carried out after 14 days.
Endpoints:	Mortality and number of juveniles
Test conditions:	Artificial soil according to OECD 226, pH 5.9 - 6.1 at test start, pH 5.8 – 5.9 at test end; water content at test start 48.17 – 49.63% of maximum water holding capacity (WHC) and 46.13 – 47.91% of maximum WHC at test end; temperature 20.0 – 21.1°C; photoperiod: 16 h light : 8 h dark; light intensity: 644 lx.
Test concentrations:	5.3, 9.6, 17.2, 31.0, 55.7, 100, 181, 325 mg test item/kg soil dry weight (spacing factor: 1.8)
Statistics:	Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm (α = 0.05, one-sided greater) for mortality; Dunnett-t-test (α = 0.05, one-sided smaller) for reproduction Statistical program: ToxRat Professional 3.2.1 (2015)

Results and discussion

All validity criteria for the study were met.

Mortality rates of 0.0 - 7.5 % were recorded in the test item treatment groups. In the control group the mortality rate was 2.5 %.
The observed mortality rates in the test item treatment groups compared to control were not statistically significant (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, α = 0.05, one-sided greater). Differences in the behaviour and the morphology of the mites between the control and the test item treatment groups could not be observed.
Fourteen days after introduction of the parental mites into the test vessels, the mean number of juveniles was 266.5, 250.0, 259.0, 257.3, 249.0, 244.3, 239.8 and 242.3 at concentrations of 5.3, 9.6, 17.2, 31.0, 55.7, 100, 181 and 325 mg test item/kg soil d.w., respectively. The mean reproduction in the control reached 245.6 juveniles. The test item showed no statistically significantly adverse effects on reproduction at all tested concentrations (Dunnett-t-test, α = 0.05, one-sided smaller).
In a separate study (BioChem project No. R 16 10 48 006 S, experimental start date: 13.01.2017), the EC50 (reproduction) of the reference item dimethoate (EC 400 g/L, nominal) was calculated to be 5.8 mg a.s./kg soil d.w. The results of the reference test demonstrate the sensitivity of the test system.

Table 1: Effects of Iodosulfuron + Mesosulfuron (0.6+3) % WG on *Hypoaspis aculeifer* mortality and reproduction (day 14)

Endpoint	Treatment group [mg test item/kg soil dry weight]								
	Control	**5.3**	**9.6**	**17.2**	**31.0**	**55.7**	**100**	**181**	**325**
Mean adult mortality [%]	2.5	2.5	2.5	7.5	0.0	2.5	5.0	2.5	2.5
Mean number of juveniles (day 14)	245.6	266.5	250.0	259.0	257.3	249.0	244.3	239.8	242.3
Coefficient of variation [%]	17.2	8.5	11.9	4.5	11.3	10.4	13.3	8.2	13.8
Reproduction in [%] of control (day 14)	**100**	**108**	**102**	**105**	**105**	**101**	**99**	**98**	**99**
	Endpoints [mg test item/kg soil dry weight]								
NOEC (mortality)	325								
NOEC (reproduction)	325								
LC_{50} (mortality)	> 325								
EC_{10} (reproduction)	> 325								
EC_{20} (reproduction)	> 325								
EC_{50} (reproduction)	> 325								

Not statistically significantly different compared to the control (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm for mortality, α = 0.05, one-sided greater and Dunnett-t-test for reproduction, α = 0.05, one-sided smaller)

Conclusion

In a 14-day Hypoaspis aculeifer reproduction study with Iodosulfuron + Mesosulfuron (0.6+3) % WG, the LC50 and the EC10, EC20 and EC50 values could not be calculated, but it can be concluded that these values are higher than 325 mg test item/kg soil dry weight. The NOEC for mortality and for reproduction was determined to be 325 mg test item/kg soil dry weight, the highest concentration tested.

A 2.4.2.1 KCP 10.4.2.1 Species level testing

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

Comments of zRMS:	The study is considered valid. All validity criteria were met. • The coefficients of variation in the control group of the nitrogen test were maximum 7.4 % and thus fulfilled the demanded range ≤ 15 %. **Agreed endpoints:** Effects on soil nitrogen transformation (measured as NO3-N-production) in the test item treatments of 0.53 and 5.3 mg test item/kg soil dry weight were below 25 % at the end of the 28-day incubation period (time interval 14-28). No adverse effects on soil nitrogen transformation are expected at concentr tions up to 5.3 mg test item/kg soil dry weight.

Reference:	KCP 10.5-01
Report	Effects of Iodosulfuron + Mesosulfuron (0.6+3)% WG on the activity of soil microflora (Nitrogen transformation test), Schulz L., 2018, 17 48 SMN 0050
Guideline(s):	Yes, OECD 216 (2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item:	Iodosulfuron + Mesosulfuron (0.6+3) % WG (GLOB289H) Batch no.: R-BAA
Reference item:	Dinoterb (purity: 98.0 % ± 0.5 analysed).
Test soil:	Biologically active agricultural soil: loamy sand (DIN 4220) / loam (USDA), pH 6.6, 1.45 % C_{org}, WHC: 37.39 g/100 g dry soil.
Test design:	The test was performed in accordance with the OECD guideline 216 (2000). Aim of the study was the determination of the nitrogen transformation (NO_3-nitrogen-production) in soil enriched with lucerne meal (concentration in soil 0.5 %) by comparison of nitrogen transformation in test item treated soil with a non-treated soil. Three replicates per treatment and concentration. NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined by using the Autoanalyzer (BRAN+LUEBBE). Sampling scheme: 0, 7, 14 and 28 days after treatment.
Endpoints :	Effects on NO_3-nitrogen-production after 28 days of exposure.
Test concentrations:	Control, 0.53 mg test item/kg soil dry weight and 5.3 mg test item/kg soil dry weight. Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3.
Test conditions:	Water content: approx. 45 % of its maximum water holding capacity; water content: 16.54 - 17.20 g/100 g dry soil; pH: 6.3 Soil samples were incubated at 19.7 - 20.9 °C, while stored in test vessels in the dark.
Statistics:	Calculation of mean values per treatment, standard deviations, coefficients of variation.

Results and discussions

No adverse effects of the test item on nitrogen transformation in soil could be observed at both test concentrations (0.53 mg/kg soil dry weight and 5.3 mg/kg soil dry weight) after 28 days (time interval 14-28). The results are summarised in the table below. As no significant effects were seen at 28 days, the test was terminated at this time.

Table 1: Effects on nitrogen transformation in soil after treatment with the test item

Time Interval (days)	Control			0.53 mg test item/kg soil dry weight				5.3 mg test item/kg soil dry weight			
	Nitrate-N[1]			Nitrate-N[1]			% difference to control	Nitrate-N[1]			% difference to control
0-7	4.00	±	0.73	4.65	±	0.65	+16.2	5.68	±	0.17	+41.9
7-14	2.05	±	0.65	2.08	±	0.92	+1.2	1.40	±	0.26	-31.6
14-28	1.40	±	0.20	1.21	±	0.11	-13.5	1.24	±	0.10	-11.4

The calculations were performed with unrounded values

1) Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation

In a separate study the reference item Dinoterb caused stimulations of nitrogen transformation of +35.4 %, +28.2 % and +126.8 % at 6.80 mg, 13.60 mg and 27.20 mg Dinoterb per kg soil dry weight, respectively, determined 28 days after application (time interval 14-28).

Conclusion

Effects on soil nitrogen transformation (measured as NO3-N-production) in the test item treatments of 0.53 and 5.3 mg test item/kg soil dry weight were below 25 % at the end of the 28-day incubation period (time interval 14-28). Thus, no adverse effects on soil nitrogen transformation are expected at concentr tions up to 5.3 mg test item/kg soil dry weight.

Comments of zRMS:	The study is considered valid. All validity criteria were met. • The coefficients of variation in the control group of the nitrogen test were maximum 2.6 % and thus fulfilled the demanded range ≤ 15 %. **Agreed endpoints:** The test item GLOB289H + Pottok (tested at 0.67 mg GLOB289H + 0.27 mg Pottok/kg soil dry weight and 3.35 mg GLOB289H + 1.335 mg Pottok/kg soil dry weight) caused no adverse effects (deviation from control <25 %, OECD 216) on soil nitrogen transformation (measured as NO3-N-production) at the end of the 28-day incubation period.

Reference: KCP 10.5-02

Report: Effects of Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (6+30+90) g/kg (GLOB289H) and the adjuvant (Pottok) on the activity of soil microflora (Nitrogen transformation test), Persdorf M., 2019, 19 48 SMN 0054

Guideline(s): Yes, OECD 216 (2000)

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

Test item: GLOB289H + Pottok

	GLOB289H Batch no.: R-BAA Iodosulfuron-methyl-sodium: 0,6% w/w (nominal); 0,5604% w/w (analysed) Mesosulfuron-methyl: 3% w/w (nominal); 3,001% w/w (analysed) Mefenpyr-diethyl: 9% w/w (nominal); 8,804% w/w (analysed) Pottok (adjuvant) Batch no.: BH-MAA	
Reference item:	Dinoterb (purity: 99.28 % (g/g) analysed). The reference item was tested in a separate study 18 46 SMO 0001) at concentrations of 6.80, 13.60 and 27.20 mg/kg soil dry weight.	
Test soil:	Biologically active agricultural soil: Silty-loamy sand (DIN 4220) / loam (USDA), pH 6.0, 1.48 % C_{org}, WHC: 37.37 g/100 g dry soil.	
Test design:	The test was performed in accordance with the OECD guideline 216 (2000). Aim of the study was the determination of the nitrogen transformation (NO_3-nitrogen-production) in soil enriched with lucerne meal (concentration in soil 0.5 %) by comparison of nitrogen transformation in test item treated soil with a non-treated soil. Three replicates per treatment and concentration. NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined by using the Autoanalyzer (BRAN+LUEBBE). Sampling scheme: 0, 7, 14 and 28 days after treatment.	
Endpoints :	Effects on NO_3-nitrogen-production after 28 days of exposure.	
Test concentrations:	Control, 0.67 mg GLOB289H + 0.27 mg Pottok/kg soil dry weight and 3.35 mg GLOB289H + 1.335 mg Pottok/kg soil dry weight. Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3.	
Test conditions:	Water content: approx. 45 % of its maximum water holding capacity; water content: 16.28 – 16.84 g/100 g dry soil; pH: 5.9 – 6.1 Soil samples were incubated at 19.6 - 20.7 °C, while stored in test vessels in the dark.	
Statistics:	Calculation of mean values per treatment, standard deviations, coefficients of variation.	
Dates of work:	23.09.2019 – 21.10.2019	

Results and discussions

No adverse effects of the test item on nitrogen transformation in soil could be observed at both test concentrations (0.67 mg GLOB289H + 0.27 mg Pottok/kg soil dry weight and 3.5 mg GLOB289H + 1.335 mg Pottok/kg soil dry weight) after 28 days (time interval 14-28). The results are summarised in the table below. As no significant effects were seen at 28 days, the test was terminated at this time.

Table 1: Effects on nitrogen transformation in soil after treatment with the test item

Time Interval (days)	Control	0.67 mg GLOB289H + 0.27 mg Pottok/ kg soil dry weight		3.35 mg GLOB289H + 1.335 mg Pottok/ kg soil dry weight	
	NO_3-N/day [mg/kg soil d.w.]	NO_3-N/day [mg/kg soil d.w.]	% difference to control [1)]	NO_3-N/day [mg/kg soil d.w.]	% difference to control [1)]
0-7	4.99	4.90	-1.7	4.44	-10.9
7-14	2.43	1.66	-31.7	2.82	+15.9
14-28	0.87	1.08	+24.1	1.05	+21.4

[1)] based on NO_3-N-production; - = inhibition; + = stimulation
The calculations were performed with unrounded values

In a separate study the reference item Dinoterb caused stimulations of nitrogen transformation of +62.7% and +120.9% at 13.60 and 27.20 mg Dinoter per kg soil dry weight, respectively, determined 28 days after application (time interval 14-28).

Conclusion

The test item GLOB289H + Pottok (tested at 0.67 mg GLOB289H + 0.27 mg Pottok/kg soil dry weight and 3.35 mg GLOB289H + 1.335 mg Pottok/kg soil dry weight) caused no adverse effects (deviation from control <25 %, OECD 216) on soil nitrogen transformation (measured as NO3-N-production) at the end of the 28-day incubation period.

Comments of zRMS:	The study is considered valid. All validity criteria were met. • The coefficients of variation in the control group of the nitrogen test were maximum 4.0% and thus fulfilled the demanded range ≤ 15 %. **Agreed endpoints:** The test item GLOB289H + Actirob B (tested at 0.53 mg GLOB289H + 1.55 mg Actirob B/kg soil dry weight and 5.3 mg GLOB289H + 15.55 mg Actirob B/kg soil dry weight) caused no adverse effects (deviation from control <25 %, OECD 216) on soil nitrogen transformation (measured as NO_3-N-production) at the end of the 56-day incubation period.

Reference: KCP 10.5-03

Report: Effects of GLOB289H + Actirob B on the activity of soil microflora (Nitrogen transformation test), Persdorf M., 2019, 19 48 SMN 0053

Guideline(s): Yes, OECD 216 (2000)

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

Test item: GLOB289H + Actirob

<u>GLOB289H</u>
Batch no.: R-BAA
Iodosulfuron-methyl-sodium: 0,6% w/w (nominal); 0,5604% w/w (analysed)
Mesosulfuron-methyl: 3% w/w (nominal); 3,001% w/w (analysed)

	Mefenpyr-diethyl:	9% w/w (nominal); 8,804% w/w (analysed)
	Actirob (adjuvant) Batch no.: LC20370511	
	Rapeseed oil methyl ester	8742 g/L (nominal)

Reference item: Dinoterb (purity: 99.28 % (g/g) analysed). The reference item was tested in a separate study 19 46 SMO 0001) at concentrations of 6.80, 13.60 and 27.20 mg/kg soil dry weight.

Test soil: Biologically active agricultural soil:
Silty-loamy sand (DIN 4220) / loam (USDA), pH 6.0, 1.48 % C_{org},
WHC: 37.37 g/100 g dry soil.

Test design: The test was performed in accordance with the OECD guideline 216 (2000).
Aim of the study was the determination of the nitrogen transformation (NO_3-nitrogen-production) in soil enriched with lucerne meal (concentration in soil 0.5 %) by comparison of nitrogen transformation in test item treated soil with a non-treated soil.
Three replicates per treatment and concentration. NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined by using the Autoanalyzer (BRAN+LUEBBE). Sampling scheme: 0, 7, 14 and 28 days after treatment.

Endpoints : Effects on NO_3-nitrogen-production after 56 days of exposure.

Test concentrations: Control, 0.53 mg GLOB289H + 1.55 mg Actirob B/kg soil dry weight and 5.3 mg GLOB289H + 15.5 mg Actirob B/kg soil dry weight. Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3.

Test conditions: Water content: approx. 45 % of its maximum water holding capacity;
water content: 16.12 – 16.82 g/100 g dry soil; pH: 5.9 – 6.1
Soil samples were incubated at 19.1 - 20.7 °C, while stored in test vessels in the dark.

Statistics: Calculation of mean values per treatment, standard deviations, coefficients of variation.

Dates of work: 23.09.2019 – 18.11.2019

Results and discussions

No adverse effects of the test item on nitrogen transformation in soil could be observed at both test concentrations (0.53 mg GLOB289H + 1.55 mg Actirob B/kg soil dry weight and 5.3 mg GLOB289H + 15.55 mg Actirob B /kg soil dry weight) after 56 days (time interval 42-56). The results are summarised in the table below.

Table 16: Effects on nitrogen transformation in soil after treatment with the test item

Time Interval (days)	Control	0.53 mg GLOB289H + 1.55 mg Actirob B/ kg soil dry weight	5.3 mg GLOB289H + 15.55 mg Actirob B/ kg soil dry weight

	NO_3-N/day [mg/kg soil d.w.]	NO_3-N/day [mg/kg soil d.w.]	% difference to control [1)]	NO_3-N/day [mg/kg soil d.w.]	% difference to control [1)]
0-7	4.99	5.61	+12.6	5.75	+15.3
7-14	2.43	1.89	-22.3	2.48	+1.8
14-28	0.87	1.06	+21.9	0.61	-30.1
28-42	-0.33	-0.15	+52.9	-0.46	-40.6
42-56	1.92	1.97	+2.7	1.86	-3.0

[1)] based on NO_3-N-production; - = inhibition; + = stimulation
The calculations were performed with unrounded values

In a separate study the reference item Dinoterb caused stimulations of nitrogen transformation of +62.7 % and +120.9 % at 13.60 and 27.20 mg Dinoterb per kg soil dry weight, respectively, determined 28 days after application (time interval 14-28).

Conclusion
The test item GLOB289H + Actirob B (tested at 0.53 mg GLOB289H + 1.55 mg Actirob B/kg soil dry weight and 5.3 mg GLOB289H + 15.55 mg Actirob B/kg soil dry weight) caused no adverse effects (deviation from control <25 %, OECD 216) on soil nitrogen transformation (measured as NO_3-N-production) at the end of the 56-day incubation period.

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

No new studies were submitted.

A 2.6.2 KCP 10.6.2 Testing on non-target plants

A 2.6.2.1 Vegetative vigour test

Comments of zRMS:

The study is considered valid. All validity criteria were met.

1. Percentage germination of each seed lot used must be >70%.

2. Untreated control plants must not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformation). Untreated control plants must only exhibit normal variation in growth and morphology for that particular species.

3. The mean survival of untreated control plants is at least 90 % for the duration of the study.

4. Environmental conditions for a particular species are identical and the growing media contain the same amount of soil matrix, support media or substrate from the same source.

<u>In the current study in the control the following parametres were noted:</u>

Germination rate

Crop	**Cultivar**	**Certified Seed Lot Number**	**Germination Rate**
Corn	LG30179	F0964X029675NZ	100%
Oats	Firth	6/2L/0175/6685	95.92%
Onion	Hyfort	E43545	97.96%
Cucumber	Carmen	L38838	100%
Oilseed rape	Vision	Not Received	90.82%
Radish	Cherry Belle	5037887603	100%
Soybean	Siverka	5/BR/7087/0510	98.98%
Sugar beet	Darnella 629	Not Received	98.98%
Sunflower	Valentine	L/1038991	94.90%
Tomato	Shirley	P9414-1-1	98.98%

Mean survival: 100%
No visiable toxic effects were noted in the control plants
Enviromental condition: thhe same as for the treated plants

It should be noted that according to OECD 227 GD the following number of seeds is recommended per 15 cm container :
- **one to two corn,** soybean, tomato, cucumber, or sugar beet plants;
- **three rape** or pea plants per 15 cm container;
- **5 to 10** onion, wheat, or other small seeds.

In the study number of seeds was higher for among of species except onion and wheat.

Agreed endpoints:

ER_{10}, ER_{25} and ER_{50} values (with corresponding R-Sq values) and NOEC levels based on shoot fresh weight reduction.

Species	ER_{10} (g product/ha)	ER_{25} (g product/ha)	ER_{50} (g product/ha)	NOEC (g product/ha)	R-Sq
Corn	>70.000	>70.000	>70.000	70.000	N/A
Oats	>70.000	>70.000	>70.000	70.000	N/A
Onion	68.117	>70.000	>70.000	70.000	0.009
Cucumber	44.258	>70.000	>70.000	70.000	0.006
Oilseed rape	28.390	>70.000	>70.000	70.000	0.065
Radish	3.047	8.250	43.393	8.381	0.64
Soybean	<1.706	>70.000	>70.000	41.176	0.06
Sugar beet	31.470	>70.000	>70.000	70.000	0.0045
Sunflower	18.631	61.441	>70.000	41.176	0.34
Tomato	7.007	>70.000	>70.000	24.221	0.253

N/A = not appropriate owing to tolerance of these species to GLOB289H

It should be noted that according to OECD 227 GD the following number of seeds is recommended per 15 cm container :

- **one to two corn,** soybean, tomato, cucumber, or sugar beet plants;
- **three rape** or pea plants per 15 cm container;
- **5 to 10** onion, wheat, or other small seeds**.**

In the study number of seeds was higher for among of species except onion and wheat.

Phytotoxicity effects:

Table 1: CORN – Mean percentage visual injury at 7 DAT, 14 DAT and 21 DAT (harvest) and mean number of dead plants (as a percentage of the number planted) at 21 DAT (harvest).

Treatment GLOB289H (g product/ha)	7 DAT % Visual Injury	14 DAT % Visual Injury	21 DAT (Harvest) % Visual Injury	21 DAT (Harvest) % Dead Plants
Untreated	0	0	0	0
1.706	0	0	0	0
2.900	0	0	0	0
4.930	0	0	0	0
8.381	0	0	0	0
14.248	0	0	0	0
24.221	0	0	0	0
41.176	0	0	0	0
70.000	0	0	0	0

% Visual Injury: 0 % = no injury; 100 % = dead

Table 2: OATS – Mean percentage visual injury at 7 DAT, 14 DAT and 21 DAT (harvest) and mean number of dead plants (as a percentage of the number planted) at 21 DAT (harvest).

Treatment GLOB289H (g product/ha)	7 DAT % Visual Injury	14 DAT % Visual Injury	21 DAT (Harvest) % Visual Injury	21 DAT (Harvest) % Dead Plants
Untreated	0	0	0	0
1.706	0	0	0	0
2.900	0	0	0	0
4.930	0	0	0	0
8.381	0	0	0	0
14.248	0	0	0	0
24.221	0	0	0	0
41.176	0	0	0	0
70.000	0	0	0	0

% Visual Injury: 0 % = no injury; 100 % = dead

Table 3: ONION – Mean percentage visual injury at 7 DAT, 14 DAT and 21 DAT (harvest) and mean number of dead plants (as a percentage of the numl planted) at 21 DAT (harvest).

Treatment GLOB289H (g product/ha)	7 DAT % Visual Injury	14 DAT % Visual Injury	21 DAT (Harvest) % Visual Injury	21 DAT (Harvest) % Dead Plants
Untreated	0	0	0	0
1.706	0	0	0	0
2.900	0	0	0	0
4.930	0	0	0	0
8.381	0	0	0	0
14.248	0	0	0	0
24.221	0	0	0	0
41.176	0	0	0	0
70.000	0	0	0	0

% Visual Injury: 0 % = no injury; 100 % = dead

Table 4: CUCUMBER – Mean percentage visual injury at 7 DAT, 14 DAT and 21 DAT (harvest) and mean number of dead plants (as a percentage of the number planted) at 21 DAT (harvest).

Treatment GLOB289H (g product/ha)	7 DAT % Visual Injury	14 DAT % Visual Injury	21 DAT (Harvest) % Visual Injury	21 DAT (Harvest) % Dead Plants
Untreated	0	0	0	0
1.706	0	0	0	0
2.900	0	0	0	0
4.930	0	0	0	0
8.381	0	0	0	0
14.248	0	0	0	0
24.221	0	0	0	0
41.176	0	0	0	0
70.000	0	0	0	0

% Visual Injury: 0 % = no injury; 100 % = dead

Table 5: OILSEED RAPE – Mean percentage visual injury at 7 DAT, 14 DAT and 21 DAT (harvest) and mean number of dead plants (as a percentage of the number planted) at 21 DAT (harvest).

Treatment GLOB289H (g product/ha)	7 DAT % Visual Injury	14 DAT % Visual Injury	21 DAT (Harvest) % Visual Injury	21 DAT (Harvest) % Dead Plants
Untreated	0	0	0	0
1.706	0	0	0	0
2.900	0	0	0	0
4.930	0	0	0	0
8.381	0	0	0	0
14.248	0	0	0	0
24.221	0	0	0	0
41.176	0	0	0	0
70.000	4.5	4.5	6.2	0

% Visual Injury: 0 % = no injury; 100 % = dead

Table 6: RADISH – Mean percentage visual injury at 7 DAT, 14 DAT and 21 DAT (harvest) and mean number of dead plants (as a percentage of the number planted) at 21 DAT (harvest).

Treatment GLOB289H (g product/ha)	7 DAT % Visual Injury	14 DAT % Visual Injury	21 DAT (Harvest) % Visual Injury	21 DAT (Harvest) % Dead Plants
Untreated	0	0	0	0
1.706	0	0	0	0
2.900	0	0	0	0
4.930	0	0	0	0
8.381	0	0	0	0
14.248	2.0	3.0	3.0	0
24.221	2.8	6.3	6.3	0
41.176	8.2	22.5	25.5	0
70.000	12.2	36.7	46.7	6.7

% Visual Injury: 0 % = no injury; 100 % = dead

Table 7: SOYBEAN – Mean percentage visual injury at 7 DAT, 14 DAT and 21 DAT (harvest) and mean number of dead plants (as a percentage of the number planted) at 21 DAT (harvest).

Treatment GLOB289H (g product/ha)	7 DAT % Visual Injury	14 DAT % Visual Injury	21 DAT (Harvest) % Visual Injury	21 DAT (Harvest) % Dead Plants
Untreated	0	0	0	0
1.706	0	0	0	0
2.900	0	0	0	0
4.930	0	0	0	0
8.381	0	0	0	0
14.248	0	0	0	0
24.221	0	0	0	0
41.176	2.5	3.0	2.5	0
70.000	3.2	3.2	3.2	0

% Visual Injury: 0 % = no injury; 100 % = dead

Table 8: SUGAR BEET – Mean percentage visual injury at 7 DAT, 14 DAT and 21 DAT (harvest) and mean number of dead plants (as a percentage of the number planted) at 21 DAT (harvest).

Treatment GLOB289H (g product/ha)	7 DAT % Visual Injury	14 DAT % Visual Injury	21 DAT (Harvest) % Visual Injury	21 DAT (Harvest) % Dead Plants
Untreated	0	0	0	0
1.706	0	0	0	0
2.900	0	0	0	0
4.930	0	0	0	0
8.381	0	0	0	0
14.248	0	0	0	0
24.221	0	0	0	0
41.176	0	0	0	0
70.000	0	5.8	7.0	0

% Visual Injury: 0 % = no injury; 100 % = dead

Table 9: SUNFLOWER – Mean percentage visual injury at 7 DAT, 14 DAT and 21 DAT (harvest) and mean number of dead plants (as a percentage of the number planted) at 21 DAT (harvest).

Treatment GLOB289H (g product/ha)	7 DAT % Visual Injury	14 DAT % Visual Injury	21 DAT (Harvest) % Visual Injury	21 DAT (Harvest) % Dead Plants
Untreated	0	0	0	0
1.706	0	0	0	0
2.900	0	0	0	0
4.930	0	0	0	0
8.381	0	0	0	0
14.248	1.0	1.0	1.0	0
24.221	1.8	2.3	2.3	0
41.176	6.5	20.2	25.2	0
70.000	8.2	25.5	33.0	0

% Visual Injury: 0 % = no injury; 100 % = dead

Table 10: TOMATO – Mean percentage visual injury at 7 DAT, 14 DAT and 21 DAT (harvest) and mean number of dead plants (as a percentage of the number planted) at 21 DAT (harvest).

Treatment GLOB289H (g product/ha)	7 DAT % Visual Injury	14 DAT % Visual Injury	21 DAT (Harvest) % Visual Injury	21 DAT (Harvest) % Dead Plants
Untreated	0	0	0	0
1.706	0	0	0	0
2.900	0	0	0	0
4.930	0	0	0	0
8.381	0	0	0	0
14.248	2.7	3.2	3.2	0
24.221	5.3	7.7	7.7	0
41.176	8.2	15.7	16.0	0
70.000	11.7	26.7	28.3	0

% Visual Injury: 0 % = no injury; 100 % = dead

Reference: KCP 10.6-01

Report: GLOB289H GLP Vegetative Vigour test terrestrial non target plants (based on OECD guideline 227) – 2017. Davies C., 2018, STC/17/E1118

Guideline(s): Yes, OECD 227

Deviations:

- On three occasions during the field phase of the study, the temperature rose above 32°C (recommended range: 22°C +/- 10°C). However, this deviation had no impact on the validity of the study.
- Throughout most of the field phase of the study, the minimum relative humidity fell below 45%. On five occasions, the maximum relative humidity went above 95% (recommended range: 95% +/- 25%). However, this deviation had no impact on the validity of the study.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): Not applicable

Materials and methods

Test item: GLOB289H. A water dispersible granule (WG) formulation containing 6

	g/kg (5.6 g/kg analysed) iodosulfuron-methyl-sodium, 30 g/kg (29.2 g/kg analysed) mesosulfuron-methyl and 90 g/kg mefenpyr-diethyl (safener).
Test species:	3 monocotyledon species (corn, oats, onion) 7 dicotyledon species (cucumber, oilseed rape, radish, soybean, sugar beet, sunflower, tomato). Seed of all ten species was obtained from commercial seed companies and was from certified seed lots.
Trial design:	The test species were grown in a glasshouse. The trial was of a randomised block design with six replicates of each treatment except for cucumber which had ten replicates. Each species was treated as a separate trial to ensure optimal watering but with the same randomisation used for each species.
Endpoints:	ER_{25}, ER_{50} and NOEC values based on shoot fresh weight reduction At 7, 14 and 21 days after treatment application, visual injury was assessed and the number of plants alive were counted. At harvest (21 days after treatment application), the number of dead plants were counted and the shoot fresh weights were evaluated.
Treatments:	Control (deionised water) Test item (GLOB289H): 1.706 g GLOB289H/ha 2.900 g GLOB289H/ha 4.930 g GLOB289H/ha 8.381 g GLOB289H/ha 14.248 g GLOB289H/ha 24.221 g GLOB289H/ha 41.176 g GLOB289H/ha 70.000 g GLOB289H/ha The test-item was applied post-emergence to all ten species at growth stage BBCH 12-14 (2 to 4 true leaves)
Test conditions:	The temperature in the glasshouse was 22°C (+/- 10°C). A daily photoperiod of 16 hours was achieved by using supplementary lighting from 4am to 7am and 6pm to 8pm (13.07.17 to 23.07.17), 4am to 8am and 6pm to 8pm (24.07.17 to 03.08.17) each day to provide a minimum of 5000 lux in the glasshouse Relative humidity, sunshine hours and light intensity were recorded daily.
Statistics:	Statistical regression analyses to determine ER10, ER25, ER50 values based on shoot fresh weight reduction and Dunnett's Tests to determine NOEC levels were carried out by Stockbridge Technology Centre Ltd, UK.

Number of seed per pot

Species	Common Name	Cultivar	Number of Plants Per Pot
Zea mays	Corn	LG30179	5
Avena sativa	Oats	Firth	5
Allium cepa	Onion	Hyfort	10
Cucumis sativus	Cucumber	Carmen	3
Brassica napus	Oilseed rape	Vision	5
Raphanus sativus	Radish	Cherry Belle	5
Glycine max	Soybean	Siverka	5
Beta vulgaris	Sugar beet	Darnella-629	5
Helianthus annuus	Sunflower	Valentine	5
Lycopersicon esculentum	Tomato	Shirley	5

According to OECD 227 GD the following number of seeds is recommended per 15 cm container :
- **one to two corn,** soybean, tomato, cucumber, or sugar beet plants;
- **three rape** or pea plants per 15 cm container;
- **5 to 10** onion, wheat, or other small seeds.

Results and discussion

ER_{10}, ER_{25} and ER_{50} values (with corresponding R-Sq values) and NOEC levels based on shoot fresh weight reduction.

Species	ER_{10} (g product/ha)	ER_{25} (g product/ha)	ER_{50} (g product/ha)	NOEC (g product/ha)	R-Sq
Corn	>70.000	>70.000	>70.000	70.000	N/A
Oats	>70.000	>70.000	>70.000	70.000	N/A
Onion	68.117	>70.000	>70.000	70.000	0.009
Cucumber	44.258	>70.000	>70.000	70.000	0.006
Oilseed rape	28.390	>70.000	>70.000	70.000	0.065
Radish	3.047	8.250	43.393	8.381	0.64
Soybean	<1.706	>70.000	>70.000	41.176	0.06
Sugar beet	31.470	>70.000	>70.000	70.000	0.0045
Sunflower	18.631	61.441	>70.000	41.176	0.34
Tomato	7.007	>70.000	>70.000	24.221	0.253

N/A = not appropriate owing to tolerance of these species to GLOB289H

Conclusions

Oilseed rape, radish, soybean, sugar beet, sunflower and tomato displayed visual injury.

Based on ER_{25} and ER_{50} values for shoot fresh weight reduction all monocotyledon species had an ER_{25} and ER_{50} value of >70.000 g product/ha (the highest rate tested) to post-emergence application of GLOB289H.

Based on ER_{25} and ER_{50} values for shoot fresh weight reduction the most sensitive dicotyledon species to post-emergence application of GLOB289H was radish with an ER_{25} value of 8.250 g product/ha and an ER_{50} value of 43.393 g product/ha.

Based on NOEC values for shoot fresh weight reduction all monocotyledon species had an NOEC >70.000 g product/ha (the highest rate tested) to post-emergence application of GLOB289H.

Based on NOEC values for shoot fresh weight reduction the most sensitive dicotyledon species to post-emergence application of GLOB289H was radish with a NOEC value of 8.381 g product/ha.

Comments of zRMS:

The study is considered valid. All validity criteria were met.

- the seedling emergence is at least 70 %
- Untreated control plants must not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformation).
  Untreated control plants must only exhibit normal variation in growth and morphology for thatparticular species.
- The mean survival of untreated control plants is at least 90 % for the duration of the study.
- Environmental conditions for a particular species are identical and the growing media contain the same amount of soil matrix, support media or substrate from the same source.

<u>In the current study in the control the following parametres were noted:</u>

Germination rate:

Crop	Cultivar	Certified Seed Lot Number	Germination Rate
Corn	LG30179	F0964X029675NZ	95.92%
Oats	Firth	6/2L/0175/6685	97.96%
Onion	Hypark	E48209	95.92%
Cucumber	Carmen	L67590	99.04%
Oilseed rape	Django	Not Received	96.94%
Radish	Cherry Belle	5037887603	100%
Soybean	Siverka	17/BR/7087/0712	83.67%
Sugar beet	Cayman	553	95.92%
Sunflower	Elite Sun F1	9664-28-1	98.98%
Tomato	Shirley	P10910-1-1	97.96%

The mean survival in the controls: 100%

- It should be noted that according to OECD 227 GD the following number of seeds is recommended per 15 cm container :
- one to two corn, soybean, tomato, cucumber, or sugar beet plants;
- three rape or pea plants per 15 cm container;
- **5 to 10** onion, wheat, or other small seeds.

In the study number of seeds was higher for among of species except onion and wheat.

Agreed endpoints:

NOEC levels and ER_{10}, ER_{25} and ER_{50} values (with corresponding R-Sq values) for GLOB289H based on shoot fresh weight reduction.

Species	NOEC (g product/ha)	ER_{10}* (g product/ha)	ER_{25} (g product/ha)	ER_{50} (g product/ha)	R-Sq
Corn	23.33	6.14	91.91	>500	0.39
Oats	5.039	1.92	4.41	17.70	0.80
Onion	13.997	4.22	26.80	>500	0.56
Cucumber	23.33	5.93	27.53	355.74	0.61
Oilseed rape	3.023	4.00	4.87	7.52	0.87
Radish	5.039	2.15	4.53	15.69	0.83
Soybean	5.039	2.97	6.90	28.15	0.84
Sugar beet	8.398	2.97	7.45	34.53	0.85
Sunflower	1.814	2.03	2.44	3.67	0.88
Tomato	3.023	<1.814	4.54	21.97	0.83

* ER_{10} values need to be treated with caution due to plant to plant variability.

Phytotoxicity effects:

Table 1: CORN – Mean percentage visual injury at 7 DAT, 14 DAT and 21 DAT (harvest) and mean number of dead plants (as a percentage of the number planted) at 21 DAT (harvest).

Treatment GLOB289H (g product/ha)	7 DAT % Visual Injury	14 DAT % Visual Injury	21 DAT (Harvest) % Visual Injury	21 DAT (Harvest) % Dead Plants
Untreated	0	0	0	0
1.706	0	0	0	0
2.900	0	0	0	0
4.930	0	0	0	0
8.381	0	0	0	0
14.248	0	0	0	0
24.221	0	0	0	0
41.176	0	0	0	0
70.000	0	0	0	0

% Visual Injury: 0 % = no injury; 100 % = dead

Table 2: OATS – Mean percentage visual injury at 7 DAT, 14 DAT and 21 DAT (harvest) and mean number of dead plants (as a percentage of the number planted) at 21 DAT (harvest).

Treatment GLOB289H (g product/ha)	7 DAT % Visual Injury	14 DAT % Visual Injury	21 DAT (Harvest) % Visual Injury	21 DAT (Harvest) % Dead Plants
Untreated	0	0	0	0
1.706	0	0	0	0
2.900	0	0	0	0
4.930	0	0	0	0
8.381	0	0	0	0
14.248	0	0	0	0
24.221	0	0	0	0
41.176	0	0	0	0
70.000	0	0	0	0

% Visual Injury: 0 % = no injury; 100 % = dead

Table 3: ONION – Mean percentage visual injury at 7 DAT, 14 DAT and 21 DAT (harvest) and mean number of dead plants (as a percentage of the number planted) at 21 DAT (harvest).

Treatment GLOB289H (g product/ha)	7 DAT % Visual Injury	14 DAT % Visual Injury	21 DAT (Harvest) % Visual Injury	21 DAT (Harvest) % Dead Plants
Untreated	0	0	0	0
1.706	0	0	0	0
2.900	0	0	0	0
4.930	0	0	0	0
8.381	0	0	0	0
14.248	0	0	0	0
24.221	0	0	0	0
41.176	0	0	0	0
70.000	0	0	0	0

% Visual Injury: 0 % = no injury; 100 % = dead

Table 4: CUCUMBER – Mean percentage visual injury at 7 DAT, 14 DAT and 21 DAT (harvest) and mean number of dead plants (as a percentage of the number planted) at 21 DAT (harvest).

Treatment GLOB289H (g product/ha)	7 DAT % Visual Injury	14 DAT % Visual Injury	21 DAT (Harvest) % Visual Injury	21 DAT (Harvest) % Dead Plants
Untreated	0	0	0	0
1.706	0	0	0	0
2.900	0	0	0	0
4.930	0	0	0	0
8.381	0	0	0	0
14.248	0	0	0	0
24.221	0	0	0	0
41.176	0	0	0	0
70.000	0	0	0	0

% Visual Injury: 0 % = no injury; 100 % = dead

Table 5: OILSEED RAPE – Mean percentage visual injury at 7 DAT, 14 DAT and 21 DAT (harvest) and mean number of dead plants (as a percentage of the number planted) at 21 DAT (harvest).

Treatment GLOB289H (g product/ha)	7 DAT % Visual Injury	14 DAT % Visual Injury	21 DAT (Harvest) % Visual Injury	21 DAT (Harvest) % Dead Plants
Untreated	0	0	0	0
1.706	0	0	0	0
2.900	0	0	0	0
4.930	0	0	0	0
8.381	0	0	0	0
14.248	0	0	0	0
24.221	0	0	0	0
41.176	0	0	0	0
70.000	4.5	4.5	6.2	0

% Visual Injury: 0 % = no injury; 100 % = dead

Table 6: RADISH – Mean percentage visual injury at 7 DAT, 14 DAT and 21 DAT (harvest) and mean number of dead plants (as a percentage of the number planted) at 21 DAT (harvest).

Treatment GLOB289H (g product/ha)	7 DAT % Visual Injury	14 DAT % Visual Injury	21 DAT (Harvest) % Visual Injury	21 DAT (Harvest) % Dead Plants
Untreated	0	0	0	0
1.706	0	0	0	0
2.900	0	0	0	0
4.930	0	0	0	0
8.381	0	0	0	0
14.248	2.0	3.0	3.0	0
24.221	2.8	6.3	6.3	0
41.176	8.2	22.5	25.5	0
70.000	12.2	36.7	46.7	6.7

% Visual Injury: 0 % = no injury; 100 % = dead

Table 7: SOYBEAN – Mean percentage visual injury at 7 DAT, 14 DAT and 21 DAT (harvest) and mean number of dead plants (as a percentage of the number planted) at 21 DAT (harvest).

Treatment GLOB289H (g product/ha)	7 DAT % Visual Injury	14 DAT % Visual Injury	21 DAT (Harvest) % Visual Injury	21 DAT (Harvest) % Dead Plants
Untreated	0	0	0	0
1.706	0	0	0	0
2.900	0	0	0	0
4.930	0	0	0	0
8.381	0	0	0	0
14.248	0	0	0	0
24.221	0	0	0	0
41.176	2.5	3.0	2.5	0
70.000	3.2	3.2	3.2	0

% Visual Injury: 0 % = no injury; 100 % = dead

Table 8: SUGAR BEET – Mean percentage visual injury at 7 DAT, 14 DAT 21 DAT (harvest) and mean number of dead plants (as a percentage of the number planted) at 21 DAT (harvest).

Treatment GLOB289H (g product/ha)	7 DAT % Visual Injury	14 DAT % Visual Injury	21 DAT (Harvest) % Visual Injury	21 DAT (Harvest) % Dead Plants
Untreated	0	0	0	0
1.706	0	0	0	0
2.900	0	0	0	0
4.930	0	0	0	0
8.381	0	0	0	0
14.248	0	0	0	0
24.221	0	0	0	0
41.176	0	0	0	0
70.000	0	5.8	7.0	0

% Visual Injury: 0 % = no injury; 100 % = dead

Table 9: SUNFLOWER – Mean percentage visual injury at 7 DAT, 14 DAT and 21 DAT (harvest) and mean number of dead plants (as a percentage of the number planted) at 21 DAT (harvest).

Treatment GLOB289H (g product/ha)	7 DAT % Visual Injury	14 DAT % Visual Injury	21 DAT (Harvest) % Visual Injury	21 DAT (Harvest) % Dead Plants
Untreated	0	0	0	0
1.706	0	0	0	0
2.900	0	0	0	0
4.930	0	0	0	0
8.381	0	0	0	0
14.248	1.0	1.0	1.0	0
24.221	1.8	2.3	2.3	0
41.176	6.5	20.2	25.2	0
70.000	8.2	25.5	33.0	0

% Visual Injury: 0 % = no injury; 100 % = dead

Table 10: TOMATO – Mean percentage visual injury at 7 DAT, 14 DAT and 21 DAT (harvest) and mean number of dead plants (as a percentage of the number planted) at 21 DAT (harvest).

Treatment GLOB289H (g product/ha)	7 DAT % Visual Injury	14 DAT % Visual Injury	21 DAT (Harvest) % Visual Injury	21 DAT (Harvest) % Dead Plants
Untreated	0	0	0	0
1.706	0	0	0	0
2.900	0	0	0	0
4.930	0	0	0	0
8.381	0	0	0	0
14.248	2.7	3.2	3.2	0
24.221	5.3	7.7	7.7	0
41.176	8.2	15.7	16.0	0
70.000	11.7	26.7	28.3	0

% Visual Injury: 0 % = no injury; 100 % = dead

Reference:	KCP 10.6-03
Report	GLOB289H + Actirob B GLP Vegetative Vigour test terrestrial non target plants (based on OECD guideline 227) – 2019. Davies C., 2019, STC/19/E1233
Guideline(s):	Yes, OECD 227
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Materials and methods

Test item:

Formulation Name	Expiry Date	Active Ingredient (name)	Active Ingredient Content Nominal % w/w	Active Ingredient Content Actual* % w/w	Formulation Type
GLOB289H	Sept.2020	Iodosulfuron-methyl-sodium Mesosulfuron-methyl Mefenpyr-diethyl	0.6 3 9	0.5604 3.001 8.804	WG (water dispersible granule)
Actirob B**	N/A	Described as: esterified rapeseed oil	842 g/L	-	EC (emulsifiable concentrate)

*As stated on Certificate of Analysis
** Actirob B is an adjuvant

Test species: 3 monocotyledon species (corn, oats, onion)
7 dicotyledon species (cucumber, oilseed rape, radish, soybean, sugar beet, sunflower, tomato). Seed of all ten species was obtained from commercial seed companies and was from certified seed lots.

Trial design: The test species were grown in a glasshouse.
The trial was of a randomised block design with six replicates of each treatment except for cucumber which had ten replicates. Each species was treated as a separate trial to ensure optimal watering but with the same randomisation used for each species.

Endpoints: ER_{10}, ER_{20}, ER_{50} and NOEC values based on shoot fresh weight reduction
At 7, 14 and 21 days after treatment application, visual injury was assessed and the number of plants alive were counted. At harvest (21 days after treatment application), the number of dead plants were counted and the shoot fresh weights were evaluated.

Treatments: Control (deionised water)
Adjuvant control (1L Actirob B/ha)
Test item (GLOB289H + Actirob B):
- 1.814 g GLOB289H/ha + 1L Actirob B/ha
- 3.023 g GLOB289H/ha + 1L Actirob B/ha
- 5.039 g GLOB289H/ha + 1L Actirob B/ha
- 8.398 g GLOB289H/ha + 1L Actirob B/ha
- 13.997 g GLOB289H/ha + 1L Actirob B/ha
- 23.33 g GLOB289H/ha + 1L Actirob B/ha
- 38.88 g GLOB289H/ha + 1L Actirob B/ha
- 64.8 g GLOB289H/ha + 1L Actirob B/ha
- 108 g GLOB289H/ha + 1 L Actriob B/ha
- 180 g GLOB289H/ha + 1 L Actirob B/ha
- 300 g GLOB289H/ha + 1 L Actriob B/ha
- 500 g GLOB289H/ha + 1 L Actirob B/ha

The test-item was applied post-emergence to all ten species at growth stage BBCH 12-14 (2 to 4 true leaves)

Test conditions: The temperature in the glasshouse was 22°C (+/- 10°C).
A daily photoperiod of 16 hours was achieved by using supplementary lighting from 4am to 8pm each day to provide a minimum of 5000 lux in the glasshouse
Relative humidity, sunshine hours and light intensity were recorded daily.

Statistics: Statistical regression analyses to determine ER10, ER25, ER50 values based on shoot fresh weight reduction and Dunnett's Tests to determine NOEC levels were carried out by Stockbridge Technology Centre Ltd, UK.

Number of plants per pot:

Species	Common Name	Cultivar	Number of Plants Per Pot
Zea mays	Corn	LG30179	5
Avena sativa	Oats	Firth	5
Allium cepa	Onion	Hyfort	10
Cucumis sativus	Cucumber	Carmen	3
Brassica napus	Oilseed rape	Vision	5
Raphanus sativus	Radish	Cherry Belle	5
Glycine max	Soybean	Siverka	5
Beta vulgaris	Sugar beet	Darnella-629	5
Helianthus annuus	Sunflower	Valentine	5
Lycopersicon esculentum	Tomato	Shirley	5

According to **one to two corn,** soybean, tomato, cucumber, or sugar beet plants per 15 cm container; three rape or pea plants per 15 cm container; and 5 to 10 onion, wheat, or other small seeds per 15 cm container are recommended.

Results and discussion

NOEC levels and ER_{10}, ER_{25} and ER_{50} values (with corresponding R-Sq values) for GLOB289H based on shoot fresh weight reduction.

Species	NOEC (g product/ha)	ER_{10}* (g product/ha)	ER_{25} (g product/ha)	ER_{50} (g product/ha)	R-Sq
Corn	23.33	6.14	91.91	>500	0.39
Oats	5.039	1.92	4.41	17.70	0.80
Onion	13.997	4.22	26.80	>500	0.56
Cucumber	23.33	5.93	27.53	355.74	0.61
Oilseed rape	3.023	4.00	4.87	7.52	0.87
Radish	5.039	2.15	4.53	15.69	0.83
Soybean	5.039	2.97	6.90	28.15	0.84
Sugar beet	8.398	2.97	7.45	34.53	0.85
Sunflower	1.814	2.03	2.44	3.67	0.88
Tomato	3.023	<1.814	4.54	21.97	0.83

* ER_{10} values need to be treated with caution due to plant to plant variability.

Conclusions

All species displayed visual injury.
Based on the ER_{25} and ER_{50} values for shoot fresh weight reduction the most sensitive monocotyledon species post-emergence application of GLOB289H (+ Actriob B) was oats with an ER_{25} value of 4.41 g product/ha and an ER_{50} value of 17.70 g product/ha.
Based on ER_{25} and ER_{50} values for shoot fresh weight reduction the most sensitive dicotyledon species to post-emergence application of GLOB289H (+ Actirob B) was sunflower with an ER_{25} value of 2.44 g product/ha and an ER_{50} value of 3.67 g product/ha.
Based on NOEC values for shoot fresh weight reduction the most sensitive monocotyledon species to post-emergence application of GLOB289H (+ Actirob B) was oats which had a NOEC value of 5.039 g product/ha.
Based on NOEC values for shoot fresh weight reduction the most sensitive dicotyledon species to post-emergence application of GLOB289H (+ Actirob B) was sunflower which had a NOEC value of 1.814 g product/ha.

Comments of zRMS:

The study is considered valid. All validity criteria were met.

- Emergence recorded was ≥ 70.00 % (observed: 87.5 % to 98.1 %)
- Mean survival of the control plants was 100.00 %
- No phytotoxic effects were detected in the control plants

Agreed endpoints:

NOER, LOER and ER $_{10, 25, 50}$ of Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (6 + 30 + 90) g/kg WG (SAP63H) + adjuvant (POTTOK) for mortality

Family	Species	Common Name	Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (6 + 30 + 90) g/kg WG (SAP63H) + adjuvant (POTTOK) [g test item/ha + 200.00 mL Pottok/ha]				
			NOER	LOER	ER_{10} (95 % Confidence Limits)	ER_{25} (95 % Confidence Limits)	ER_{50} (95 % Confidence Limits)
Dicotyledonous species							
Brassicaceae	*Raphanus sativus*	Radish	***10.720***	***24.660***	36.573 (15.236, 59.375)	88.892 (53.806, 143. 356)	238.455 (147.251, s.n.r.)
Asteraceae	*Helianthus annuus*	Sunflower	≥300.00	--	>300.000	>300.000	>300.000
Solanaceae	*Lycopersicon esculentum*	Tomato	≥300.00	--	>300.000	>300.000	>300.000
Amaranthaceae	*Beta vulgaris*	Sugar beet	≥300.00	--	>300.000	>300.000	>300.000
Fabaceae	*Glycine max*	Soybean	≥300.00	--	>300.000	>300.000	>300.000
Monocotyledonous species							
Amaryllidaceae	*Allium cepa*	Onion	≥300.00	--	>300.000	>300.000	>300.000

s.n.r: statistics not reliable

NOER, LOER and ER 10, 25, 50 of Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (6 + 30 + 90) g/kg WG (SAP63H) + adjuvant (POTTOK) for shoot height after 21 days

Family	Species	Common Name	Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (6 + 30 + 90) g/kg WG (SAP63H) + adjuvant (POTTOK) [g test item/ha + 200.000 mL Pottok/ha]				
			NOER	LOER	ER_{10} (95 % Confidence Limits)	ER_{25} (95 % Confidence Limits)	ER_{50} (95 % Confidence Limits)
Dicotyledonous species							
Brassicaceae	*Raphanus sativus*	Radish	56.710	130.430	***11.788 (5.948, 27.605)***	***>300.000***	>300.000
Asteraceae	*Helianthus annuus*	Sunflower	0.880	0.380	2.970 (2.048, 4.259)	10.134 (7.990, 12.655)	39.625 (34.643, 43.963)
Solanaceae	*Lycopersicon esculentum*	Tomato	2.030	4.660	3.449 (2.509, 5.095)	10.703 (8.613, 13.278)	37.666 (32.992, 41.326)
Amaranthaceae	*Beta vulgaris*	Sugar beet	10.720	24.660	3.028 (1.874, 4.748)	23.309 (17.387, 29.866)	225.055 (184.333, 280.747)
Fabaceae	*Glycine max*	Soybean	4.660	10.720	2.965 (2.485, 3.525)	8.618 (7.603, 9.751)	28.202 (25.997, 30.704)
Monocotyledonous species							
Amaryllidaceae	*Allium cepa*	Onion	4.660	10.720	2.038 (0.804, 3.817)	14.032 (8.616, 20.438)	119.678 (82.914, 191.055)

s.n.r: statistics not reliable

NOER, LOER and ER 10, 25, 50 of Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (6 + 30 + 90) g/kg WG (SAP63H) + adjuvant (POTTOK) for shoot dry weight after 21 days

Family	Species	Common Name	Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (6 + 30 + 90) g/kg WG (SAP63H) + adjuvant (POTTOK) [g test item/ha + 200.000 mL Pottok/ha]				
			NOER	LOER	ER_{10} (95 % Confidence Limits)	ER_{25} (95 % Confidence Limits)	ER_{50} (95 % Confidence Limits)
Dicotyledonous species							
Brassicaceae	*Raphanus sativus*	Radish	***0.880***	***2.030***	***0.247 (0.065, 0.570)***	***1.881 (0.772, 2.874)***	***17.909 (12.016, 22.892)***
Asteraceae	*Helianthus annuus*	Sunflower	4.660	10.720	1.362 (0.809, 3.484)	6.722 (4.673, 12.849)	39.612 (28.901, 49.785)
Solanaceae	*Lycopersicon esculentum*	Tomato	24.660	56.710	20.738 (15.241, 28.450)	28.871 (23.956, 34.241)	41.697 (38.298, 44.294)
Amaranthaceae	*Beta vulgaris*	Sugar beet	10.720	24.660	5.897 (3.200, 10.430)	38.765 (27.987, 52.582)	>300.000
Fabaceae	*Glycine max*	Soybean	10.720	24.660	5.041 (3.625, 7.258)	14.643 (11.352, 18.425)	47.884 (39.375, 54.825)
Monocotyledonous species							
Amaryllidaceae	*Allium cepa*	Onion	4.660	10.720	1.159 (0.655, 2.140)	4.020 (2.783, 5.754)	16.014 (12.396, 20.007)

The overall lowest ER_{50} =16.014 g test item/ha (equivalent to 0.07 g Iodosulfuron-methyl-sodium/ha, 0.44 g Mesosulfuron-methyl/ha and 1.44 g Mefenpyr-diethyl/ha) for the species Allium cepa (onion) in shoot dry weight.
The overall lowest NOER was estimated to be 0.880 g test item/ha (equivalent to 0.004 g Iodosulfuron-methyl-sodium/ha, 0.02 g Mesosulfuron-methyl/ha and 0.08 g Mefenpyr-diethyl/ha), based on nominal treatment levels for shoot height of Helianthus annuus (sunflower) and for shoot dry weight of Raphanus sativus (radish).

Phytotoxicity effects:

NOER and LOER relative to parameter phytotoxicity (based on nominal rates)

	Raphanus sativus	Helianthus annuus	L. esculentum	Beta vulgaris	Glycine max	Allium cepa
	Rate (g test item/ha+ 200.000 mL adjuvant /ha)					
LOER	10.720	10.720	10.720	24.660	10.720	10.720
NOER	4.660	4.660	4.660	10.720	4.660	4.660

Injury symptoms were observed on all the tested species. Injury symptoms included epinastic growth of the stem and petiole, abnormal leaf development (enlargement, blistering and downward curvature). The stoppage of growth of the apical meristem also occurred at the higher rates. Chlorosis and necrosis were observed, and in some cases develop with plant death.
No symptoms were observed in the control plants.

The content of iodosulfuron-methyl-sodium (analysed) was only 4.6 g/kg, which is appr. 24 % below the nominal content stated for the formulated product GLOB289H. Due to the overall reduced content of active substance of the test substance in the study (Davies C.; 2018). Therfore, the study should be considered with caution as the effects could be underestimated.

Reference: KCP 10.6-04

Report: Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (6 + 30 + 90) g/kg WG (SAP63H) + adjuvant (POTTOK): Effects on the Vegetative Vigour of Six Non-Target Terrestrial Plant Species under Greenhouse Conditions. Huerta F., 2019, S19-00811

Guideline(s): Yes, OECD 227

Deviations: Temperature was out of the range one period longer than 2 hours: 17 Mar 2019 (Less than 2.5 hours, Maximum 35.40 ºC). This had no impact on the outcome of the study.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): Not applicable

Materials and methods

Test item: Test item: Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (6 + 30 + 90) g/kg WG (SAP63H).
Batch No.: F-DBA
Active ingredients (a.i.): Iodosulfuron-methyl-sodium, mesosulfuron-methyl and mefenpyr-diethyl
Content of iodosulfuron-methyl-sodium (analysed): 4.6 [g/kg]
Content of mesosulfuron-methyl (analysed): 27.2 [g/kg]
Content of mefenpyr-diethyl (analysed): 89.9 [g/kg]
Adjuvant POTTOK

Test species: Dicotyledonous species: Raphanus sativus (radish), Helianthus annuus (sunflower), Lycopersicon esculentum (tomato), Beta vulgaris (sugar beet), Glycine max (soybean).
Monocotyledonous species: Allium cepa (onion).

Trial design: Five dicotyledonous and one monocotyledonous species were cultivated in soil. Iodosulfuron-methyl-sodium + Mesosulfuron- methyl + Mefenpyr-diethyl (6 + 30 + 90) g/kg WG (SAP63H) + adjuvant (POTTOK) was applied at nine defined application rates ranging from 0.380 to 300.000 g test

	item/ha; the adjuvant POTTOK at a rate of 200.000 mL/ha will be added to each treatment. In each treatment group a total of 20 plants at BBCH growth stage 12 – 14 was applied. The test observation period was 21 days following application. During this period, plants were assessed for mortality and phytotoxicity symptoms on day 7, 14 and 21. The effects on plant shoot height and shoot dry weight were determined for day 21. Results were compared to the tap water treated control.
Endpoints:	Mortality, phytotoxicity, growth stage, shoot height and shoot dry weight; NOER (No Observed Effect Rate), LOER (Lowest Observed Effect Rate) and ER25, 50 (Effect Rate for 10, 25, 50 %) for mortality or effect on shoot height and shoot dry weight on day 21, where possible.
Treatments:	0 (control – C1) 0 + 200.000 mL adjuvant/ha (control – C2) 0.380, 0.880, 2.030, 4.660, 10.720, 24.660, 56.710, 130.430 and 300.000 g test item/ha + 200.000 mL adjuvant/ha in 200 L/ha
Test conditions:	Air temperature (min/max) [°C]: 13.40 / 35.40 Relative humidity (min/max) [%]: 25.0 / 90.0 Photoperiod (light /dark) [h]: 16/8 Light intensity (min) [µE/m2/s]: 524
Statistics:	Mean mortality, mean final shoot height and mean final shoot dry weight of the surviving plants were determined for each test rate and the control. Mean mortality, mean final shoot height and mean final shoot dry weight were compared using a suitable statistical test in order to obtain the NOER and LOER values. Calculations were performed using unrounded raw data, numbers are rounded for presentation purposes and therefore manual re-calculation may result in slightly different values. Comparison between treatment C2 and the relative control was performed for all the plant species, using Fisher Exact Binomial test (α=0.05) for quantal data, and a Student-t test for metric data. Comparison between each rate of the test item assayed, with at least three replicates with surviving individuals and the relative control, was performed for all the plant species. For quantal data, when the monotone rate-response is evident and homogeneity of the data was not obtained a Step-down-Rao-Scott-Cochran-Armitage Test (α=0.05) was performed, when the rate-response is not evident a Fisher Exact Binomial test (α=0.05) was performed. Metric data was tested for normality of data with the Shapiro-Wilk's test and for homoscedasticity with the Levene's test before performing the appropriate statistical test. For metric data, when normal distribution and homogeneity of variance of the data was obtained, and a monotone rate-response was evident, a Williams test (α=0.05) was performed. When normal distribution of the data was obtained and homogeneity of variance of the data was not obtained, Multiple Sequentially rejective Welcht-test after Bonferroni-Holm (α=0.05) was performed. When normal distribution of the data was not obtained, Step-down-Jonckeere Terpstra test (α=0.05) or Multiple sequentially-rejective U test (α=0.05) after Bonferroni-Holm was performed. Statistical analyses of mortality, shoot height and shoot dry weight also included the determination of effect concentrations (ER 10, 25, 50) and their 95 % confidence limits by Probit analysis using linear max. likelihood re-

gression, where possible.

Dates of work: 25 February – 21 March 2018

Validity criteria: The study was considered valid for all species; emergence recorded was ≥ 70.00 % (actually: 87.5 % to 98.1 %) and mean survival of the control plants was 100.00 %, and moreover, no phytotoxic effects were detected in the control plants. Correct dose preparation and application was confirmed by calibration of the spray equipment.

Number of plants per pot:

Family	Species (Variety)	Seed supplier	Lot No.	Seeds/Rep.	Rep./TG	No. of TG*
Dicotyledonous species						
Brasicaceae	*Raphanus sativus* (Famox)	Hild	11800.607	2	10	11
Asteraceae	*Helianthus annuus* (Sunspot)	Bingenheimer	11651-17	2	10	11
Solanaceae	*Lycopersicon esculentum* (Malpica)	Monsanto	0180959713	2	10	11
Amaranthaceae	*Beta vulgaris* (Ariestea)	Trialcamp	RmTRC-18	2	10	11
Fabaceae	*Glycine max* (Pollux)	Baywa	2015-GM-002	2	10	11
Monocotyledonous species						
Amaryllidaceae	*Allium cepa* (Amarilla Parma)	Batlle	002/GE017	4	5	11

* 9 test item treatment groups, 1 adjuvant treatment and 1 control group
Rep. = Replicate (pot), TG = Treatment group

According to **one to two corn,** soybean, tomato, cucumber, or sugar beet plants per 15 cm container; three rape or pea plants per 15 cm container; and 5 to 10 onion, wheat, or other small seeds per 15 cm container are recommended.

Results and discussion

Mortality: No significant effects on mortality were observed for all the species with exception of Raphanus sativus (radish).
The estimated ER50-value for mortality was 238.455 g test item/ha (equivalent to 1.10 g Iodosulfuron-methyl-sodium/ha, 6.49 g Mesosulfuron-methyl/ha and 21.44 g Mefenpyr-diethyl/ha).

NOER, LOER and ER $_{10, 25, 50}$ of Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (6 + 30 + 90) g/kg WG (SAP63H) + adjuvant (POTTOK) for mortality

Family	Species	Common Name	Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (6 + 30 + 90) g/kg WG (SAP63H) + adjuvant (POTTOK) [g test item/ha + 200.00 mL Pottok/ha]				
			NOER	LOER	ER_{10} (95 % Confidence Limits)	ER_{25} (95 % Confidence Limits)	ER_{50} (95 % Confidence Limits)
Dicotyledonous species							
Brassicaceae	*Raphanus sativus*	Radish	*10.720*	*24.660*	36.573 (15.236, 59.375)	88.892 (53.806, 143. 356)	238.455 (147.251, s.n.r.)
Asteraceae	*Helianthus annuus*	Sunflower	≥300.00	--	>300.000	>300.000	>300.000
Solanaceae	*Lycopersicon esculentum*	Tomato	≥300.00	--	>300.000	>300.000	>300.000
Amaranthaceae	*Beta vulgaris*	Sugar beet	≥300.00	--	>300.000	>300.000	>300.000
Fabaceae	*Glycine max*	Soybean	≥300.00	--	>300.000	>300.000	>300.000
Monocotyledonous species							
Amaryllidaceae	*Allium cepa*	Onion	≥300.00	--	>300.000	>300.000	>300.000

s.n.r.: statistics not reliable

Phytotoxicity: Injury symptoms were observed on all the tested species. Injury symptoms included epinastic growth of the stem and petiole, abnormal leaf development (enlargement, blistering and downward curvature). The stoppage of growth of the apical meristem also occurred at the higher rates. Chlorosis and necrosis were observed, and in some cases develop with plant death.
No symptoms were observed in the control plants.

Growth Stage: All the tested species showed differences in the growth stage between several treatment rates and the control.

Shoot Height: Significant effects on shoot height were observed at several treatment rates for all the tested species. The estimated ER50-values for shoot height were 39.625 g test item/ha (equivalent to 0.18 g Iodosulfuron-methyl-sodium/ha, 1.08 g Mesosulfuron-methyl/ha and 3.56 g Mefenpyr-diethyl/ha) for *Helianthus annuus* (sunflower), 37.666 g test item/ha (equivalent to 0.17 g Iodosulfuron-methyl-sodium/ha, 1.02 g Mesosulfuron-methyl/ha and 3.39 g Mefenpyr-diethyl/ha) for Lycopersicon esculentum (tomato), 225.055 g test item/ha (equivalent to 1.04 g Iodosulfuron-methyl-sodium/ha, 6.12 g Mesosulfuron-methyl/ha and 20.23 g Mefenpyr-diethyl/ha) for Beta vulgaris (sugar beet), 28.202 g test item/ha (equivalent to 0.13 g Iodosulfuron-methyl-sodium/ ha, 0.77 g Mesosulfuron-methyl/ha and 2.54 g Mefenpyr-diethyl/ha) for Glycine max (soybean) and 119.678 g test item/ha (equivalent to 0.55 g Iodosulfuron-methyl-sodium/ha, 3.26 g Mesosulfuron-methyl/ha and 10.76 g Mefenpyr-diethyl/ha) for Allium cepa (onion).

NOER, LOER and ER $_{10,\ 25,\ 50}$ of Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (6 + 30 + 90) g/kg WG (SAP63H) + adjuvant (POTTOK) for shoot height after 21 days

Family	Species	Common Name	Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (6 + 30 + 90) g/kg WG (SAP63H) + adjuvant (POTTOK) [g test item/ha + 200.000 mL Pottok/ha]				
			NOER	LOER	ER_{10} (95 % Confidence Limits)	ER_{25} (95 % Confidence Limits)	ER_{50} (95 % Confidence Limits)
Dicotyledonous species							
Brassicaceae	*Raphanus sativus*	Radish	56.710	130.430	***11.788 (5.948, 27.605)***	***>300.000***	>300.000
Asteraceae	*Helianthus annuus*	Sunflower	0.880	0.380	2.970 (2.048, 4.259)	10.134 (7.990, 12.655)	39.625 (34.643, 43.963)
Solanaceae	*Lycopersicon esculentum*	Tomato	2.030	4.660	3.449 (2.509, 5.095)	10.703 (8.613, 13.278)	37.666 (32.992, 41.326)
Amaranthaceae	*Beta vulgaris*	Sugar beet	10.720	24.660	3.028 (1.874, 4.748)	23.309 (17.387, 29.866)	225.055 (184.333, 280,747)
Fabaceae	*Glycine max*	Soybean	4.660	10.720	2.965 (2.485, 3.525)	8.618 (7.603, 9.751)	28.202 (25.997, 30.704)
Monocotyledonous species							
Amaryllidaceae	*Allium cepa*	Onion	4.660	10.720	2.038 (0.804, 3.817)	14.032 (8.616, 20.438)	119.678 (82.914, 191.055)

s.n.r: statistics not reliable

Shoot Dry Weight: Significant effects on shoot dry weight were observed at several treatment rates for all the species.

The estimated ER50-values for shoot dry weight were 17.909 g test item/ha (equivalent to 0.08 g Iodosulfuron-methyl-sodium/ha, 0.49 g Mesosulfuron-methyl/ha and 1.61 g Mefenpyr-diethyl/ha) for Raphanus sativus (radish), 39.612 g test item/ha (equivalent to 0.18 g Iodosulfuron-methyl-sodium/ha, 1.08 g Mesosulfuron-methyl/ ha and 3.56 g Mefenpyr-diethyl/ha) for Helianthus annuus (sunflower), 41.697 g test item/ha (equivalent to 0.19 g Iodosulfuron-methyl-sodium/ha, 1.13 g Mesosulfuron-methyl/ha and 3.75 g Mefenpyr-diethyl/ha) for Lycopersicon esculentum (tomato), 47.884 g test item/ha (equivalent to 0.22 g Iodosulfuron-methyl-sodium/ha, 1.30 g Mesosulfuron-methyl/ha and 4.30 g Mefenpyr-diethyl/ha) for Glycine max (soybean) and 16.014 g test item/ha (equivalent to 0.07g Iodosulfuron-methyl-sodium/ha, 0.44 g Mesosulfuron-methyl/ha and 1.44 g Mefenpyr-diethyl/ha) for Allium cepa (onion).

NOER, LOER and ER $_{10, 25, 50}$ of Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (6 + 30 + 90) g/kg WG (SAP63H) + adjuvant (POTTOK) for shoot dry weight after 21 days

Family	Species	Common Name	Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (6 + 30 + 90) g/kg WG (SAP63H) + adjuvant (POTTOK) [g test item/ha + 200.000 mL Pottok/ha]				
			NOER	LOER	ER_{10} (95 % Confidence Limits)	ER_{25} (95 % Confidence Limits)	ER_{50} (95 % Confidence Limits)
Dicotyledonous species							
Brassicaceae	*Raphanus sativus*	Radish	***0.880***	***2.030***	***0.247 (0.065, 0.570)***	***1.881 (0.772, 2.874)***	***17.909 (12.016, 22.892)***
Asteraceae	*Helianthus annuus*	Sunflower	4.660	10.720	1.362 (0.809, 3.484)	6.722 (4.673, 12.849)	39.612 (28.901, 49.785)
Solanaceae	*Lycopersicon esculentum*	Tomato	24.660	56.710	20.738 (15.241, 28.450)	28.871 (23.956, 34.241)	41.697 (38.298, 44.294)
Amaranthaceae	*Beta vulgaris*	Sugar beet	10.720	24.660	5.897 (3.200, 10.430)	38.765 (27.987, 52.582)	>300.000
Fabaceae	*Glycine max*	Soybean	10.720	24.660	5.041 (3.625, 7.258)	14.643 (11.352, 18.425)	47.884 (39.375, 54.825)
Monocotyledonous species							
Amaryllidaceae	*Allium cepa*	Onion	4.660	10.720	1.159 (0.655, 2.140)	4.020 (2.783, 5.754)	16.014 (12.396, 20.007)

Conclusions

It can be concluded that Iodosulfuron-methyl-sodium + Mesosulfuron-methyl + Mefenpyr-diethyl (6 + 30 + 90) g/kg WG (SAP63H) + adjuvant (POTTOK) had significant effects in mortality (radish) and for shoot height and on shoot dry weight for all the species at the tested rates. The most sensitive end point proved to be shoot dry weight, where numeric endpoints (ER50 values) could be estimated for five of the tested plant species.

The overall lowest ER50 was nominally 16.014 g test item/ha (equivalent to 0.07 g Iodosulfuron-methyl-sodium/ha, 0.44 g Mesosulfuron-methyl/ha and 1.44 g Mefenpyr-diethyl/ha) for the species Allium cepa (onion) in shoot dry weight.

The overall lowest NOER was estimated to be 0.880 g test item/ha (equivalent to 0.004 g Iodosulfuron-methyl-sodium/ha, 0.02 g Mesosulfuron-methyl/ha and 0.08 g Mefenpyr-diethyl/ha), based on nominal treatment levels for shoot height of Helianthus annuus (sunflower) and for shoot dry weight of Raphanus sativus (radish).

A 2.6.2.2 Seedling emergence test

Comments of zRMS:

The study is considered valid. All validity criteria were met.

- Emergence in the untreated control pots must exceed 70 %.
- Untreated control seedlings must not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformation). Untreated control seedlings must only exhibit normal variation in growth and morphology for that particular species.
- The mean survival of emerged untreated control seedlings is at least 90 % for the duration of the study.
- Environmental conditions for a particular species are identical and the growing media contain the same amount of soil matrix, support media or substrate from the same source.

In the current study:

- Emergence : 96-100 %
- Plant survival: 100%

It should be noted that according to OECD 227 GD the following number of seeds is recommended per 15 cm container :
- one to two corn, soybean, tomato, cucumber, or sugar beet plants;
- three rape or pea plants per 15 cm container;
- 5 to 10 onion, wheat, or other small seeds.
In the study number of seeds was higher for among of species except onion and wheat.

Agreed endpoints:

Species	ER_{10}# (g product/ha)	ER_{25} (g product/ha)	ER_{50} (g product/ha)	R-Sq	NOEC (g product/ha)
Corn	34.395	>70.00	>70.00	0.021	70.00
Oats	>70.00	>70.00	>70.00	0.042	70.00
Onion	34.047	>70.00	>70.00	0.63	70.00
Cucumber	>70.00	>70.00	>70.00	N/A	70.00
Oilseed rape	12.686	43.718	>70.00	0.42	41.176
Radish	17.591	54.934	>70.00	0.37	41.176
Soybean	>70.00	>70.00	>70.00	0.32	70.00
Sugar beet	6.270	23.730	52.831	0.55	41.176
Sunflower	2.817	21.067	51.483	0.65	14.248
Tomato	10.195	29.902	62.748	0.56	41.176

ER_{10} values should be treated with caution due to natural plant to plant variability.
N/A = not appropriate owing to tolerance of these species to GLOB289H.

Phytotoxicity effects:

Table 1: CORN - Mean percentage visual injury score at 14 and 21 days after 50% of the untreated plants had emerged, mean highest percentage emergence (as a percentage of the number sown) and mean survival of plants at harvest (as a percentage of the highest number emerged).

Treatment: GLOB289H: g product/ha	14 days % Visual Injury	21 days (Harvest) % Visual Injury	% Emergence	21 days (Harvest) % Survival
Untreated	0	0	100	100
1.706	0	0	100	100
2.900	0	0	100	100
4.930	0	0	100	100
8.381	0	0	100	100
14.248	0	0	100	100
24.221	0	0	100	100
41.176	0	0	100	100
70.00	0	0	100	100

Visual Injury: 0 % = no injury; 100 % = all plants dead/not emerged

Table 2: OATS: - Mean percentage visual injury score at 14 and 21 days after 50% of the untreated plants had emerged, mean highest percentage emergence (as a percentage of the number sown) and mean survival of plants at harvest (as a percentage of the highest number emerged).

Treatment: GLOB289H: g product/ha	14 days % Visual Injury	21 days (Harvest) % Visual Injury	% Emergence	21 days (Harvest) % Survival
Untreated	0	0	100	100
1.706	0	0	100	100
2.900	0	0	100	100
4.930	0	0	100	100
8.381	0	0	100	100
14.248	0	0	100	100
24.221	0	1	100	100
41.176	3	3	100	100
70.00	8	11	100	100

Visual Injury: 0 % = no injury; 100 % = all plants dead/not emerged

Table 3: ONION - Mean percentage visual injury score at 14 and 21 days afte[r] 50% of the untreated plants had emerged, mean highest percentage emergenc[e] (as a percentage of the number sown) and mean survival of plants at harvest ([a]s a percentage of the highest number emerged).

Treatment: GLOB289H: g product/ha	14 days % Visual Injury	21 days (Harvest) % Visual Injury	% Emergence	21 days (Harvest) % Survival
Untreated	0	0	100	100
1.706	0	0	100	100
2.900	0	0	100	100
4.930	0	0	100	100
8.381	0	0	100	100
14.248	0	0	100	100
24.221	0	0	100	100
41.176	3	10	100	96
70.00	6	12	100	98

Visual Injury: 0 % = no injury; 100 % = all plants dead/not emerged

Table 4: CUCUMBER - Mean percentage visual injury score at 14 and 21 days after 50% of the untreated plants had emerged, mean highest percentage emergence (as a percentage of the number sown) and mean survival of plants at harvest (as a percentage of the highest number emerged).

Treatment: GLOB289H: g product/ha	14 days % Visual Injury	21 days (Harvest) % Visual Injury	% Emergence	21 days (Harvest) % Survival
Untreated	0	0	100	100
1.706	0	0	100	100
2.900	0	0	100	100
4.930	0	0	100	100
8.381	0	0	100	100
14.248	0	0	100	100
24.221	0	0	98	100
41.176	0	0	100	100
70.00	0	0	100	100

Visual Injury: 0 % = no injury; 100 % = all plants dead/not emerged

Table 5: OILSEED RAPE - Mean percentage visual injury score at 14 and 21 days after 50% of the untreated plants had emerged, mean highest percentage emergence (as a percentage of the number sown) and mean survival of plants at harvest (as a percentage of the highest number emerged).

Treatment: GLOB289H: g product/ha	14 days % Visual Injury	21 days (Harvest) % Visual Injury	% Emergence	21 days (Harvest) % Survival
Untreated	0	0	100	100
1.706	0	0	100	100
2.900	0	0	100	100
4.930	0	0	100	100
8.381	0	0	100	100
14.248	0	0	100	100
24.221	0	0	100	100
41.176	5	4	100	100
70.00	18	26	100	100

Visual Injury: 0 % = no injury; 100 % = all plants dead/not emerged

Table 6: RADISH - Mean percentage visual injury score at 14 and 21 days after 50% of the untreated plants had emerged, mean highest percentage emergence (as a percentage of the number sown) and mean survival of plants at harvest (as a percentage of the highest number emerged).

Treatment: GLOB289H: g product/ha	14 days % Visual Injury	21 days (Harvest) % Visual Injury	% Emergence	21 days (Harvest) % Survival
Untreated	0	0	96	100
1.706	0	0	100	100
2.900	0	0	98	100
4.930	0	0	100	100
8.381	0	0	100	100
14.248	2	0	98	100
24.221	4	6	100	100
41.176	9	14	98	98
70.00	15	21	100	100

Visual Injury: 0 % = no injury; 100 % = all plants dead/not emerged

Table 7: SOYBEAN - Mean percentage visual injury score at 14 and 21 days after 50% of the untreated plants had emerged, mean highest percentage emergence (as a percentage of the number sown) and mean survival of plants at harvest (as a percentage of the highest number emerged).

Treatment: GLOB289H: g product/ha	14 days % Visual Injury	21 days (Harvest) % Visual Injury	% Emergence	21 days (Harvest) % Survival
Untreated	0	0	100	100
1.706	0	0	100	100
2.900	0	0	100	100
4.930	0	0	100	100
8.381	0	0	100	100
14.248	0	0	100	100
24.221	0	0	100	100
41.176	0	0	100	100
70.00	0	0	100	100

Visual Injury: 0 % = no injury; 100 % = all plants dead/not emerged

Table 8: SUGAR BEET - Mean percentage visual injury score at 14 and 21 days after 50% of the untreated plants had emerged, mean highest percentage emergence (as a percentage of the number sown) and mean survival of plants at harvest (as a percentage of the highest number emerged).

Treatment: GLOB289H: g product/ha	14 days % Visual Injury	21 days (Harvest) % Visual Injury	% Emergence	21 days (Harvest) % Survival
Untreated	0	0	100	100
1.706	0	0	100	100
2.900	0	0	100	100
4.930	0	0	100	100
8.381	0	0	100	100
14.248	0	0	98	100
24.221	4	13	100	100
41.176	9	18	100	98
70.00	25	56	100	86

Visual Injury: 0 % = no injury; 100 % = all plants dead/not emerged

Table 9: SUNFLOWER - Mean percentage visual injury score at 14 and 21 day after 50% of the untreated plants had emerged, mean highest percentage emergence (as a percentage of the number sown) and mean survival of plants at harvest (as a percentage of the highest number emerged).

Treatment: GLOB289H: g product/ha	14 days % Visual Injury	21 days (Harvest) % Visual Injury	% Emergence	21 days (Harvest) % Survival
Untreated	0	0	96	100
1.706	0	0	100	100
2.900	0	0	100	100
4.930	0	0	98	100
8.381	0	0	98	100
14.248	0	0	98	100
24.221	7	12	86	100
41.176	12	23	96	100
70.00	17	29	100	98

Visual Injury: 0 % = no injury; 100 % = all plants dead/not emerged

Table 10: TOMATO - Mean percentage visual injury score at 14 and 21 days after 50% of the untreated plants had emerged, mean highest percentage emergence (as a percentage of the number sown) and mean survival of plants at harvest (as a percentage of the highest number emerged).

Treatment: GLOB289H: g product/ha	14 days % Visual Injury	21 days (Harvest) % Visual Injury	% Emergence	21 days (Harvest) % Survival
Untreated	0	0	100	100
1.706	0	0	98	100
2.900	0	0	100	100
4.930	0	0	100	100
8.381	0	0	94	100
14.248	0	0	100	100
24.221	0	0	98	100
41.176	3	7	88	100
70.00	12	20	96	98

Visual Injury: 0 % = no injury; 100 % = all plants dead/not emerged

Reference: KCP10.6-02

Report: GLP Seedling emergence and seedling growth test Terrestrial non-target plants (based on OECD guideline 208) – 2017. Stead A., 2018, STC/17/E1119.

Guideline(s): OECD 208

Deviations:

- For oats, onion, oilseed rape, soybean and sunflower, on one day during the field phase, the temperature went to 35,1°C. For carrot, on two days the temperature went to 32.4°C and 35.6°C respectively (recommended range: 22°C ±). However, this deviation had no impact on the validity of the study.
- On a number of occasions during the field phase of the study, relative humidity went above 95% and also fell below 45% for all species (recommended range: 70% ± 25%). However, this deviation had no impact on the validity of the study.
- Oilseed rape was assessed at 15 days instead of 14 days. However, this deviation had no impact on the validity of the study.

GLP: Yes

Acceptability: Yes

Duplication: Not applicable

(if vertebrate study)

Materials and methods

Test item: GLOB289H. A water dispersible granule (WG) formulation containing 6 g/kg (5.6 g/kg analysed) iodosulfuron-methyl-sodium, 30 g/kg (29.2 g/kg analysed) mesosulfuron-methyl and 90 g/kg mefenpyr-diethyl (safener).

Test species: 3 monocotyledon species (corn, oats, onion)
7 dicotyledon species (cucumber, oilseed rape, radish, soybean, sugar beet, sunflower, tomato). Seed of all ten species was obtained from commercial seed companies and was from certified seed lots.

Trial design: The test species were grown in a glasshouse.
The trial was of a randomised block design with five replicates of each treatment except for cucumber which had eight replicates. Each species was treated as a separate trial to ensure optimal watering.

Endpoints: ER_{10}, ER_{25}, ER_{50} and NOEC values based on shoot fresh weight reduction
At 14, 21 and 28 days after 50% of the untreated control plants have emerged, visual injury and emergence were. At harvest (21 days after treatment application), the shoot fresh weights were evaluated.

Treatments: Control (deionised water)
Test item (GLOB289H):
- 1.706 g GLOB289H/ha
- 2.900 g GLOB289H/ha
- 4.930 g GLOB289H/ha
- 8.381 g GLOB289H/ha
- 14.248 g GLOB289H/ha
- 24.221 g GLOB289H/ha
- 41.176 g GLOB289H/ha
- 70.000 g GLOB289H/ha

The test-item was applied pre-emergence of the 6 test species.

Test conditions: The temperature in the glasshouse was 22°C (+/- 10°C).
A daily photoperiod of 16 hours was maintained with a light intensity of > 300 µE/m²/s.
Relative humidity, sunshine hours and light intensity were recorded daily.

Statistics: Statistical regression analyses to determine ER10, ER25, ER50 values based on shoot fresh weight reduction and Dunnett's Tests to determine NOEC levels were carried out by Stockbridge Technology Centre Ltd, UK.

Number of plats per pot

Species	Common Name	Number of seeds sown per pot	Number of replicates	Number of plants per treatment
Zea mays	Corn	10	5	50
Avena cepa	Oats	10	5	50
Allium cepa	Onion	10	5	50
Cucumis sativus	Cucumber	5	8	40
Brassica napus	Oilseed rape	10	5	50
Raphanus sativus	Radish	10	5	50
Glycine max	Soybean	10	5	50
Beta vulgaris	Sugar beet	10	5	50
Helianthus annuus	Sunflower	10	5	50
Lycopersicon esculentum	Tomato	10	5	50

According to **one to two corn,** soybean, tomato, cucumber, or sugar beet plants per 15 cm container; three rape or pea plants per 15 cm container; and 5 to 10 onion, wheat, or other small seeds per 15 cm container are recommended.

Results and discussion

Species	ER_{10}# (g product/ha)	ER_{25} (g product/ha)	ER_{50} (g product/ha)	R-Sq	NOEC (g product/ha)
Corn	34.395	>70.00	>70.00	0.021	70.00
Oats	>70.00	>70.00	>70.00	0.042	70.00
Onion	34.047	>70.00	>70.00	0.63	70.00
Cucumber	>70.00	>70.00	>70.00	N/A	70.00
Oilseed rape	12.686	43.718	>70.00	0.42	41.176
Radish	17.591	54.934	>70.00	0.37	41.176
Soybean	>70.00	>70.00	>70.00	0.32	70.00
Sugar beet	6.270	23.730	52.831	0.55	41.176
Sunflower	2.817	21.067	51.483	0.65	14.248
Tomato	10.195	29.902	62.748	0.56	41.176

\# ER_{10} values should be treated with caution due to natural plant to plant variability.
N/A = not appropriate owing to tolerance of these species to GLOB289H.

Conclusions

Visual Injury

- Visual injury was displayed on oats, oilseed rape, onion, radish, sugar beet, sunflower and tomato.

Based on shoot fresh weight reduction

- Based on shoot fresh weight reduction, the ER_{25} and ER_{50} values for all monocotyledon species were 70.00g product/ha (the highest rate tested).
- The most sensitive dicotyledon species to pre-emergence application of GLOB289H were sugar beet and sunflower, with ER_{25} values of 23.730 and 21.067 g product/ha and ER_{50} values of 52.831 and 51.483 g product/ha respectively.
- Based on shoot fresh weight reduction, the NOEC values for all monocotyledon species were 70.00 g product/ ha (the highest rate tested).
- Based on shoot fresh weight reduction sunflower, with a NOEC value of 14.248 g product/ha was the most sensitive dicotyledon species to pre-emergence application of GLOB289H.

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