FINAL REGISTRATION REPORT

# Part B

# Section 9

# Ecotoxicology

Detailed summary of the risk assessment

Product code: SAE053H/01

Product name(s): KAGURA / GENKI

Chemical active substance:

Mesotrione, 80 g/L
Nicosulfuron, 30 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

**Document number - SAEDoc-00020 CEU**

(authorization)

Applicant: Sumi Agro Europe Limited

Submission date: November 2019

Updated July 2021

MS Finalisation date: 18/02/2022

# Version history

| When | What |
|---|---|
| November 2019 | dRR submitted by applicant to the Polish Ministry of Agriculture and Rural Development |
| August 2020 | Submission to the evaluation unit: Merit Mark (PL) |
| July 2021 | First update of dRR on request of zRMS<br>Main changes:<br>• Adapting endpoints for nicosulfuron and nicosulfuron degradation products to the EU agreed endpoints instead of endpoints from SSSD and update the risk assessment according to the changes in endpoints (Sections 9.2, 9.3, 9.5, 9.6, 9.8, 9.9)<br>• Adapting the residue definition for nicosulfuron and its degradation products based on EU agreed studies and current guidance and corresponding update of risk assessment (Section 9.1.3, 9.5, 9.8 and 9.9).<br>• Change of nicosulfuron mammalian long-term endpoint to the one from the developmental study (300 mg a.s./kg bw/day; Section 9.3.2).<br>• Inclusion of further data on PT of wood mice and change of PT for mesotrione risk assessment to more conservative value; inclusion of alternative approaches based on other NOAEL of mesotrione; clarification on publications used for refinements (Section 9.3.2.2). Update of drinking water assessment with new PECs and endpoint (Section 9.3.2.3).<br>• Update of $PEC_{SW}$ and $PEC_{soil}$ values due to changes in Document B8 (Section 9.5, 9.8 and 9.9); change to 1.2 L product/ha as risk envelope.<br>• Inclusion of VFSmod option for aquatic risk assessment (Section 9.5.2).<br>• Addition of two new aquatic macrophyte studies which deliver the worst-case endpoint and at the same time are used for a geometric mean approach (Section 9.5.1, 9.5.2 and Appendix 1 and 2 as KCP 10.2.1/08 and KCP 10.2.1/09).<br>• Justification added for larval bee study design (Section 9.6.1) |
| October 2021 | zRMS finalised evaluation |
| January 2022 | Final version prepared by zRMS after Commenting period |
| February 2022 | Final version prepared by zRMS after Commenting period |

# Table of Contents

# 9 Ecotoxicology (KCP 10)

**Review Comments:**

This document describes the acceptable use conditions required for registration of SAE053H/01, an oil dispersion formulation (OD) containing 80 g/L mesotrione and 30 g/L nicosulfuron, for use as a herbicide for controls weeds in maize.

This Part B document only reviews data and additional information that has not previously been considered within the EU review process.

Since this document is based on the information provided by the applicant, all review comments, additions and corrections have been made using commenting boxes or highlighted in grey. Any incorrect data or text not evaluated by the zRMS has been crossed out.

## 9.1 Critical GAP and overall conclusions

### Table 9.1-1: Table of critical GAPs

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Use-No. * | Member state(s) | Crop and/or situation (crop destination / purpose of crop) | F, Fn, Fpn G, Gn, Gpn or I ** | Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group) | **Application** | | | | **Application rate** | | | PHI (days) | Remarks: e.g. g safener/ synergist per ha | **Conclusion** | | | | | | |
| | | | | | Method / Kind | Timing / Growth stage of crop & season | Max. number a) per use b) per crop/ season | Min. interval between applications (days) | kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season | g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season | Water L/ha min/max | | | Birds | Mammals | Aquatic organisms | Bees | Non-target | Soil organisms | Non-target plants |
| **Zonal uses (field or outdoor uses, certain types of protected crops)** | | | | | | | | | | | | | | | | | | | | |
| 1 | SK, PL, RO, HU, CZ, UK, IE, DE, BE, NL, AT, SI | Maize | F | *Broadleaved weeds and grasses* | foliar spray | BBCH 12-~~19~~ 18 | a, b) 1 | - | a, b) 1.2 L/ha | a, b) mesotrione: 96 g/ha nicosulfuron: 36 g/ha | 200-400 | n.a. | | | | | | | | |

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

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

Explanation for column 15 – 21 “Conclusion”

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

**Remarks table:**

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

## 9.1.1 Overall conclusions

The intended maximal application rate to be registered is 1.2 L product/ha, which is equivalent to 96 g mesotrione/ha and 36 g nicosulfuron/ha. Nevertheless, the dossier has been prepared for a maximal application rate of 1.5 L product/ha, and thus all risk and exposure assessments presented have been performed with that exaggerated application rate, unless otherwise stated. An application rate of 1.5 L product/ha is regarded as worst case and is therefore covering the intended rate of 1.2 L product/ha.

### 9.1.1.1 a) Not relevant, acute rat toxicity similar compared to parent ($LD_{50}$ > 5000 mg/kg bw)

[b)] Not relevant, acute rat toxicity similar to parent ($LD_{50}$ > 2000 mg/kg bw)

The relevant degradation products of mesotrione for terrestrial organisms are MNBA and AMBA. For aquatic organisms, all degradation products, i.e. MNBA, AMBA and SYN546974, need to be considered.

Degradation products of mesotrione identified in the plant metabolism are 4-hydroxy-mesotrione, AMBA and MNBA, however only AMBA and MNBA occurred in amounts of > 10% TRR and are therefore considered as major degradation products (see table above). Both of these major degradation products were tested in acute studies with rats and did not show a higher toxicity compared to the parent compound mesotrione ($LD_{50}$ > 5000 mg/kg bw).

The relevant degradation products of nicosulfuron for terrestrial and aquatic organisms are HMUD, AUSN, UCSN, ASDM and ADMP. ~~For aquatic organisms, additionally DUDN and ADHP are relevant. Furthermore, aquatic toxicity data on MU-466 is available, although the maximum occurrence was < 1% in water/sediment systems, and is therefore included in the risk assessment.~~

Degradation products of nicosulfuron in the plant metabolism are ASDM, AUSN, HMUD, ADMP, DMPU, 5-HDUD and 5-GDUD. Only ASDM and AUSN occurred in amounts of > 10% TRR and are therefore considered as major degradation products. However, both were tested in acute toxicity studies with rats and did not show an increased toxicity compared to the parent compound ($LD_{50}$ > 2000 mg/kg bw). Furthermore, ASDM was found in significant amounts in the animal metabolism study with lactating goats and is therefore also considered to be covered by avian and mammalian studies on the parent.

### 9.1.1.2 Effects on birds (KCP 10.1.1),

### 9.1.1.3

9.1.1.4 Review Comments:

The acute and chronic risks of SAE053H/01 to birds were assessed from toxicity exposure ratios between toxicity endpoints, estimated from study with active ingredients and maximum residues occurring on food items. No acute toxicity test with the formulation was required.

All TER values exceed the relevant triggers indicating that SAE053H/01 does not pose an unacceptable risk to birds following applications according to recommended use pattern.

Evaluation of exposing to birds through the drinking water demonstrated the acceptable risk. Since the log $P_{ow}$ value of mesotrione, nicosulfuron and their relevant metabolites are all below the trigger of 3, the evaluation of the risk of secondary poisoning is not triggered.

#### 9.1.1.5 Effects on terrestrial vertebrates other than birds (KCP 10.1.2), The risk from dietary exposure to mesotrione and nicosulfuron applied as SAE053H/01 in maize (shown ~~for both, the risk envelope: 1 x 120 g mesotrione/ha and 45 g nicosulfuron/ha as well as~~ the actual application rate: 1 x 96 g mesotrione/ha and 36 g nicosulfuron/ha) is indicated to be acceptable for mammals based on acute screening risk assessments for the single substance exposure and for the mixture exposure. The reproductive risk is indicated to be acceptable for nicosulfuron based on screening assessments and for mesotrione ~~and the mixture~~ based on higher tier assessment. The risk assessment from drinking water was not triggered to be investigated further for nicosulfuron and the acute risk for mesotrione and therefore the risk was considered low. For the reproductive risk of mesotrione from consumption of drinking water the refined assessment did indicate an acceptable risk for mammals. The risk from secondary poisoning and biomagnification in terrestrial food chains was not triggered and is therefore indicated to be low.

**Review Comments:**

In the screening step the $TER_A$ and $TER_{LT}$ values for nicosulfuron and the $TER_A$ mesotrione exceeds the trigger value set by Commission regulation (EU) 546/2011 for acceptability of effects. For mesotrione the $TER_{LT}$ values from the tier 1 reproductive risk assessment are below the trigger for all scenarios.

A higher tier risk assessment was based on the following refinement parameters: focal species, foliage residue dissipation ($DT_{50}$) and ecological data on PT value. Based on these refinements the quantitative higher tier risk assessments show that the dietary reproductive risks to mammals from the intended use of SAE053H/01 are acceptable for post-emergence (at 96 g a.s./ha) use in maize.

Evaluation of exposing to birds through the drinking water demonstrated the acceptable risk. Since the log $P_{ow}$ value of mesotrione, nicosulfuron and their relevant metabolites are all below the trigger of 3, the evaluation of the risk of secondary poisoning is not triggered.

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

The risk from dietary exposure to mesotrione and nicosulfuron applied as SAE053H/01 in maize (risk envelope: 1 x 120 g mesotrione and 45 g nicosulfuron per ha) is indicated to be acceptable for birds based on screening risk assessments. The risk assessment from drinking water was not triggered to be investigated further and therefore the risk was considered low.

The risk from dietary exposure to mesotrione and nicosulfuron applied as SAE053H/01 in maize (shown for both, the risk envelope: 1 x 120 g mesotrione/ha and 45 g nicosulfuron/ha as well as the actual application rate: 1 x 96 g mesotrione/ha and 36 g nicosulfuron/ha) is indicated to be acceptable for mammals based on acute screening risk assessments for the single substance exposure and for the mixture exposure. The reproductive risk is indicated to be acceptable for nicosulfuron based on screening assessments and for mesotrione ~~and the mixture of active substances~~ based on higher tier assessment. The risk assessment from drinking water was not triggered to be investigated further for nicosulfuron and the acute risk for mesotrione and therefore the risk was considered low. For the reproductive risk of mesotrione from consumption of

drinking water the refined assessment did indicate an acceptable risk for mammals.

The risk from secondary poisoning and biomagnification in terrestrial food chains was not triggered and is therefore indicated to be low.

No relevant data on effects on other terrestrial vertebrate wildlife were reported during EU review of the active substances.

### 9.1.1.7 Effects on aquatic organisms (KCP 10.2)

~~The risk from exposure to mesotrione and nicosulfuron applied as SAE053H/01 in maize at the actual application rate of 1 x 1.2 L product/ha is indicated to be acceptable for the individual active substances and the mixture based on higher tier data and FOCUS Step 4 calculations with 20 m vegetated buffer zone.~~ The risk from the active substances mesotrione and nicosulfuron as well as the mixture is indicated to be acceptable based on worst-case Tier 1 data and FOCUS Step 4 calculations when considering risk mitigation options. An overview on the country-specific requirements is given below. For those countries for which specific national modelling was considered, reference is made to the corresponding national addenda (i.e. Germany, The Netherlands, Slovenia and United Kingdom).

**Relevant FOCUS scenarios for CEU countries included in the GAP and required risk mitigation measures.**

| CEU Country | FOCUS scenarios | | | | | | | | National modelling | Comment |
|---|---|---|---|---|---|---|---|---|---|---|
| | D3 | D4 | D5 | D6 | R1 | R2 | R3 | R4 | | |
| Austria (AT) | | X | | | X | | X | | | Passes with 5 m VFSmod |
| Belgium (BE) | X | X | | | X | | | | | Passes with Step 4, 20 m VFS |
| Czech Republik (CZ) | | X | | | X | | | | | Passes with Step 4, 20 m VFS |
| Germany (DE) | | | | | X [a)] | | | | X | Refer to national addendum |
| Hungary (HU) | X | | X | | X | | X | X | | Passes with 5 m VFSmod |
| Ireland (IE) | | | | | | | | | X | Refer to national addendum UK |
| The Netherlands (NL) | | | | | | | | | X | Refer to national addendum |
| Poland (PL) | X | X | | | X | | | | | Passes with Step 4, 20 m VFS or 5 m VFSmod |
| Romania (RO) | | | X | | X | | | | | Passes with Step 4, 20 m VFS or 5 m VFSmod |
| Slovakia (SK) | | X | X | | X | | | | | Passes with Step 4, 20 m VFS or 5 m VFSmod |
| Slovenia (SI) | | | | | | | | | X | Refer to national addendum |
| United Kingdom (UK) | | | | | | | | | X | Refer to national addendum |

| | |
|---|---|
| | FOCUS scenario not relevant for this country |
| | FOCUS scenario is passed without VFSmod |
| | FOCUS scenario is passed using VFSmod |

[a)] The higher assessment factor for primary producers of 30 was considered for the calculations, reference is made to the German National Addendum.

The risk from the product via spray drift exposure is indicated to be acceptable when applying a ≥ 5 m buffer zone. The risk from metabolites of mesotrione and nicosulfuron is indicated to be acceptable based on Tier 1 data and FOCUS Step 1 calculations.

9.1.1.8 Review Comments:

The relevant predicted environmental concentrations in water ($PEC_{sw}$) for risk assessments covering the proposed use pattern are taken from Part B Section 8 (Environmental Fate). The risk assessment was based on the worst case PEC values and the results of laboratory toxicity testing.

SAE053H/01 pose no unacceptable risk to aquatic organisms according to the label with appropriate buffer zone.

The acceptability of risk mitigation measures used in refined risk assessment for aquatic plants should be checked on national level (width of buffer zones, VFSmod).

### 9.1.1.9 Effects on bees (KCP 10.3.1)

The risk from oral and contact exposure to mesotrione and nicosulfuron applied as SAE053H/01 in maize (risk envelope: 1 x 1.5 L product/ha, i.e. 120 g a.s./h mesotrione and 45 g a.s./ha nicosulfuron) is indicated to be acceptable for bees based on active substance and product data.

**9.1.1.10**

9.1.1.11 Review Comments:

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 submitted risk assessment, based on laboratory studies, has been accepted. It can therefore be concluded that there will be negligible risk associated with the exposure of bees to SAE053H/01.

### 9.1.1.12 Effects on arthropods other than bees (KCP 10.3.2)

The in-field and off-field risk from exposure to mesotrione and nicosulfuron applied as SAE053H/01 in maize (risk envelope: 1 x 1.5 L product/ha, i.e. 120 g a.s./ha mesotrione and 45 g a.s./ha nicosulfuron) is indicated to be acceptable for non-target arthropods other than bees based on Tier 2 data.

**9.1.1.13**

**9.1.1.14**

9.1.1.15 Review Comments:

Based on the results of the conducted risk assessment it can be concluded that low risk for non-target arthropods is expected from the use of SAE053H/01 according to the proposed use pattern. No unacceptable effects on non-target arthropods are expected in in-field and off-field habitats.

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

**9.1.1.17**

9.1.1.18 Review Comments:

All TER values for SAE053H/01, the active substances and relevant metabolites for chronic exposure of earthworms and other non-target soil organisms (meso- and macrofauna) are considerably higher than the Commission Regulation (EU) 546/2011 trigger value of 5. This indicates that SAE053H/01 poses no unacceptable risk to earthworms and other non-target soil organisms (meso- and macrofauna) when applied according to the proposed use pattern.

### 9.1.1.19 Effects on soil microbial activity (KCP 10.5)

### 9.1.1.20 The risk from exposure to mesotrione and nicosulfuron applied as SAE053H/01 in maize (~~risk envelope: 1 x 1.5 L product/ha, i.e. 120 g a.s./ha mesotrione and 45 g a.s./ha nicosulfuron~~ 1 x 1.2 L product/ha, i.e. 96 g mesotrione/ha and 36 g nicosulfuron/ha) is indicated to be acceptable for earthworms and the soil macro- and mesofauna as well as the soil microflora. The risk from the product itself and from relevant soil degradation products is indicated to be acceptable as well.Effects on non-target terrestrial plants (KCP 10.6)

The risk from exposure to mesotrione and nicosulfuron applied as SAE053H/01 in maize (risk envelope: 1 x 1.5 L product/ha; i.e. 120 g a.s./ha mesotrione and 45 g a.s./ha nicosulfuron) is indicated to be acceptable for non-target plants based on Tier 2 data (SSD) if risk mitigation is accounted for. The possible mitigation options are either a drift buffer zone of 10 m or a combination of 5 m drift buffer and 50% drift-reducing nozzles, or 90% drift-reducing nozzles with the default buffer zone of 1 m**,** or 90% drift-reducing nozzles.

### 9.1.1.21

9.1.1.22 Review Comments:

The risk assessment is based on the “Guidance Document on Terrestrial Ecotoxicology”, (SANCO/10329/2002 rev.2 final, 2002).

Based on the probabilistic risk assessment it can be concluded that the proposed use of SAE053H/01 poses acceptable risk to non-target plants, if applied according to the recommended use pattern. Particular precautions to reduce the environmental concentrations resulting from SAE053H/01 applications are required (10 m buffer zone or 5 m with 50% or 1 m with 90% drift reduction techniques).

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

No other relevant data were identified in the EU review of the active substances mesotrione and nicosulfuron.

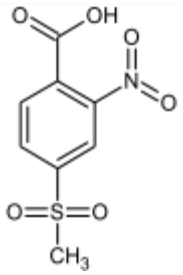
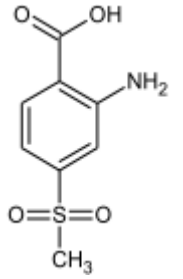
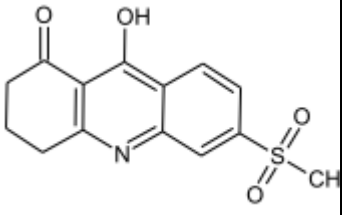
### 9.1.2 Grouping of intended uses for risk assessment

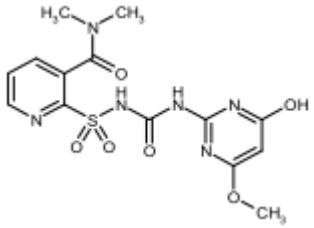
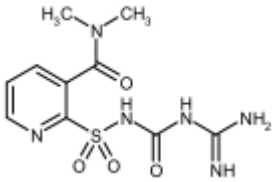
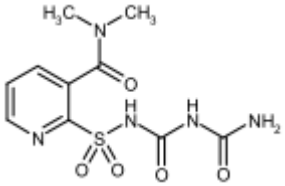
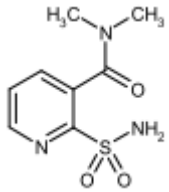
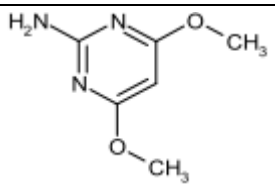
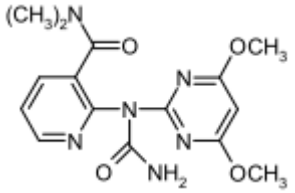
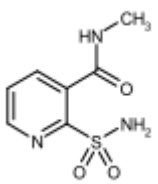
**The intended maximal application rate to be registered is 1.2 L product/ha, which is equivalent to 96 g mesotrione/ha and 36 g nicosulfuron/ha. Nevertheless, the dossier has been prepared for a maximal application rate of 1.5 L product/ha, and thus all risk and exposure assessments presented have been performed with that exaggerated application rate, unless otherwise stated. An application rate of 1.5 L product/ha is regarded as worst case and is therefore covering the intended rate of 1.2 L product/ha.**

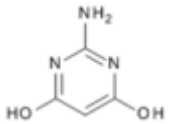
### 9.1.3 Consideration of metabolites

A list of degradation products found in environmental compartments is provided below. The need for conducting a degradation-product-specific risk assessment in the context of the evaluation of SAE053H/01 is indicated in the table.

**Table 0-1 Degradation products of mesotrione and nicosulfuron**

| Degradation product | Chemical structure | Molar mass | Maximum occurrence in compartments | Risk assessment required? |
|---|---|---|---|---|
| **Mesotrione** | | | | |
| MNBA |  | 245 | Soil: 57.2 % after 28 d<br><br>Water/sediment systems: 7.4 % after 3 d<br><br>Plants: 19.7% TRR in maize forage after 27 d pre-emergence | Yes, for aquatic and soil organisms<br><br>No, for birds & mammals [a)] |
| AMBA |  | 215 | Soil: 9.3 % after 13 d (max 9.7%; aerobic lab)<br><br>Water: 15.8 % after 46 d<br><br>Sediment: 8.8 % after 46 d<br><br>Plants: 28.2% TRR in maize fodder after 125 d post-emergence | Yes, for aquatic and soil organisms<br><br>No, for birds & mammals [a)] |
| SYN546974 |  | 291 | Water: 9.4 % after 29 d<br><br>Sediment: 25.6 % after 102 d | Yes, for aquatic organisms |

| Degradation product | Chemical structure | Molar mass | Maximum occurrence in compartments | Risk assessment required? |
|---|---|---|---|---|
| **Nicosulfuron** | | | | |
| HMUD |  | 396.4 | Soil: ~~35.9~~ 14.4 %<br><br>Water/sediment systems: ~~26.7~~ 19.3 %<br><br>Plants: < 10% TRR | Yes, for aquatic and soil organisms<br><br>No, for birds & mammals |
| AUSN |  | 314.3 | Soil: ~~53.8~~ 26.8 %<br><br>Water/sediment systems: 11.1 %<br><br>Plants: 20.4% TRR in whole maize plants at 0 d | Yes, for aquatic and soil organisms<br><br>No, for birds & mammals [b)] |
| UCSN |  | 315.3 | Soil: ~~15.6~~ 11.0 %<br><br>Water/sediment systems: 6.5 % | Yes, for aquatic and soil organisms |
| ASDM |  | 229.3 | Soil: ~~72.4~~ 63.4 %<br><br>Water/sediment systems: ~~60.9~~ 9.4 %<br><br>Plants: ~~39.9~~ 16.7 % TRR in ~~whole maize plants~~ straw/grain at ~~113~~ 60 d | Yes, for aquatic and soil organisms<br><br>No, for birds & mammals [b)] |
| ADMP |  | 127.1 | Soil: ~~48.8~~ 9.8 %<br><br>Water/sediment systems: -<br><br>Plants: < 10% TRR | Yes, for aquatic and soil organisms<br><br>No, for birds & mammals |
| ~~DUDN~~ |  | ~~346.3~~ | ~~Soil: 4.6 %~~<br><br>~~Water/sediment systems: 22.3 %~~ | ~~Yes, for aquatic organisms~~ |
| ~~MU 466~~ |  | ~~215.2~~ | ~~Soil: 2 %~~<br><br>~~Water/sediment systems: < 1 %~~ | ~~No, but aquatic data available~~ |

| Degradation product | Chemical structure | Molar mass | Maximum occurrence in compartments | Risk assessment required? |
|---|---|---|---|---|
| ~~ADHP~~ |  | ~~127.1~~ | ~~Water/sediment systems: 14.2%~~ | ~~Yes, for aquatic organisms~~ |

[a)] Not relevant, acute rat toxicity similar compared to parent ($LD_{50}$ > 5000 mg/kg bw)
[b)] Not relevant, acute rat toxicity similar to parent ($LD_{50}$ > 2000 mg/kg bw)

The relevant degradation products of mesotrione for terrestrial organisms are MNBA and AMBA. For aquatic organisms, all degradation products, i.e. MNBA, AMBA and SYN546974, need to be considered.

Degradation products of mesotrione identified in the plant metabolism are 4-hydroxy-mesotrione, AMBA and MNBA, however only AMBA and MNBA occurred in amounts of > 10% TRR and are therefore considered as major degradation products (see table above). Both of these major degradation products were tested in acute studies with rats and did not show a higher toxicity compared to the parent compound mesotrione ($LD_{50}$ > 5000 mg/kg bw).

The relevant degradation products of nicosulfuron for terrestrial and aquatic organisms are HMUD, AUSN, UCSN, ASDM and ADMP. ~~For aquatic organisms, additionally DUDN and ADHP are relevant. Furthermore, aquatic toxicity data on MU-466 is available, although the maximum occurrence was < 1% in water/sediment systems, and is therefore included in the risk assessment.~~

Degradation products of nicosulfuron in the plant metabolism are ASDM, AUSN, HMUD, ADMP, DMPU, 5-HDUD and 5-GDUD. Only ASDM and AUSN occurred in amounts of > 10% TRR and are therefore considered as major degradation products. However, both were tested in acute toxicity studies with rats and did not show an increased toxicity compared to the parent compound ($LD_{50}$ > 2000 mg/kg bw). Furthermore, ASDM was found in significant amounts in the animal metabolism study with lactating goats and is therefore also considered to be covered by avian and mammalian studies on the parent.

## 9.2 Effects on birds (KCP 10.1.1)

### 9.2.1 Toxicity data

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

The provision of further data on SAE053H/01 is not considered essential, as the mammalian acute oral toxicity endpoint for the product does not indicate increased product toxicity.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process of mesotrione and ~~with the supplementary dossier for the approval renewal of~~ nicosulfuron ~~(N2 document; 2016)~~.

**9Table 9.2-1: Endpoints and effect values relevant for the risk assessment for birds**

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| **Mesotrione** | | | | |
| Bobwhite quail (*Colinus virginianus*) | mesotrione | Oral<br>1 d<br>Acute | $LD_{50}$ > 2000 mg a.s./kg bw<br>**$LD_{50,\ extrapolated}$ = 3776 mg a.s./kg bw** | EFSA conclusion [a)]<br>Rodgers, 1995a, ISN 347/951557 |
| Bobwhite quail (*Colinus virginianus*) | mesotrione | Dietary<br>5 d<br>Short-term | $LC_{50}$ > 5200 mg a.s./kg diet | EFSA conclusion [a)]<br>Rodgers, 1995b, ISN 345/951542 |
| Mallard duck (*Anas platyrhynchos*) | mesotrione | Dietary<br>5 d<br>Short-term | $LC_{50}$ > 5200 mg a.s./kg diet | EFSA conclusion [a)]<br>Rodgers, 1995c, ISN 346/951543 |
| Bobwhite quail (*Colinus virginianus*) | mesotrione | Dietary<br>Reproductive toxicity | NOEC = 3000 mg a.s./kg diet | EFSA conclusion [a)]<br>Johnson, 1997a, ISN 359/961596 |
| Mallard duck (*Anas platyrhynchos*) | mesotrione | Dietary<br>Reproductive toxicity | NOEC = 120 mg a.s./kg diet<br>**NOEL = 20.6 mg a.s./kg bw/d** | EFSA conclusion [a)]<br>Johnson, 1997b, ISN 358/961595 |
| **Nicosulfuron** | | | | |
| Bobwhite quail (*Colinus virginianus*) | nicosulfuron | Oral<br>1 d<br>Acute | **$LD_{50}$ > 2000 mg a.s./kg bw**<br>**$LD_{50,\ extrapolated}$ = 3776 mg a.s./kg bw** | EFSA conclusion [b)]<br>Cummings, 1991b, 90/ISK147/1196 |
| Mallard duck (*Anas platyrhynchos*) | nicosulfuron | Oral<br>1 d<br>Acute | **$LD_{50}$ > 2000 mg a.s./kg bw** | EFSA conclusion [b)]<br>Cummings, 1991a, 90/ISK146/1227 |
| Bobwhite quail (*Colinus virginianus*) | nicosulfuron | Dietary<br>8 d<br>Short-term | $LC_{50}$ > 5000 mg a.s./kg diet<br>$LD_{50}$ > 1603 mg a.s./kg bw/d | EFSA conclusion [b)]<br>Cummings, 1991d, 90/ISK149/1228 |
| Mallard duck (*Anas platyrhynchos*) | nicosulfuron | Dietary<br>8 d<br>Short-term | $LC_{50}$ > 5000 mg a.s./kg diet<br>$LD_{50}$ > 911 mg a.s./kg bw/d | EFSA conclusion [b)]<br>Cummings, 1991c, 90/ISK148/1229 |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| ~~Bobwhite quail (*Colinus virginianus*)~~ | ~~nicosulfuron~~ | ~~Dietary Reproductive toxicity~~ | ~~NOEC ≥ 1250 mg a.s./kg diet~~ **~~NOEL ≥ 125 mg a.s./kg bw/d~~** [c)] | ~~Renewal dossier~~ [d)] ~~Author censored, 1996a, AMR 3371-95~~ |
| ~~Mallard duck (*Anas platyrhynchos*)~~ | ~~nicosulfuron~~ | ~~Dietary Reproductive toxicity~~ | ~~NOEC ≥ 1250 mg a.s./kg diet~~ **~~NOEL ≥ 125 mg a.s./kg bw/d~~** [c)] | ~~Renewal dossier~~ [d)] ~~Author censored, 1996b, AMR 3372-95~~ |
| Japanese quail (*Cortunix japonica*) | nicosulfuron | Dietary Reproductive toxicity | NOEC = 1000 mg a.s./kg diet **NOEL = 171 mg a.s./kg bw/d** | EFSA conclusion [b)] Burri, 1999, 696060 |

[a)] EFSA Journal 2016; 14(3):4419
[b)] EFSA Scientific Report 2007; 120, 1-91
[c)] A conversion factor of 0.1 was used to convert mg a.s./kg diet into mg a.s./kg bw/d in accordance with EFSA Guidance on birds and mammals (2009).
~~[d)] Supplementary dossier for the approval renewal of nicosulfuron (N2 document; 2016)~~
Endpoints in **bold** were used for the risk assessment.

Assessments of the dietary risk (short-term exposure) are not required in accordance with the EFSA guidance (2009) unless a higher toxicity is indicated from the feeding study. As all dietary studies resulted in limit endpoints, no increased toxicity is indicated for either mesotrione or nicosulfuron.

#### 9.2.1.1 Justification for new endpoints

The acute risk assessment is based on the EU agreed endpoints for mesotrione ($LD_{50}$ = 3776 mg a.s./kg bw; Rodgers 1995a) and nicosulfuron ($LD_{50}$ > 2000 mg a.s./kg bw; Cummings 1991 a, b).

The chronic risk assessment for mesotrione is also based on the EU agreed NOEL of 20.6 mg a.s./kg bw/d (Johnson 1997b). For nicosulfuron, ~~new endpoints are available from the supplementary dossier for the approval renewal of nicosulfuron (N2 document; 2016), which are lower than~~ the previously EU agreed NOEL of 171 mg a.s./kg bw/d is used~~. In a conservative approach, the lower NOEL of 125 mg a.s./kg bw/d for bobwhite quail and mallard duck is used for the chronic risk assessment.~~

Both active substances are metabolized in plants and some of their degradation products are present in relevant amounts. For mesotrione, MNBA and AMBA occur in amounts of > 10% TRR and for nicosulfuron ASDM and AUSN are above 10% TRR. However, all of these degradation products were tested in acute toxicity studies with rats and did not indicate a higher toxicity compared to their respective parent compound. Furthermore, in tests with earthworms and aquatic organisms, a higher toxicity of these degradation products was not found, indicating the loss of the toxophore. With lower residue levels as in comparison to the parent compound, risk assessments for these degradation products are not required.

### 9.2.2 Risk assessment for spray applications

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

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

The formulation SAE053H/01 is a combination product containing two active substances (mesotrione and nicosulfuron). For this reason, potential mixture toxicity has to be accounted for.

With reference to EFSA/2009/1438, a ´toxicity per fraction´ assessment was performed (see table below).

**Table 9.2-2: Toxicity per fraction assessment for birds**

| Active substance | A.s. content in the product [g a.s./L] | Fraction in mixture $X_{(a.s.)}$ | $LD_{50}$/NOEL of active substance [mg a.s./kg bw(/d)] | Toxicity per fraction for CA | $LD_{50}$ (mix), surrogate endpoint [mg/kg bw] | Contribution to overall toxicity [%] |
|---|---|---|---|---|---|---|
| ~~Acute effects~~ | | | | | | |
| ~~mesotrione~~ | ~~81.7~~ | ~~0.7269~~ | ~~3776~~ | ~~5194.9~~ | ~~> 3038.9~~ | ~~58.5~~ |
| ~~nicosulfuron~~ | ~~30.7~~ | ~~0.2731~~ | ~~> 2000~~ | ~~> 7322.5~~ | | ~~41.5~~ |
| **Chronic/reproductive effects** | | | | | | |
| mesotrione | 81.7 | 0.7269 | 20.6 | 28.3 | ~~26.7~~ 27.1 | **~~94.2~~ 95.7** |
| nicosulfuron | 30.7 | 0.2731 | ~~≥ 125~~ 171 | ~~≥ 457.7~~ 626.1 | | ~~5.8~~ 4.3 |

CA: Concentration Addition

**Review Comments:**
According to the toxicity data of the two active substances ($LD_{50}$ >2000 mg/kg bw and NOEL = 2000 mg/kg bw for both the active substances), an increase of the toxicity of the product is not expected. Therefore, the assessment of acute combined toxicity is presented as supportive data only. The LD mix is 3776 mg/kg bw.

Here, the deviation of the two factors “toxicity per fraction” and “$LD_{50}$ (mix)” indicates that the acute risk for birds is driven equally by both of the active substances, mesotrione and nicosulfuron, ~~with mesotrione having a slightly higher contribution~~. Therefore the risk assessment is shown for both active substances and additionally the mixture toxicity is assessed by comparing the $LD_{50}$ of the mixture with the combined daily dietary doses (DDD).

For the chronic mixture toxicity assessment, it is indicated that nicosulfuron does not significantly contribute to the predicted reproductive mixture toxicity.
Apart from the proportion of normal hatchlings of viable embryos and live three week embryos, which was statistically significantly reduced at 600 and 3000 ppm compared to the control, no reproductive or other treatment-related effects have been observed in the chronic bird study with mesotrione. The NOAEL from the study with nicosulfuron represents the highest feed concentration tested and therefore also indicating no reproductive toxicity at the relevant feed concentrations. As there is no known common mode of action interfering with reproductive parameters at relevant feed concentrations, chronic mixture toxicity assessments are not considered necessary for terrestrial vertebrates.
However, in a comprehensive approach, reproductive mixture toxicity assessments are presented by calculating the sum TER-triggers divided by TER for the individual active substances. This approach is considered to be most appropriate to account for potential combined effects not disregarding the actual toxicity data of the individual active substances, rather than the worst-case approach proposed by EFSA/2009/1438 which relates exposure of all actives expressed in equivalents of the active with the lowest available endpoint.

The lower tier acute and reproductive risk assessments for birds based on the risk envelope of 1.5 L product/ha are summarized in the following table. As there is no indication for short-term effects on reproductive performance, reproductive risk assessments are based on time-weighted average dietary doses over 21 days (i.e. $f_{TWA}$ = 0.53 based on the default (pseudo) $DT_{50}$ of 10 days).

Since for the reproductive mixture toxicity assessment the Tier 1 TERs are needed, the reproductive Tier 1 assessment will be additionally presented in the tables below although the risk assessment indicates an acceptable risk for the single active substances based on a screening assessment, already.

**Table 9.2-3: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of SAE053H/01 in maize (1.5 L product/ha) - mesotrione**

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | **Maize** | | | | |
| **Active substance/product** | mesotrione / SAE053H/01 | | | | |
| **Application rate (g/ha)** | 1.5 L product/ha, i.e. 120 g a.s./ha mesotrione and 45 g a.s./ha nicosulfuron | | | | |
| **Acute toxicity (mg/kg bw)** | 3776 | | | | |
| **TER criterion** | 10 | | | | |
| **Crop scenario<br>Growth stage** | **Indicator species for screening** | **$SV_{90}$** | **$MAF_{90}$** | **$DDD_{90}$ (mg/kg bw/d)** | **$TER_a$** |
| Maize<br>BBCH n.a. | Small omnivorous bird | 158.8 | 1.0 | 19.05 | 198.2 |
| **Reprod. toxicity (mg/kg bw/d)** | 20.6 | | | | |
| **TER criterion** | 5 | | | | |
| **Screening assessment** | | | | | |
| **Crop scenario<br>Growth stage** | **Indicator species for screening** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$ (mg/kg bw/d)** | **$TER_{lt}$** |
| Maize<br>BBCH n.a. | Small omnivorous bird | 64.8 | 1.0 × 0.53 | 4.12 | 5.0 |
| **Tier 1 assessment** | | | | | |
| **Crop scenario<br>Growth stage** | **Indicator/generic focal species** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$ (mg/kg bw/d)** | **$TER_{lt}$** |
| Maize<br>BBCH 10 – 29 | Medium granivorous gamebird | 3 | 1.0 × 0.53 | 0.19 | 108.6 |
| Maize<br>leaf development<br>BBCH 10 – 29 | Small insectivorous/worm feeding thrush | 5.7 | 1.0 × 0.53 | 0.36 | 57.2 |
| Maize<br>BBCH 10 – 29 | Small omnivorous lark | 10.9 | 1.0 × 0.53 | 0.69 | 29.9 |
| Maize<br>BBCH 10 – 29 | Medium herbivorous/granivorous pigeon | 22.7 | 1.0 × 0.53 | 1.43 | 14.4 |
| Maize<br>BBCH 10 – 19 | Small insectivorous wagtail | 11.3 | 1.0 × 0.53 | 0.71 | 28.8 |

n.a.: not applicable; 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. Exposure estimates are rounded to 2 significant figures; TERs represent accurate values underlying the actual exposure estimates

**Table 9.2-4: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of SAE053H/01 in maize (1.5 L product/ha) - nicosulfuron**

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | **Maize** | | | | |
| **Active substance/product** | nicosulfuron / SAE053H/01 | | | | |
| **Application rate (g/ha)** | 1.5 L product/ha, i.e. 45 g a.s./ha nicosulfuron and 120 g a.s./ha mesotrione | | | | |
| **Acute toxicity (mg/kg bw)** | ~~> 2000~~ 3776 | | | | |
| **TER criterion** | 10 | | | | |
| **Crop scenario<br>Growth stage** | **Indicator species for screening** | **$SV_{90}$** | **$MAF_{90}$** | **$DDD_{90}$ (mg/kg bw/d)** | **$TER_a$** |
| Maize<br>BBCH n.a. | Small omnivorous bird | 158.8 | 1.0 | 7.14 | ~~> 280.0~~<br>529 |
| **Reprod. toxicity (mg/kg bw/d)** | ~~≥ 125~~ 171 | | | | |
| **TER criterion** | 5 | | | | |
| **Screening assessment** | | | | | |
| **Crop scenario<br>Growth stage** | **Indicator species for screening** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$ (mg/kg bw/d)** | **$TER_{lt}$** |
| Maize<br>BBCH n.a. | Small omnivorous bird | 64.8 | 1.0 × 0.53 | 1.55 | ~~≥ 80.9~~<br>110.6 |
| **Tier 1 assessment** | | | | | |
| **Crop scenario<br>Growth stage** | **Indicator/generic focal species** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$ (mg/kg bw/d)** | **$TER_{lt}$** |
| Maize<br>BBCH 10 – 29 | Medium granivorous gamebird | 3 | 1.0 × 0.53 | 0.07 | ~~≥ 1757.8~~<br>2404.7 |
| Maize<br>leaf development<br>BBCH 10 – 29 | Small insectivorous/worm feeding thrush | 5.7 | 1.0 × 0.53 | 0.14 | ~~≥ 925.2~~<br>1265.6 |
| Maize<br>BBCH 10 – 29 | Small omnivorous lark | 10.9 | 1.0 × 0.53 | 0.26 | ~~≥ 483.8~~<br>661.8 |
| Maize<br>BBCH 10 – 29 | Medium herbivorous/granivorous pigeon | 22.7 | 1.0 × 0.53 | 0.54 | ~~≥ 232.3~~<br>317.8 |
| Maize<br>BBCH 10 – 19 | Small insectivorous wagtail | 11.3 | 1.0 × 0.53 | 0.27 | ~~≥ 466.7~~<br>638.4 |

n.a.: not applicable; 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. Exposure estimates are rounded to 2 significant figures; TERs represent accurate values underlying the actual exposure estimates

### Mixture toxicity assessment for acute risk

The surrogate toxicity endpoint for the mixture ($LD_{50}$ > 3038.9 mg/kg bw) is compared to the combined daily dietary doses of both active substances (*cf.* following table).

**Table 9.2-5: Acute mixture toxicity assessment for birds due to the use of SAE053H/01 in maize (1.5 L product/ha) based on screening tier assessments**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Intended use** | **Maize** | | | | | | |
| **Active substance/product** | mesotrione + nicosulfuron / SAE053H/01 | | | | | | |
| **Application rate (g/ha)** | 1.5 L product/ha, i.e. 120 g a.s./ha mesotrione and 45 g a.s./ha nicosulfuron | | | | | | |
| **Acute toxicity (mg/kg bw)** | ~~≥ 3038.9~~ 3776 | | | | | | |
| **TER criterion** | 10 | | | | | | |
| **Crop scenario Growth stage** | **Indicator species for screening** | **$SV_{90}$** | **$MAF_{90}$** | **$DDD_{90}$ (mg/kg bw/d)** | | **∑ $DDD_{90}$ (mg/kg bw/d)** | **$TER_a$** |
| | | | | **mesotrione** | **nicosulfuron** | | |
| Maize BBCH n.a. | Small omnivorous bird | 158.8 | 1.0 | 19.05 | 7.14 | 26.19 | ~~≥ 116.0~~ 144.1 |

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

Accordingly, with a TER greater than the trigger of 10, an acceptable acute risk for birds is indicated for exposure to SAE053H/01 for the intended use in maize based on screening assessments.

**Mixture toxicity assessment for reproductive risk**

Reproductive mixture toxicity based on the sum of TER-triggers divided by TER is presented in the following table based on Screening tier and Tier 1 TERs.

**Table 9.2-6: Reproductive mixture toxicity assessment for birds due to the use of SAE053H/01 in maize (1.5 L product/ha) based on screening tier and Tier 1 assessments**

| | | | | |
|---|---|---|---|---|
| **Intended use** | **Maize** | | | |
| **Active substance/product** | mesotrione + nicosulfuron / SAE053H/01 | | | |
| **Application rate (g/ha)** | 1.5 L product/ha, i.e. 120 g a.s./ha mesotrione and 45 g a.s./ha nicosulfuron | | | |
| **Reprod. toxicity (mg/kg bw/d)** | 20.6 (mesotrione) / ~~≥ 125~~ 171 (nicosulfuron) | | | |
| **TER criterion** | 5 | | | |
| **Screening assessment** | | | | |
| **Crop scenario Growth stage** | **Indicator species for screening** | **$TER_{lt}$** | | **∑ (TER-trigger/TER)** |
| | | **mesotrione** | **nicosulfuron** | |
| Maize BBCH n.a. | Small omnivorous bird | 5.0 | ~~≥ 116.0~~ 110.6 | ~~**1.04**~~ **1.05** |

| Tier 1 assessment | | | | |
|---|---|---|---|---|
| **Crop scenario Growth stage** | **Generic focal species** | **$TER_{lt}$** | | **∑ (TER-trigger/TER)** |
| | | **mesotrione** | **nicosulfuron** | |
| Maize BBCH 10 – 29 | Medium granivorous gamebird | 108.6 | ~~≥ 1757.8~~ 2404.7 | ~~≤ 0.049~~ 0.048 |
| Maize leaf development BBCH 10 – 29 | Small insectivorous/worm feeding thrush | 57.2 | ~~≥ 925.2~~ 1265.6 | ~~≤ 0.093~~ 0.091 |
| Maize BBCH 10 – 29 | Small omnivorous lark | 29.9 | ~~≥ 483.8~~ 661.8 | ~~≤ 0.178~~ 0.175 |
| Maize BBCH 10 – 29 | Medium herbivorous/granivorous pigeon | 14.4 | ~~≥ 232.3~~ 317.8 | ~~≤ 0.369~~ 0.363 |
| Maize BBCH 10 – 19 | Small insectivorous wagtail | 28.8 | ~~≥ 466.7~~ 638.4 | ~~≤ 0.184~~ 0.181 |

The sum of (TER-trigger/TER) shown in bold exceed the relevant trigger of 1.

Accordingly, with the sum of TER-trigger divided by TERs below the trigger of 1, an acceptable reproductive risk for birds is indicated for exposure to SAE053H/01 for the intended use in maize based on Tier 1 assessments.

#### 9.2.2.2 Higher-tier risk assessment

An acceptable risk was already shown using Tier 1 risk assessment and therefore no higher tier risk assessment is required.

#### 9.2.2.3 Drinking water exposure

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

**Leaf scenario**

Since SAE053H/01 is applied at BBCH 12 – 19 in maize, the leaf scenario does not have to be considered, as no relevant leaf axils are formed in maize at this growth stage.

**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 ($K_{oc}$ < 500 L/kg) or 3000 in the case of more sorptive substances ($K_{oc}$ ≥ 500 L/kg).

With a $K(f)_{oc}$ of 14 to 156.6 L/kg (pH depended), mesotrione belongs to the group of less sorptive substances. Nicosulfuron has a $K(f)_{oc}$ of 25 L/kg and therefore also belongs to the group of less sorptive substances.

| | | | | |
|---|---|---|---|---|
| **Mesotrione** | | | | |
| Effective application rate [g/ha] | 120 | | | |
| Acute toxicity [mg/kg bw] | 3766 | quotient | = | 0.032 |
| Reproductive toxicity [mg/kg bw] | 20.6 | quotient | = | 5.83 |
| **Nicosulfuron** | | | | |
| Effective application rate [g/ha] | 45 | | | |
| Acute toxicity [mg/kg bw] | ~~> 2000~~ 3776 | quotient | < | ~~0.023~~ 0.012 |
| Reproductive toxicity [mg/kg bw] | ~~≥ 125~~ 171 | quotient | ~~≤~~ = | ~~0.36~~ 0.26 |

Accordingly, no specific calculations of exposure and TER are necessary with ratios not exceeding the trigger of 50 for both, mesotrione and nicosulfuron.

#### 9.2.2.4 Effects of secondary poisoning

The log $P_{ow}$ of mesotrione amounts to 0.11 and for nicosulfuron to maximum 0.61 (pH dependent) and thus both active substances do not exceed the trigger value of 3. Furthermore, no indication of bioaccumulation was found in the EFSA conclusions for both active substances. A risk assessment for effects due to secondary poisoning is not required.

All major degradation products in soil and water for both active substances have log $P_{OW}$ values below the relevant trigger of 3. Therefore, a risk assessment is not required.

**Risk assessment for earthworm-eating birds via secondary poisoning**

Not required.

**Risk assessment for fish-eating birds via secondary poisoning**

Not required.

#### 9.2.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

### 9.2.4 Overall conclusions

The risk from dietary exposure to mesotrione and nicosulfuron applied as SAE053H/01 in maize (risk envelope: 1 x 120 g mesotrione and 45 g nicosulfuron per ha) is indicated to be acceptable for birds based on screening risk assessments for the single substance exposure and based on Tier 1 assessments for the mixture exposure. The risk assessment from drinking water was not triggered to be investigated further and therefore the risk was considered low. The risk from secondary poisoning and biomagnification in terrestrial food chains was not triggered and is therefore indicated to be low.

**Review Comments:**

The acute and chronic risks of SAE053H/01 to birds were assessed from toxicity exposure ratios between toxicity endpoints, estimated from study with active ingredients and maximum residues occurring on food items. No acute toxicity test with the formulation was required.

All TER values exceed the relevant triggers indicating that SAE053H/01 does not pose an unacceptable risk to birds following applications according to recommended use pattern.

Evaluation of exposing to birds through the drinking water demonstrated the acceptable risk. Since the log $P_{ow}$ value of mesotrione, nicosulfuron and their relevant metabolites are all below the trigger of 3, the evaluation of the risk of secondary poisoning is not triggered.

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

### 9.3.1 Toxicity data

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

Effects on mammals of SAE053H/01 were not evaluated as part of the EU assessment of mesotrione or nicosulfuron. New data submitted with this application are listed in Appendix 1 and summarised in Section 6 (Mammalian Toxicology) of this report.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review processes of mesotrione and ~~the supplementary dossier for the approval renewal of~~ nicosulfuron ~~(N2 document; 2016)~~.

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

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| **SAE053H/01** | | | | |
| Rat | SAE053H/01 | Oral<br>1 d<br>Acute | $LD_{50}$ > 2000 mg product/kg bw | xxx, 2016,<br>401-1-01-15025 |
| **Mesotrione** | | | | |
| Rat | mesotrione | Oral<br>1 d<br>Acute | **$LD_{50}$ > 5000 mg a.s./kg bw** | EFSA conclusion [a)]<br>xxx, 1994a, CTL/P/4502 |
| Rat | degradation product MNBA | Oral<br>1 d<br>Acute | $LD_{50}$ > 5000 mg a.s./kg bw | EFSA conclusion [a)]<br>xxx, 1996, CTL/P/5210 |
| Rat | degradation product AMBA | Oral<br>1 d<br>Acute | $LD_{50}$ > 5000 mg a.s./kg bw | EFSA conclusion [a)]<br>xxx, 1996a, CTL/P/5282 |
| Rat | mesotrione | Oral<br>Multi-generation, reproduction | NOEC = 2.5 mg a.s./kg diet<br>**NOAEL = 0.3 mg a.s./kg bw/d**<br>~~(reduced litter size in F2)~~ [~~d)~~]<br>~~NOAEL = 1.2 mg a.s./kg bw/d~~<br>~~(changes in reproduction and development)~~ [~~e)~~] | EFSA conclusion [a)]<br>xxx, 1997a, CTL/P/5147 |
| ~~Several mammalian species~~ | ~~mesotrione~~ | ~~Review of data from several long-term studies~~ | ~~NOAEL = 2.0 mg a.s./kg bw/d~~ [~~g)~~] | ~~KCP 10.12.2/01 xxxx, 2019, 10002, pTC~~ |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| **Nicosulfuron** | | | | |
| Rat & Mouse | nicosulfuron | Oral<br>1 d<br>Acute | **$LD_{50}$ > 5000 mg a.s./kg bw** | EFSA conclusion [b]<br>xxx, 1991a,<br>89/ISK126/0912 (mouse)<br>xxx, 1991b,<br>89/ISK127/0913 (rat) |
| Rat & Mouse | degradation product ASDM | Oral<br>1 d<br>Acute | $LD_{50}$ > 5000 mg a.s./kg bw | EFSA conclusion [b]<br>xxx, 1993a,<br>93/ISK195/0591 (rat), xxx, 1992a, 92-0103 (mouse) |
| Rat | degradation product AUSN | Oral<br>1 d<br>Acute | $LD_{50}$ > 2000 mg a.s./kg bw | EFSA conclusion [b]<br>xxx, 1996a, 601863 |
| Rat | nicosulfuron | Oral<br>Two-generation, reproduction | NOAEL ≥ 3861 mg a.s./kg bw/d<br>(no effects on reproductive performance) | EFSA conclusion [b]<br>xxx, 1992,<br>91/ISK130/0054 |
| Rat | nicosulfuron | Oral<br>Developmental toxicity | **NOAEL = 300 mg a.s./kg bw/d**<br>(developmental; marginally increased incidence of skeletal findings) | EFSA conclusion [b]<br>xxx, 1990c,<br>90/ISK125/0221 |
| Rabbit | nicosulfuron | Oral<br>Developmental toxicity | **NOAEL = 300 mg a.s./kg bw/d**<br>(maternal and developmental; slightly increased incidence of skeletal findings) | EFSA conclusion [b]<br>xxx, 1990c,<br>89/ISK132/0368 |
| ~~Rat~~ | ~~nicosulfuron~~ | ~~Oral<br>Developmental toxicity~~ | ~~NOAEL = 1000 mg/kg bw/d<br>(no developmental effects)~~ | ~~Renewal dossier [b]<br>xxx, 1990c,<br>90/ISK125/0221~~ |
| ~~Rat~~ | ~~degradation product ASDM~~ | ~~Oral<br>One-generation reproduction~~ | ~~NOAEL ≥ 1000 mg/kg bw/d~~ | ~~Renewal dossier [c]<br>xxx., 1998a, 16041~~ |
| ~~Rat~~ | ~~degradation product ASDM~~ | ~~Oral<br>Developmental toxicity~~ | ~~NOAEL = 200 mg/kg bw/d<br>(increased incidence of dilated ureter)~~ | ~~Renewal dossier [b]<br>xxx., 1998b, 15251~~ |

[a] EFSA Journal 2016; 14(3):4419
[b] EFSA Scientific Report 2007, 120, 1-91
~~[c] Supplementary dossier for the approval renewal of nicosulfuron (N2 document; 2016)~~
[d] Endpoint considered for Screening and Tier 1 risk assessment.
[e] Endpoint considered more relevant compared to 0.3 mg a.s./kg bw/day by zRMS.
[f] Endpoint considered as most appropriate for the relevant focal species in maize by the applicant.
Endpoints in **bold** were used for the risk assessment.

#### 9.3.1.1 Justification for new endpoints

The acute ~~and chronic~~ toxicity endpoints were chosen in line with the EU reviews on mesotrione and nicosulfuron ~~and based on the supplementary dossier for the approval renewal of nicosulfuron (N2 document; 2016)~~.

Acute oral mammalian testing does not indicate an increased toxicity of the formulated product SAE053H/01 compared to the active substances, with an $LD_{50}$ greater than the highest dose tested of 2000 mg product/kg bw.

For the long-term toxicity endpoints, for mesotrione in a first approach the EU agreed endpoint of NOAEL = 0.3 mg a.s./kg bw/day was considered. However, in a refined risk assessment approach, other NOAELs of 1.2 and 2.0 mg a.s./kg bw/day where also considered, please refer to the higher tier risk assessment below. The developmental studies did not indicate higher toxicity compared to the data from multi-generation studies.

**Review Comments:**

The applicant's proposal to change the mammalian endpoint was not accepted. This issue was discussed at Pesticides Peer Review experts Meeting 136 in December 2015, where it was decided that the observed effects (e.g., litter size and pup survival) on the F2 generation should not disregard. Therefore the meeting agreed that the NOAEL of 0.3 mg/kg bw/day should be used in the risk assessment.

In zRMS opinion, the endpoint can be re-evaluated by using the benchmark dose approach. Further details can be found in the EFSA Journal 2017;15(1):4658.

Without additional data (BMD approach), it is not possible to change the mammalian endpoint.

Both active substances are metabolized in plants and some of their degradation products are present in relevant amounts. For mesotrione, MNBA and AMBA occur in amounts of > 10% TRR and for nicosulfuron ASDM and AUSN are above 10% TRR. However, all of these degradation products have been tested in acute toxicity studies with rats and did not have higher toxicity than their respective parent compound. Furthermore, in tests with earthworms and aquatic organisms, a higher toxicity of these degradation products was not found. With lower residue levels as in comparison to the parent compound, risk assessments for these degradation products are not required.

### 9.3.2 Risk assessment for spray applications

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

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

The formulation SAE053H/01 is a combination product containing two active substances (mesotrione and nicosulfuron). For this reason, potential mixture toxicity has to be accounted for.

With reference to EFSA/2009/1438, a ´toxicity per fraction´ assessment was performed (see table below). As already shown for the assessment for birds, the results indicate that the acute risk for mammals is driven by both active substances with mesotrione contributing more to the overall toxicity.

**Table 9.3-2: Toxicity per fraction assessment for mammals**

| Active substance | A.s. content in the product [g a.s./kg] | Fraction in mixture $X_{(a.s.)}$ | $LD_{50}$/NOEL of active substance [mg a.s./kg bw] | Toxicity per fraction for CA | $LD_{50}$ (mix), surrogate endpoint [mg/kg bw] | Contribution to overall toxicity [%] |
|---|---|---|---|---|---|---|
| **Acute effects** | | | | | | |
| mesotrione | 81.7 | 0.7269 | > 5000 | > 6878.8 | > 5000 | **72.7** |
| nicosulfuron | 30.7 | 0.2731 | > 5000 | > 18306.2 | | **27.3** |
| **Chronic/reproductive effects** | | | | | | |
| mesotrione | 81.7 | 0.7269 | 0.3 | 0.4 | 0.4 | **100.0** |
| nicosulfuron | 30.7 | 0.2731 | ~~≥ 3861~~ 300 | ~~≥ 14136.0~~ 1098.4 | | 0.0 |

CA: Concentration Addition
Values in **bold** indicate significant contribution (≥ 10%) to overall toxicity

The comparison of observed and predicted mixture toxicity shows that formally acute risk assessments are to be based on the product endpoint by xxx (2016) of $LD_{50}$ > 2000 mg product/kg bw, which is formally lower than the predicted endpoint for CA. However, the measured endpoint corresponds to a limit dose and therefore no increased product toxicity is indicated. Consequently, it is considered justified to present mixture toxicity risk assessments based on active substance data as was already performed for birds.

For the chronic mixture toxicity assessment, it is indicated that nicosulfuron does not contribute significantly to the predicted reproductive mixture toxicity.
~~Apart from the reduction in litter size (which was not statistically significant but was considered ecologically relevant at the Pesticides Peer Review Experts Meeting 136, 2015), no reproductive or other treatment-related effects have been observed in the two-generation mammalian study with mesotrione. The NOAEL from the study with nicosulfuron represents the highest feed concentration tested, indicating no reproductive toxicity at relevant feed concentrations. As there is no known common mode of action for mesotrione and nicosulfuron interfering with reproductive parameters at relevant feed concentrations~~ Thus, chronic mixture toxicity assessments are not considered necessary for terrestrial vertebrates.
~~However, in a comprehensive approach, reproductive mixture toxicity assessments are presented by calculating the sum TER triggers divided by TER for the individual active substances. This approach is considered to be most appropriate to account for potential combined effects not disregarding the actual toxicity data of the individual active substances, rather than the worst-case approach proposed by EFSA/2009/1438 which relates exposure of all actives expressed in equivalents of the active with the lowest available endpoint.~~

The lower tier acute and reproductive risk assessments for terrestrial vertebrates other than birds (mammals) are summarised in the following table. As there is no indication for short-term effects on reproductive performance, reproductive risk assessments are based on time-weighted average dietary doses over 21 days (i.e. $f_{TWA}$ = 0.53 based on the default (pseudo) $DT_{50}$ of 10 days).

The risk assessments are shown based on the risk envelope of 1.5 L product/ha as well as based on the actual application rate of 1.2 L product/ha.

**Table 9.3-3: Screening and first-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of SAE053H/01 in maize (1.5 L product/ha) - mesotrione**

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | **Maize** | | | | |
| **Active substance/product** | mesotrione / SAE053H/01 | | | | |
| **Application rate (g/ha)** | 1.5 L product/ha, i.e. 120 g a.s./ha mesotrione and 45 g a.s./ha nicosulfuron | | | | |
| **Acute toxicity (mg/kg bw)** | > 5000 | | | | |
| **TER criterion** | 10 | | | | |
| **Screening assessment** | | | | | |
| **Crop scenario<br>Growth stage** | **Indicator species for screening** | **$SV_{90}$** | **$MAF_{90}$** | **$DDD_{90}$<br>(mg/kg bw/d)** | **$TER_a$** |
| Maize<br>BBCH n.a. | Small herbivorous mammal | 136.4 | 1.00 | 16.36 | > 305.6 |
| **Reprod. toxicity (mg/kg bw/d)** | 0.3 | | | | |
| **TER criterion** | 5 | | | | |
| **Screening assessment** | | | | | |
| **Crop scenario<br>Growth stage** | **Indicator species for screening** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$<br>(mg/kg bw/d)** | **$TER_{lt}$** |
| Maize<br>BBCH n.a. | Small herbivorous mammal | 72.3 | 1.00 | 4.60 | **0.1** |
| **Tier 1 assessment** | | | | | |
| **Crop scenario<br>Growth stage** | **Indicator/generic focal species** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$<br>(mg/kg bw/d)** | **$TER_{lt}$** |
| Maize<br>BBCH 10 – 19 | Small insectivorous shrew | 4.2 | 1.0 × 0.53 | 0.27 | **1.1** |
| Maize<br>BBCH 10 – 29 | Small herbivorous vole | 72.3 | 1.0 × 0.53 | 4.57 | **0.1** |
| Maize<br>BBCH 10 – 29 | Small omnivorous mouse | 7.8 | 1.0 × 0.53 | 0.49 | **0.6** |

n.a.: not applicable; 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. Exposure estimates are rounded to 2 significant figures; TERs represent accurate values underlying the actual exposure estimates

**Table 9.3-4: Screening and first-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of SAE053H/01 in maize (actual application rate: 1.2 L product/ha) - mesotrione**

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | **Maize** | | | | |
| **Active substance/product** | mesotrione / SAE053H/01 | | | | |
| **Application rate (g/ha)** | 1.2 L product/ha, i.e. 96 g a.s./ha mesotrione and 36 g a.s./ha nicosulfuron | | | | |
| **Acute toxicity (mg/kg bw)** | > 5000 | | | | |
| **TER criterion** | 10 | | | | |
| **Screening assessment** | | | | | |
| **Crop scenario<br>Growth stage** | **Indicator species for screening** | **$SV_{90}$** | **$MAF_{90}$** | **$DDD_{90}$<br>(mg/kg bw/d)** | **$TER_a$** |
| Maize<br>BBCH n.a. | Small herbivorous mammal | 136.4 | 1.00 | 13.09 | > 382.0 |
| **Reprod. toxicity (mg/kg bw/d)** | 0.3 | | | | |
| **TER criterion** | 5 | | | | |
| **Screening assessment** | | | | | |
| **Crop scenario<br>Growth stage** | **Indicator species for screening** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$<br>(mg/kg bw/d)** | **$TER_{lt}$** |
| Maize<br>BBCH n.a. | Small herbivorous mammal | 72.3 | 1.0 × 0.53 | 3.68 | **0.1** |
| **Tier 1 assessment** | | | | | |
| **Crop scenario<br>Growth stage** | **Indicator/generic focal species** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$<br>(mg/kg bw/d)** | **$TER_{lt}$** |
| Maize<br>BBCH 10 – 19 | Small insectivorous shrew | 4.2 | 1.0 × 0.53 | 0.21 | **1.4** |
| Maize<br>BBCH 10 – 29 | Small herbivorous vole | 72.3 | 1.0 × 0.53 | 3.66 | **0.1** |
| Maize<br>BBCH 10 – 29 | Small omnivorous mouse | 7.8 | 1.0 × 0.53 | 0.39 | **0.8** |

n.a.: not applicable; 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. Exposure estimates are rounded to 2 significant figures; TERs represent accurate values underlying the actual exposure estimates

**Table 9.3-5: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of SAE053H/01 in maize (1.5 L product/ha) - nicosulfuron**

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | **Maize** | | | | |
| **Active substance/product** | nicosulfuron / SAE053H/01 | | | | |
| **Application rate (g/ha)** | 1.5 L product/ha, i.e. 45 g a.s./ha nicosulfuron and 120 g a.s./ha mesotrione | | | | |
| **Acute toxicity (mg/kg bw)** | > 5000 | | | | |
| **TER criterion** | 10 | | | | |
| **Crop scenario<br>Growth stage** | **Indicator species for screening** | **$SV_{90}$** | **$MAF_{90}$** | **$DDD_{90}$<br>(mg/kg bw/d)** | **$TER_a$** |
| Maize<br>BBCH n.a. | Small herbivorous mammal | 136.4 | 1.00 | 6.14 | > 814.9 |
| **Reprod. toxicity (mg/kg bw/d)** | ~~≥ 3861~~ 300 | | | | |
| **TER criterion** | 5 | | | | |
| **Crop scenario<br>Growth stage** | **Indicator species for screening** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$<br>(mg/kg bw/d)** | **$TER_{lt}$** |
| Maize<br>BBCH n.a. | Small herbivorous mammal | 72.3 | 1.00 × 0.53 | 1.72 | ~~2239.1~~ 174.0 |

n.a.: not applicable; 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. Exposure estimates are rounded to 2 significant figures; TERs represent accurate values underlying the actual exposure estimates

**Table 9.3-6: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of SAE053H/01 in maize (actual application rate: 1.2 L product/ha) - nicosulfuron**

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | **Maize** | | | | |
| **Active substance/product** | nicosulfuron / SAE053H/01 | | | | |
| **Application rate (g/ha)** | 1.2 L product/ha, i.e. 36 g a.s./ha nicosulfuron and 96 g a.s./ha mesotrione | | | | |
| **Acute toxicity (mg/kg bw)** | > 5000 | | | | |
| **TER criterion** | 10 | | | | |
| **Crop scenario<br>Growth stage** | **Indicator species for screening** | **$SV_{90}$** | **$MAF_{90}$** | **$DDD_{90}$<br>(mg/kg bw/d)** | **$TER_a$** |
| Maize<br>BBCH n.a. | Small herbivorous mammal | 136.4 | 1.00 | 4.91 | > 1018.6 |
| **Reprod. toxicity (mg/kg bw/d)** | ~~≥ 3861~~ 300 | | | | |
| **TER criterion** | 5 | | | | |
| **Crop scenario<br>Growth stage** | **Indicator species for screening** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$<br>(mg/kg bw/d)** | **$TER_{lt}$** |
| Maize<br>BBCH n.a. | Small herbivorous mammal | 72.3 | 1.00 × 0.53 | 1.38 | ~~2798.9~~ 217.5 |

n.a.: not applicable; 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. Exposure estimates are rounded to 2 significant figures; TERs represent accurate values underlying the actual exposure estimates

Accordingly, the acute and reproductive risk for mammals is indicated to be acceptable for nicosulfuron based on screening assessments for the risk envelope of 1.5 L product/ha and the actual application rate of 1.2 L product/ha. For mesotrione, the acute risk is indicated to be acceptable based on screening data while the reproductive risk requires further refinement, reference is made to Section 9.3.2.2 below.

**Mixture toxicity assessment for acute risk**

The surrogate toxicity endpoint for the mixture ($LD_{50}$ > 5000 mg/kg bw) is compared to the combined daily dietary doses of both active substances (*cf.* following table).

**Table 9.3-7: Acute mixture toxicity assessment for mammals due to the use of SAE053H/01 in maize (1.5 L product/ha) based on screening tier assessments**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Intended use** | **Maize** | | | | | | |
| **Active substance/product** | mesotrione + nicosulfuron / SAE053H/01 | | | | | | |
| **Application rate (g/ha)** | 1.5 L product/ha, i.e. 120 g a.s./ha mesotrione and 45 g a.s./ha nicosulfuron | | | | | | |
| **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)** | | **∑ $DDD_{90}$ (mg/kg bw/d)** | **$TER_a$** |
| | | | | **mesotrione** | **nicosulfuron** | | |
| Maize BBCH n.a. | Small herbivorous mammal | 136.4 | 1.00 | 16.36 | 6.14 | 22.5 | > 222.2 |

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.

Accordingly, based on screening assessments with a TER greater than the trigger of 10, an acceptable acute risk for mammals is indicated for exposure to SAE053H/01 in maize for the risk envelope of 1.5 L product/ha, which covers the actual application rate of 1.2 L product/ha.

The mixture toxicity assessment for the reproductive risk is presented under 9.3.2.2 below, as the refined daily dietary doses of mesotrione have to be considered for the calculations.

### 9.3.2.2 Higher-tier risk assessment

The refined reproductive risk assessment for mammals is based on the following main approaches:

a) Refinement of focal species in maize BBCH 12 – 19
b) Refinement of mammalian reproduction endpoint
c) Refinement of proportion of diet (PD)
d) Refinement of proportion of time spent in the treated area (PT)
e) Refinement of deposition values on feed items
f) Refinement of residue decline ($DT_{50}$) in maize plants

*a) Refinement of focal species in maize BBCH 12 – 19*

A generic field study is available from the RAR on mesotrione (2015[1] as CP 10.1.2.2/05; Grimm et al. 2013; *cf.* Letter of Access from Sipcam Oxon) which monitored the presence of small mammals and nocturnal mammals in four to five maize fields in Germany at BBCH 00 – 12 and BBCH 10 – 16, respectively. For small mammals, data from 3040 trap nights is available and additionally 20 individual wood mice were radio-tracked in 17 tracking sessions. For nocturnal mammals, 186 thermographic scan sessions were performed.

The most abundant small mammal species found during the trapping was the wood mouse (*Apodemus sylvaticus*; 121 captures, 33 individuals). Additionally, bank voles (*Myodes glareolus*; 110 captures, 26 individuals) and yellow-necked mice (*Apodemus flavicollis*; 25 captures, 10 individuals) were captured, however, only in the surrounding field area and not within the maize fields. One house mouse (*Mus musculus*) was captured once, but no common voles and shrews were captured.

This data was confirmed by the nocturnal scan sessions, which identified the majority of observed mammals as mice species (i.e. *Apodemus* or *Mus* species but no voles). Additionally three observations of medium sized/large mammals were made, i.e. rabbit (*Oryctolagus cuniculus*), marten (*Martes* sp.) and fox (*Vulpes vulpes*).

The wood mouse and, as alternative herbivorous species replacing the common vole, the rabbit/brown hare were identified as focal species in maize for BBCH 12 – 19. This choice is further supported by the guidance documents on birds and mammals risk assessment from the Northern zone countries (~~xxx. 2015[2];~~ Northern zone 2015[3]), which identified the wood mouse and the brown hare (for early application) as focal species in maize. Additionally, Jahn et al. (2014); as published by the German Environmental Agency, UBA)[4] ~~performed studies for the German Federal Environmental Agency (Umweltbundesamt, UBA) and~~ found that maize is the third most preferred habitat of the wood mouse (after cereals and beets) with wood mouse being therefore the most abundant species in maize. Also brown hares were present in maize, but to a smaller extent compared to wood mice. Shrews were not found to be present in maize and the common vole to a small extent, only.

Finally, during the EU review of mesotrione (2016) the wood mouse and the brown hare were accepted as relevant focal species in maize for early stages after germination.
It is therefore considered justified to use **the wood mouse and the brown hare as focal species** for the refined reproductive risk assessment.

**Review Comments:**

Agree that the focal species for maize at BBCH growth stages of 12-18 (according to the GAP) are wood mouse and brown hare.

*b) Refinement of mammalian reproduction endpoint*

The EU agreed reproductive endpoint of mesotrione for mammals was determined as 0.3 mg a.s./kg bw/d based on the three-generation study in rats (Milburn 1997a) where a non-statistically significant reduction

[1] Renewal Assessment Report (RAR) on mesotrione, Volume 3, B.9 (PPP), Callisto 100SC

[2] ~~Aae, R., Hage, M., Heen, G.S., Bakken, V. and Isaksen, K. (2015). Risk assessment of agricultural pesticides for birds and mammals in Southeast Norway – Recommendations for focal species. Report to the Norwegian Food Safety Authority, December, 2015~~

[3] Northern zone (2015). Pesticide Risk Assessment For Birds And Mammals. Selection of relevant species and development of standard scenarios for higher tier risk assessment in the Northern Zone in accordance with Regulation EC 1107/2009. Version 1.4, April 2016

[4] Jahn, T., Hötker, H., Oppermann, R., Bleil, R. and Vele, L. (2014). Protection of biodiversity of free living birds and mammals in respect of the effects of pesticides. Published by: Umweltbundesamt, Dessau-Roßlau, Texte 30/2014, April 2014.

in litter size was observed in the F2 generation.

Considering the short duration of the season of use, during which SAE053H/01 is applied together with the limited number of applications (only one) and the fast degradation of mesotrione in feed items ($DT_{50}$ = 0.46 days) as shown by residue studies (see Section 9.3.2.2 *f)* below), the continuous exposure of individuals during three generations is not considered relevant for the intended application of SAE053H/01. Therefore, it is reasonable to consider data from the first generation (F0 parents, F1 pups) from the multi-generation study.

Additionally, an expert statement is available (Guckland et al., 2019, KCP 10.1.2.2/01) which underlines the difference in sensitivity for rats and mice and extrapolates the sensitivity of lagomorphs as well. With regard to the selection of wood mouse and brown hare as focal species as discussed above, it is considered justified to take this expert statement into account.
One of the approaches in the statement was to compare the available multi-generation data on rats (Milburn 1997a) and mice (Anonymous, 1997a) and to point out the high difference in sensitivity, since for rats effects on litter size were observed at 1.2 mg a.s./kg bw/d, whereas for mice no effects had been found up to the highest dose tested (7000 ppm in the diet), but instead the NOAEL in the study was based on increased organ weights (testis and kidney).
In addition, the mode of action of mesotrione was explained in detail and put into perspective with regard to the relevance of rat and mouse data. As mesotrione inhibits one step of the tyrosine catabolism which leads to elevated levels of tyrosine in plasma, the tyrosine-response of mouse is more relevant for the assessment of toxicity to humans and wild mammals. Rats on the contrary, have a much higher sensitivity to hypertyrosinemia. Consequently, for the focal species wood mouse, the endpoint from the multi-generation study with mice is considered the most relevant.
For lagomorphs, no multi-generation data is available, however the expert statement compared relative sensitivities of rats, mice and lagomorphs to be able to extrapolate the most likely sensitivity of lagomorphs compared to rats and mice. For this purpose, sensitivities to an HPPD-inhibitor much more potent than mesotrione, namely nitisinone, were compared and found that mouse and rabbit are much less sensitive than rat. This was also confirmed for mesotrione. Additionally, the concentrations required to raise the tyrosine levels in the blood plasma had been compared and showed that here again mouse and rabbit required much higher concentrations to reach the same level of tyrosine in the blood plasma compared to rat.
As a consequence, sensitivity of mouse and lagomorphs have been concluded to be similar whereas sensitivity of rats is much higher. Considering the focal species being wood mouse and lagomorph, the endpoint from the multi-generation study on mice is equally relevant for lagomorphs, whereas the endpoint from the study in rats is considered less relevant for this specific risk assessment.

In conclusion, **the NOAEL of 2 mg a.s./kg bw/d from the multi-generation study in mice is considered the most relevant one for the risk assessment of wild mammals with wood mouse and lagormoph being the identified focal species** and common shrew being shown for completeness. In an alternative and **very conservative approach**, the refined risk assessment will also be shown based on the NOAEL of 1.2 mg a.s./kg bw/d.

**Review Comments:**

The applicant's proposal to change the mammalian endpoint was not accepted. This issue was discussed at Pesticides Peer Review experts Meeting 136 in December 2015, where it was decided that the observed effects (e.g., litter size and pup survival) on the F2 generation should not disregard. Therefore the meeting agreed that the NOAEL of 0.3 mg/kg bw/day should be used in the risk assessment.

In zRMS opinion, the endpoint can be re-evaluated by using the benchmark dose approach. Further details can be found in the EFSA Journal 2017;15(1):4658.

Without additional data (BMD approach), it is not possible to change the mammalian endpoint.

*c) Refinement of proportion of diet*

The default diets of the identified focal species wood mouse and brown hare have been refined to represent a more realistic situation.

For the wood mouse, detailed data is available from the Northern Zone Guidance (2015)[5], which refers to data from Pelz (1989)[6], Green (1979)[7] and Rogers & Gorman (1995b)[8] and was specifically adjusted for maize. The table including PD values for maize from BBCH 00 to 39 is shown below.

**Table 9.3-8: Proportions of diet for the wood mouse feeding in maize (Northern Zone 2015, Appendix 4)**

| Food category | BBCH 0-9 (April) | BBCH 0-9 (May) | BBCH 10-29 (May) | BBCH 10-29 (June) | BBCH 30-39 (June) | BBCH 30-39 (July) |
|---|---|---|---|---|---|---|
| Grasses and cereals | - | - | 22 % | 12 % | 8 % | 10 % |
| Non-grass herbs | - | - | - | - | 4 % | 5 % |
| Cereal grain/ large seeds | 29 % | 29 % | 5 % | 5 % | 5 % | 5 % |
| Weed seeds / small seeds | - | 5 % | 5 % | 35 % | 35 % | 29 % |
| Ground-dwelling arthropods and soil invertebrates | 71 % | 66 % | 68 % | 48 % | 48 % | 51 % |

The relevant BBCH for the application of SAE053H/01 in maize is 12 – 19, therefore two different possibly relevant PD were identified for the wood mouse (BBCH 10 – 29 in May and in June). To determine the worst-case exposure, both PD refinements were examined for the reproductive risk assessment.

[5] Northern zone (2015). Pesticide Risk Assessment For Birds And Mammals. Selection of relevant species and development of standard scenarios for higher tier risk assessment in the Northern Zone in accordance with Regulation EC 1107/2009. Version 1.4, April 2016.

[6] Pelz, H. J. (1989) Ecological aspects of damage to sugar beet seeds by *Apodemus sylvaticus*. In: *Mammals as Pests* (ed. Putman, R.J.) 34-48, Chapman and Hall, London.

[7] Green, R. (1979). The ecology of wood mice (*Apodemus sylvaticus*) on arable farmland. *J. Zool*. 118: 357-377.

[8] Rogers, L. M. and Gorman, M. L. (1995). The diet of the wood mouse *Apodemus sylvaticus* on set-aside land. *J. Zool., Lond.* 235: 77-83.

**Review Comments:**

The evaluation of PD refinements for mouse, proposed by the applicant, is not necessary as safe use can be identified using standard SVmean (maize BBCH 10-29, combination diet) and PT value of 0.139. Thus, the risk assessment is updated accordingly.

The realistic diet for the brown hare was considered to be 100 % maize shoots which belong to the food category 'grasses and cereals'.

**Review Comments:**

It should be highlighted that the default RUD values, diet composition and body weight of focal species (in result - FIR/bw) were taken from EFSA B&M guidance. Only $f_{TWA}$ and PT values were refined. Thus, there was no need to carry out such detailed calculations as was done by the Applicant in tables below. Therefore, zRMS performed new calculations using accepted $f_{TWA}$ and PT values in a simplified format.

~~The respective FIR/bw calculations for the wood mouse and the brown hare based on the PD refinements are shown below.~~

~~**Table 9.3-9: Calculation of FIR/bw for the wood mouse and the brown hare**~~

| ~~Feed item~~ | ~~[%]~~ | ~~$FE_i$ [kJ/g dry]~~ | ~~$AE_i$ [%]~~ | ~~$MC_i$ [%]~~ | ~~$FE_i$ fresh [kJ/g fresh]~~ | ~~$FE_{i,\ total}$ fresh [kJ/g fresh]~~ | ~~DEE [kJ/d]~~ | ~~$FIR_{i,\ total}$ fresh [g fresh weight/d]~~ | ~~Body weight [g]~~ | ~~FIR / bw~~ |
|---|---|---|---|---|---|---|---|---|---|---|
| ~~Wood mouse, BBCH 10 – 29, May~~ | | | | | | | | | | |
| ~~Grasses & cereals~~ | ~~22~~ | ~~17.6~~ | ~~47~~ | ~~76.4~~ | ~~0.43~~ | ~~6.10~~ | ~~58.83~~ | ~~9.64~~ | ~~21.7~~ | ~~0.44~~ |
| ~~Cereal grain~~ | ~~5~~ | ~~18.4~~ | ~~84~~ | ~~14.7~~ | ~~0.66~~ | | | | | |
| ~~Weed seeds~~ | ~~5~~ | ~~21.7~~ | ~~84~~ | ~~9.9~~ | ~~0.82~~ | | | | | |
| ~~Ground-dw. arthropods~~ | ~~68~~ | ~~22.7~~ | ~~87~~ | ~~68.8~~ | ~~4.19~~ | | | | | |
| ~~Wood mouse, BBCH 10 – 29, June~~ | | | | | | | | | | |
| ~~Grasses & cereals~~ | ~~12~~ | ~~17.6~~ | ~~47~~ | ~~76.4~~ | ~~0.23~~ | ~~9.60~~ | ~~58.83~~ | ~~6.13~~ | ~~21.7~~ | ~~0.28~~ |
| ~~Cereal grain~~ | ~~5~~ | ~~18.4~~ | ~~84~~ | ~~14.7~~ | ~~0.66~~ | | | | | |
| ~~Weed seeds~~ | ~~35~~ | ~~21.7~~ | ~~84~~ | ~~9.9~~ | ~~5.75~~ | | | | | |
| ~~Ground-dw. arthropods~~ | ~~48~~ | ~~22.7~~ | ~~87~~ | ~~68.8~~ | ~~2.96~~ | | | | | |
| ~~Brown hare~~ | | | | | | | | | | |
| ~~Maize shoots (grasses & cereals)~~ | ~~100~~ | ~~17.6~~ | ~~47~~ | ~~76.4~~ | ~~1.95~~ | ~~1.95~~ | ~~2363.4~~ | ~~1210.66~~ | ~~3800~~ | ~~0.32~~ |

~~$FE_i$: Food Energy; $AE_i$: Assimilation Efficiency; $MC_i$: Moisture Content; DEE: Daily Energy Expenditure; FIR: Food Intake Rate; bw: body weight~~

*d) Refinement of PT*

The proportion of time that is spent in the treated area can be refined for the wood mouse based on the study

by Grimm et al. (2013; *cf.* Letter of Access from Sipcam Oxon), as submitted in the RAR on mesotrione (2015)[9] as CP 10.1.2.2/05, which tracked 14 individual wood mice in 17 tracking sessions in freshly germinated maize fields from BBCH 10 to 16. This BBCH range includes most of the intended use of SAE053H/01 in maize at BBCH 12 – 19 and therefore the study is considered applicable. The resulting PT values ranged from 0.4 to 13.9%. As the data set was relatively small, it is considered appropriate to use the worst-case PT value rather than the 90th percentile or a mean value. **For the wood mouse a PT value of 0.139 was therefore used** for the refined reproductive risk assessment of wood mice~~. as supportive information in a weight-of-evidence approach but not quantitative for the risk assessment.~~

~~Additional data is available from the Northern Zone Guidance (2018)[Ref! No bookmarks exist!]. Since no data for maize in spring is available, the best fitting scenario was considered to be newly drilled winter cereals (September – November), where a similar coverage compared to maize at BBCH 12 – 18 may be assumed. In the Northern Zone Guidance document it was noted that for this scenario "all animals" instead of "consumers only" should be considered, since animals in the original study had been trapped in or adjacent to newly drilled cereal fields (reference is made to Table 6.77 in the guidance). The corresponding 90th percentile PT value is 0.37. In a worst-case approach, and considering that the monitoring study by Grimm et al. (2013) may have underestimated the PT since BBCH 17 and 18 were not covered, the PT from the Northern Zone guidance of 0.37 was chosen for the risk assessment refinement as the most appropriate value. For comparison, the refined risk assessment will also be shown based on the "consumers only" 90th percentile PT value of 0.51, although this value is considered overly conservative as mentioned above.~~

No specific PT data was acquired for the brown hare in maize fields, however, the Guidance document for higher tier risk assessment in the Northern Zone (2015)[10] indicated that hares are abundant in arable crops with 90th percentile PT values between 47 and 100% depending on the season and crop ~~(data from Prosser 2010, UK Food and Environment Research Agency[11])~~. The application of SAE053H/01 in maize is intended at BBCH 12 – 19. Optimal maize sowing dates across the central zone range from late March to May (Rüdelsheim & Smets 2011[12]) with BBCH 12 – 19 being passed latest at the end of May. It is therefore considered appropriate to take into account the PT data on brown hares for spring (March to May) which, however, indicate a 90th percentile PT value for all crops of 1.00 (consumers only). Therefore, **no refined PT value was taken into account for the brown hare** but the default of 1 was used.

**Review Comments:**

The PT value of 0.139 for wood mouse was accepted at the EU level during mesotrione evaluation and, thus, will be used in the current assessment.

*e) Refinement of deposition values*

According to EFSA/2009/1438, deposition values might be refined based on FOCUS groundwater values[13]. Due to the application at BBCH 12 – 19, the deposition factor in maize was refined to 0.75 for the feed items 'cereal grain', 'small seeds' and 'ground-dwelling arthropods' for the wood mouse. The deposition values were not refined for the feed category 'grasses and cereals' as this category might not be intercepted by the crop.

[9] Renewal Assessment Report (RAR) on mesotrione, Volume 3, B.9 (PPP), Callisto 100SC

[10] Northern zone (2015). Pesticide Risk Assessment For Birds And Mammals. Selection of relevant species and development of standard scenarios for higher tier risk assessment in the Northern Zone in accordance with Regulation EC 1107/2009. Version 1.4, April 2016.

~~[11] Prosser, P. (2010). Consolidation of bird and mammal PT data for use in risk assessment. Food and Environmental Research Agency, UK, March 2010.~~

[12] Rüdelsheim, P.L.J and Smets, G. (2011). Baseline information on agricultural practices in the EU – Maize (*Zea mays* L.). Study performed for EuropaBio aisbl, July 2011.

[13] FOCUS (2014). Generic Guidance for Tier 1 FOCUS Ground Water Assessments. Version 2.2, May 2014.

For the brown hare, no deposition refinement was performed as it is assumed that the diet comprises 100% early maize shoots, i.e. 'grasses and cereals', and these will not be intercepted by other plants.

**Review Comments:**

The applicant proposal to use refined DF was not accepted for maize BBCH 12-18. For early crop' growth stages is not recommended to change the default value. Thus, the default value of 1 from B&M guidance will be used in the risk assessment.

*f) Refinement of residue decline in maize*

Two residue decline studies were performed to establish realistic $DT_{50}$ values for mesotrione in maize.

The first study was performed by Bakker (2016, KCP 8.10/01) to determine the amount and kinetics of residues of mesotrione in maize plants after application of a 10% SC product at 1 x 1.5 L product/ha under field conditions at BBH 12 – 18. The study was performed in South West France and The Netherlands with three trials in each country. For a detailed study summary please refer to Section B7 (Residues) of this submission.

The second study was performed by van de Sandt (2019, KCP 8.10/02) to determined the amount and decline of residues of mesotrione in maize plants after application of a 10% SC product at 1 x 1.5 L product/ha under field conditions at BBCH 12 – 18. This study was performed in The Netherlands with four trials. A detailed summary is presented in Section B7 (Residues).

Both studies have been used for the determination of a combined $DT_{50}$ value (Cooke 2019, KCP 10.1.1.2/02), however, from the first study two residue trials in The Netherlands had to be excluded as the measured residues show a period of rapid dissipation in the first 6 – 12 hours after application which might have been caused by rainfall. The data used for the combined $DT_{50}$ determination is shown below.

**Table 9.3-10: Residue decline of mesotrione in maize plants (Bakker 2016 and van de Sandt 2019)**

| Study reference | Trial | $DT_{50}$ [d] | $\chi^2$ error [%] | Kinetic model |
|---|---|---|---|---|
| Bakker (2016) | JS001LRM-01 (France) | 0.69 | 13.6 | SFO |
| | JS001LRM-02 (France) | 0.42 | 6.8 | SFO |
| | JS001LRM-03 (France) | 0.33 | 6.7 | SFO |
| | JS001LRM-06 (The Netherlands) | 0.45 | 4.6 | SFO |
| van de Sandt (2019) | S17-05218-01 (The Netherlands) | 0.92 | 12.5 | SFO |
| | S17-05218-02 (The Netherlands) | 0.70 | 10.3 | SFO |
| | S17-05218-03 (The Netherlands) | 0.54 | 13.7 | FOMC |
| | S17-05218-04 | 0.13 | 10.4 | FOMC |

| Study reference | Trial | $DT_{50}$ [d] | $\chi^2$ error [%] | Kinetic model |
|---|---|---|---|---|
| | (The Netherlands) | | | |
| **Geometric mean** | | **0.46** | | |

SFO: Single first order model; FOMC: First-order multi compartment model

As a result, the **geometric mean $DT_{50}$ of 0.46 days** can be used for the refined risk assessment. This translates into an **$f_{TWA}$ value of 0.03**.

**Review Comments:**

The residue trials reported by Bakker (2016) no. JS001LRM-06 and van de Sandt (2019) were assessed and accepted. Based on the results of those 5 trials (highlighted in grey for transparency) new mean value was calculated which is 0.459.

Thus, the $DT_{50}$ of 0.46 days proposed by applicant can be use in the risk assessment ($f_{TWA}$ for grasses and cereal shoots is 0.031).

Although not considered acceptable for determination of a specific $DT_{50}$ during EU review of mesotrione, the study by White (2001) on residues of mesotrione in maize performed on fields in Canada, which was submitted for the EU review of mesotrione, does confirm the results of Bakker (2016, KCP 8.10/01) and van de Sandt (2019) as the residues in the White (2001) study were considered to be less than 1% after 3-4 days in maize plants. In the study by Bakker (2016), the residues were ≤ 10% after a maximum of 2.33 days and in the study by van de Sandt (2019) the residues were in the range of 0.8 – 2.2% after four days.

The overall refined risk assessment for mesotrione for the wood mouse and the brown hare based on the refinements mentioned above is presented in the following table for ~~the risk envelope of 1.5 L product/ha as well as for~~ the actual application rate of 1.2 L product/ha.

~~Two different scenarios are presented, first the realistic worst-case scenario (a) which includes the refined NOAEL of 2.0 mg a.s./kg bw/day and the PT of 0.37 for the wood mouse, and second the overly conservative scenario (b) which considers the NOAEL of 1.2 mg a.s./kg bw/day and the PT of 0.51 for the wood mouse.~~

**Table 9.3-11: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of SAE053H/01 in maize (1.5 L product/ha) – mesotrione – scenario (a)**

| | |
|---|---|
| **Intended use** | **Maize** |
| **Active substance/product** | mesotrione / SAE053H/01 |
| **Application rate (g/ha)** | 1.5 L product/ha, i.e. 120 g a.s./ha mesotrione and 45 g a.s./ha nicosulfuron |
| **Reprod. toxicity (mg/kg bw/d)** | 2.0* |
| **TER criterion** | 5 |

| **Focal species** | **Food category, % in diet** | **FIR/bw** | **$RUD_m$ × DF** (mg/kg food) | **$MAF_m$ × TWA** | **PT** | **$DDD_m$** (mg/kg bw/d) | **$TER_{lt}$** |
|---|---|---|---|---|---|---|---|
| "omnivorous" *Apodemus sylvaticus* (Wood mouse) BBCH 12 – 19<br><br>based on PD refinement for BBCH 10-29, **May** | Grasses and cereals, 22 %* | 0.44* | 54.2 × 1.0 | 1.0 × 0.03* | 0.139<br>0.27* | 0.02-0.07 | 77.48<br>39.14 |
| | Cereal grain, 5 %* | | 15 × 0.75* | 1.0 × 0.53 | | | |
| | Small seeds, 5 %* | | 40.2 × 0.75* | | | | |
| | Ground-dwelling invertebrates without interception, 68 %* | | 7.5 × 0.75* | | | | |
| "omnivorous" *Apodemus sylvaticus* (Wood mouse) BBCH 12 – 19<br><br>based on PD refinement for BBCH 10-29, **June** | Grasses and cereals, 12 %* | 0.28* | 54.2 × 1.0 | 1.0 × 0.03* | 0.139<br>0.27* | 0.04-0.09 | 86.48<br>21.22 |
| | Cereal grain, 5 %* | | 15 × 0.75* | 1.0 × 0.53 | | | |
| | Small seeds, 35 %* | | 40.2 × 0.75* | | | | |
| | Ground-dwelling invertebrates without interception, 48 %* | | 7.5 × 0.75* | | | | |
| "herbivorous" *Lepus europaeus* (Brown hare) * BBCH 12 – 19 | Maize shoots (grasses and cereals), 100 %* | 0.32* | 54.2 × 1.00 | 1.0 × 0.03* | 1.00 | 0.06 | 32.17 |

FIR/bw: Food intake rate per body weight; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger. Exposure estimates are rounded to 2 significant figures; TERs represent accurate values underlying the actual exposure estimates.
* refined parameters, further details in the text

**Table 9.3-12: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of SAE053H/01 in maize (1.5 L product/ha) – mesotrione – scenario (b)**

| | |
|---|---|
| Intended use | Maize |
| Active substance/product | mesotrione / SAE053H/01 |
| Application rate (g/ha) | 1.5 L product/ha, i.e. 120 g a.s./ha mesotrione and 45 g a.s./ha micotiafuret |
| Reprod. toxicity (mg/kg bw/d) | 1.2* |
| TER criterion | 5 |

| Focal species | Food category; % in diet | FIR/bw | $RUD_{st} \times DF$ (mg/kg food) | $MAF_{st} \times cPWA$ | PT | $DDD_{st}$ (mg/kg bw/d) | $TER_{st}$ |
|---|---|---|---|---|---|---|---|
| "omnivorous" *Apodemus sylvaticus* (Wood mouse) BBCH 12 – 19<br><br>based on PD refinement for BBCH 10-29, May | Grasses and cereals; 22 %* | 0.44* | 54.2 × 1.0 | 1.0 × 0.03* | 0.51* | 0.09 | 42.67 |
| | Cereal grain; 5 %* | | 15 × 0.75* | 1.0 × 0.53 | | | |
| | Small seeds; 5 %* | | 40.2 × 0.75* | | | | |
| | Ground-dwelling invertebrates without interception; 68 %* | | 7.5 × 0.75* | | | | |
| "omnivorous" *Apodemus sylvaticus* (Wood mouse) BBCH 12 – 19<br><br>based on PD refinement for BBCH 10-29, June | Grasses and cereals; 22 %* | 0.28* | 54.2 × 1.0 | 1.0 × 0.03* | 0.51* | 0.12 | 9.24 |
| | Cereal grain; 5 %* | | 15 × 0.75* | 1.0 × 0.53 | | | |
| | Small seeds; 5 %* | | 40.2 × 0.75* | | | | |
| | Ground-dwelling invertebrates without interception; 68 %* | | 7.5 × 0.75* | | | | |
| "herbivorous" *Lepus europaeus* (Brown hare) – BBCH 12 – 19 | Maize sheet; Grasses and cereals; 100 %* | 0.22* | 54.2 × 1.00 | 1.0 × 0.03* | 1.00 | 0.06 | 10.20 |

FIR/bw: Food intake rate per body weight; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger. Exposure estimates are rounded to 2 significant figures. TERs represent accurate values underlying the actual exposure estimates.
* refined parameters, further details in the text

**Table 9.3-13: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of SAE053H/01 in maize (1.2 L product/ha) – mesotrione – scenario (a)**

| | |
|---|---|
| **Intended use** | **Maize** |
| **Active substance/product** | mesotrione / SAE053H/01 |
| **Application rate (g/ha)** | 1.2 L product/ha, i.e. 96 g a.s./ha mesotrione and 36 g a.s./ha nicosulfuron |
| **Reprod. toxicity (mg/kg bw/d)** | 2.0* |
| **TER criterion** | 5 |

| **Focal species** | **Food category, % in diet** | **FIR/bw** | **$RUD_m$ × DF** (mg/kg food) | **$MAF_m$ × TWA** | **PT** | **$DDD_m$** (mg/kg bw/d) | **$TER_{lt}$** |
|---|---|---|---|---|---|---|---|
| "omnivorous" *Apodemus sylvaticus* (Wood mouse) BBCH 12—19<br><br>based on PD refinement for BBCH 10-29, **May** | Grasses and cereals, 22 %* | 0.44* | 54.2 × 1.0 | 1.0 × 0.03* | 0.439<br>1.27* | 0.02-0.05 | 96-85<br>36-39 |
| | Cereal grain, 5 %* | | 15 × 0.75* | 1.0 × 0.53 | | | |
| | Small seeds, 5 %* | | 40.2 × 0.75* | | | | |
| | Ground-dwelling invertebrates without interception, 68 %* | | 7.5 × 0.75* | | | | |
| "omnivorous" *Apodemus sylvaticus* (Wood mouse) BBCH 12—19<br><br>based on PD refinement for BBCH 10-29, **June** | Grasses and cereals, 12 %* | 0.28* | 54.2 × 1.0 | 1.0 × 0.03* | 0.439<br>0.27* | 0.02-0.08 | 70-60<br>26-52 |
| | Cereal grain, 5 %* | | 15 × 0.75* | 1.0 × 0.53 | | | |
| | Small seeds, 35 %* | | 40.2 × 0.75* | | | | |
| | Ground-dwelling invertebrates without interception, 48 %* | | 7.5 × 0.75* | | | | |
| "herbivorous" *Lepus europaeus* (Brown hare) * BBCH 12—19 | Maize shoots (grasses and cereals), 100 %* | 0.32* | 54.2 × 1.00 | 1.0 × 0.03* | 1.00 | 0.05 | 40.22 |

FIR/bw: Food intake rate per body weight; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger. Exposure estimates are rounded to 2 significant figures; TERs represent accurate values underlying the actual exposure estimates.
* refined parameters, further details in the text

Table 9.3.14: Higher tier assessment of the long-term/reproductive risk for mammals due to the use of SAE053H/01 in maize (1.2 L product/ha) – mesotrione – scenario (b)

| | |
|---|---|
| Intended use | Maize |
| Active substance/product | mesotrione / SAE053H/01 |
| Application rate (g/ha) | 1.2 L product/ha, i.e. 96 g a.s./ha mesotrione and 36 g a.s./ha nicosulfuron |
| Repro. toxicity (mg/kg bw/d) | 1.2* |
| TER criterion | 5 |

| Focal species | Food category, % in diet | FIR/bw | $RUD_{st} \times DF$ (mg/kg food) | $MAF_{st} \times cPWA$ | PT | $DDD_{st}$ (mg/kg bw/d) | $TER_{st}$ |
|---|---|---|---|---|---|---|---|
| "omnivorous" *Apodemus sylvaticus* (Wood mouse) BBCH 12 – 19<br><br>based on PD refinement for BBCH 10-29, May | Grasses and cereals, 22 %* | 0.44* | 54.2 × 1.0 | 1.0 × 0.03* | 0.51* | 0.08 | 45.48 |
| | Cereal grain, 5 %* | | 15 × 0.75* | 1.0 × 0.53 | | | |
| | Small seeds, 5 %* | | 40.2 × 0.75* | | | | |
| | Ground dwelling invertebrates without interception, 68 %* | | 7.5 × 0.75* | | | | |
| "omnivorous" *Apodemus sylvaticus* (Wood mouse) BBCH 12 – 19<br><br>based on PD refinement for BBCH 10-29, June | Grasses and cereals, 12 %* | 0.28* | 54.2 × 1.0 | 1.0 × 0.03* | 0.51* | 0.10 | 71.55 |
| | Cereal grain, 5 %* | | 15 × 0.75* | 1.0 × 0.53 | | | |
| | Small seeds, 35 %* | | 40.2 × 0.75* | | | | |
| | Ground dwelling invertebrates without interception, 48 %* | | 7.5 × 0.75* | | | | |
| "herbivorous" *Lepus europaeus* (Brown hare) – BBCH 12 – 19 | Maize sheet, Grasses and cereals, 100 %* | 0.22* | 54.2 × 1.00 | 1.0 × 0.03* | 1.00 | 0.05 | 64.12 |

FIR/bw: Food intake rate per body weight; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger. Exposure estimates are rounded to 2 significant figures. TERs represent accurate values underlying the actual exposure estimates.
*: refined parameters, further details in the text

| | | |
|---|---|---|
| **Intended use** | | **Maize** |
| **Active substance/product** | | mesotrione / SAE053H/01 |
| **Application rate (g/ha)** | | 1.2 L product/ha, i.e. 96 g a.s./ha mesotrione and 36 g a.s./ha nicosulfuron |
| **Reprod. toxicity (mg/kg bw/d)** | | 0.3 |
| **TER criterion** | | 5 |

| **Growth stage** | **Focal species** | **$SV_m$** | **$MAF_m$ × TWA** | **PT** | **$DDD_m$ (mg/kg bw/d)** | **$TER_{lt}$** |
|---|---|---|---|---|---|---|
| BBCH 12-19 | *Lepus europaeus* (Brown hare) * | 17.3[1)] (100% grass) | 1.0 × 0.031* | 1 | 0.051 | 5.9 |
| BBCH 12-19 | Small omnivorous mouse | 7.8[2)] | 1.0 × 0.53 | 0.139 * | 0.055 | 5.4 |

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.
1) SVm from EFSA B&M guidance (2009) for Brown hare (grassland scenario)
2) SVm from EFSA B&M guidance (2009) for Wood mouse (maize scenario)
* refined parameters

Consequently, even when assuming overly conservative assumptions, an acceptable reproductive risk is indicated for all focal species from the exposure to mesotrione at ~~120 g a.s./ha (risk envelope) as well as~~ 96 g a.s./ha (actual application rate) in maize with TERs clearly exceeding the relevant trigger of 5.

~~**Mixture toxicity assessment for reproductive risk**~~

~~Reproductive mixture toxicity based on the sum of TER triggers divided by TER is presented based on refined TERs for mesotrione and additionally calculated Tier 1 TERs for nicosulfuron for the wood mouse and the brown hare.~~

~~As the screening risk assessment for nicosulfuron performed above did not include TER calculations for the wood mouse and the brown hare, these calculations are shown in the following table. For the assessments the PD values and respective FIR/bw were adapted as determined for mesotrione to ensure comparability of the two data sets, however, no further refinements were included in the calculations in a conservative approach.~~

~~Reproductive mixture toxicity is presented for the risk envelope of 1.5 L product/ha, which covers the actual application rate of 1.2 L product/ha, and both scenarios for mesotrione, once based on a realistic worst-case (a) and once based on overly conservative assumptions (b).~~

**Table 9.3.1.5: Tier 1 assessment of the long-term/reproductive risk for wood mouse and brown hare due to the use of SAE053H/01 in maize (1.5 L product/ha) – nicosulfuron**

| | |
|---|---|
| **Intended use**<br>**Active substance/product**<br>**Application rate (g/ha)** | **Maize**<br>nicosulfuron / SAE053H/01<br>1.5 L product/ha, i.e. 45 g a.s./ha nicosulfuron and 120 g a.s./ha mesotrione |
| **Reprod. toxicity (mg/kg bw/d)**<br>**TER criterion** | ≥ 2861-300<br>5 |

| **Focal species** | **Food category, % in diet** | **FIR/bw** | **$RUD_m$ × DF** (mg/kg food) | **$MAF_m$ × TWA** | **PT** | **$DDD_m$** (mg/kg bw/d) | **$TER_{lt}$** |
|---|---|---|---|---|---|---|---|
| "omnivorous" *Apodemus sylvaticus* (Wood mouse) BBCH 12—19<br><br>based on PD refinement for BBCH 10-29, **May** | Grasses and cereals, 22 %* | 0.44* | 54.2 × 1.0 | 1.0 × 0.53 | 1.0 | 0.21 | ≥ 1841.2–1430.6 |
| | Cereal grain, 5 %* | | 15 × 1.0 | | | | |
| | Small seeds, 5 %* | | 40.2 × 1.0 | | | | |
| | Ground-dwelling invertebrates without interception, 68 %* | | 7.5 × 1.0 | | | | |
| "omnivorous" *Apodemus sylvaticus* (Wood mouse) BBCH 12—19<br><br>based on PD refinement for BBCH 10-29, **June** | Grasses and cereals, 12 %* | 0.28* | 54.2 × 1.0 | 1.0 × 0.53 | 1.0 | 0.17 | ≥ 22900.7–1787.1 |
| | Cereal grain, 5 %* | | 15 × 1.0 | | | | |
| | Small seeds, 35 %* | | 40.2 × 1.0 | | | | |
| | Ground-dwelling invertebrates without interception, 48 %* | | 7.5 × 1.0 | | | | |
| "herbivorous" *Lepus europaeus* (Brown hare) BBCH 12—19 | Maize shoots (grasses and cereals), 100 %* | 0.32* | 54.2 × 1.00 | 1.0 × 0.53 | 1.0 | 0.41 | ≥ 9275.0–723.4 |

n.a.: not applicable; 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. Exposure estimates are rounded to 2 significant figures; TERs represent accurate values underlying the actual exposure estimates

**Table 9.3-16: Reproductive mixture toxicity assessment for mammals due to the use of SAE053H/01 in maize (1.5 L product/ha) based on higher tier assessments scenario (a)**

| | |
|---|---|
| **Intended use** | **Maize** |
| **Active substance/product** | mesotrione + nicosulfuron / SAE053H/01 |
| **Application rate (g/ha)** | 1.5 L product/ha, i.e. 120 g a.s./ha mesotrione and 45 g a.s./ha nicosulfuron |
| **Reprod. toxicity (mg/kg bw/d)** | 2.0 (mesotrione) / ≥ 3961.300 (nicosulfuron) |
| **TER criterion** | 5 |

**Higher tier assessment**

| **Crop scenario Growth stage** | **Generic focal species** | **$TER_{lt}$** | | **Σ (TER trigger/TER)** |
|---|---|---|---|---|
| | | **mesotrione** | **nicosulfuron** | |
| Maize BBCH 10 – 29, May | "omnivorous" *Apodemus sylvaticus* (Wood mouse) | 77.48 29.14 | ≥ 18412.1 1430.6 | ≤ 0.065 0.175 |
| Maize BBCH 10 – 29, June | "omnivorous" *Apodemus sylvaticus* (Wood mouse) | 56.48 21.22 | ≥ 22000.7 1787.3 | ≤ 0.089 0.238 |
| Maize BBCH 12 – 19 | "herbivorous" *Lepus europaeus* (Brown hare) | 32.17 | ≥ 9273.0 728.4 | ≤ 0.156 0.162 |

The sum of (TER trigger/TER) shown in **bold** exceed the relevant trigger of 1.

Table 9.3.17: Reproductive mixture toxicity assessment for mammals due to the use of SAE053H/01 in maize (1.5 L product/ha) based on higher tier assessments scenario (b)

| | | | | |
|---|---|---|---|---|
| Intended use | Maize | | | |
| Active substance/product | mesotrione + nicosulfuron / SAE053H/02 | | | |
| Application rate (g/ha) | 1.5 L product/ha, i.e. 120 g a.s./ha mesotrione and 45 g a.s./ha nicosulfuron | | | |
| Reprod. toxicity (mg/kg bw/d) | 1.2 (mesotrione) / ≥ 3961.200 (nicosulfuron) | | | |
| TER criterion | 5 | | | |
| Higher tier assessment | | | | |
| Crop scenario<br>Growth stage | Generic focal species | TERs | | Σ (TER trigger/TER) |
| | | mesotrione | nicosulfuron | |
| Maize<br>BBCH 10—29,<br>May | Omnivorous<br>*Apodemus sylvaticus*<br>(Wood mouse) | 12.67 | 1439.6 | 0.398 |
| Maize<br>BBCH 10—29,<br>June | Omnivorous<br>*Apodemus sylvaticus*<br>(Wood mouse) | 9.24 | 1787.1 | 0.544 |
| Maize<br>BBCH 12—19 | Herbivorous<br>*Lepus europaeus*<br>(Brown hare) | 19.20 | 728.4 | 0.966 |

The sum of (TER trigger/TER) shown in bold exceed the relevant trigger of 1.

Consequently, even when assuming overly conservative input parameters, with the sum of TER trigger divided by TERs being clearly below the trigger of 1, an acceptable reproductive risk for mammals is indicated for exposure to SAE053H/01 in maize based on the risk envelope of 1.5 L product/ha which covers the actual application rate of 1.2 L product/ha.

### 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 ($K_{oc}$ < 500 L/kg) or 3000 in the case of more sorptive substances ($K_{oc}$ ≥ 500 L/kg).

Due to the risk indicated for the reproductive dietary risk assessment for mammals, for the drinking water already the actual application rate of 1.2 L product/ha is applied, i.e. 96 g a.s./ha mesotrione and 36 g a.s./ha nicosulfuron.

With a $K(f)_{oc}$ of 14 to 156.6 L/kg (pH depended), mesotrione belongs to the group of less sorptive substances. Nicosulfuron has a $K(f)_{oc}$ of 25 L/kg and therefore also belongs to the group of less sorptive substances.

| | | | | |
|---|---|---|---|---|
| **Mesotrione** | | | | |
| Effective application rate (g/ha) | ~~120~~ 96 | | | |
| Acute toxicity (mg/kg bw) | > 5000 | quotient | < | ~~0.024~~ 0.019 |
| Reprod. toxicity (mg/kg bw/d) | 0.3 (Tier 1) | quotient | = | **~~400~~ 320** |
| | ~~1.2 (higher Tier)~~ | ~~quotient~~ | ~~=~~ | ~~80~~ |
| **Nicosulfuron** | | | | |
| Effective application rate (g/ha) | 45 | | | |
| Acute toxicity (mg/kg bw) | > 5000 | quotient | < | 0.009 |
| Reprod. toxicity (mg/kg bw/d) | ~~≥ 3861~~ 300 | quotient | ~~≤~~ = | ~~0.012~~ 0.15 |

Values in **bold** exceed the relevant screening trigger of 50 or 3000.

Accordingly, no specific calculations of exposure and TER are necessary with ratios not exceeding the trigger of 50 for nicosulfuron, and the acute exposure to mesotrione. For the reproductive exposure to mesotrione, a detailed assessment is required. ~~For the detailed assessment, the conservative NOEC of 1.2 mg a.s./kg bw/day from the higher Tier risk assessment is applied.~~

As the $K_{OC}$ is a relevant parameter for the drinking water assessment and as it is pH-dependant for mesotrione, the risk assessment is shown for all three available pH values of 5.1, 6.5 and 7.9 with the respective $K_{OC}$ values of 156.6, 52.2 and 17.39 L/kg.

**Table 9.3-18: Assessment of the risk for mammals due to exposure to mesotrione via contaminated drinking water in puddles**

| | | | | | |
|---|---|---|---|---|---|
| ~~Intended use~~<br>~~Active substance~~<br>~~Application rate (g/ha)~~ | ~~Maize~~<br>~~mesotrione~~<br>~~1 × 120 96 g a.s./ha~~ | | | | |
| ~~Reprod. toxicity (mg/kg bw/d)~~<br>~~TER criterion~~ | ~~0.3 1.2~~<br>~~5~~ | | | | |
| ~~Soil-relevant application rate (g/ha)~~ | ~~Koc (L/kg)~~ | ~~$PEC_{puddle}$ *) (mg/L)~~ | ~~DW uptake (L/kg bw/d)~~ | ~~Daily dose (mg/kg bw/d)~~ | ~~$TER_{lt}$~~ |
| ~~120~~ | ~~for pH 5.1: 156.6~~ | ~~0.047 0.038~~ | ~~0.24~~ | ~~0.0113 0.0091~~ | ~~26.6 131.5~~ |
| | ~~for pH 6.5: 52.2~~ | ~~0.122 0.098~~ | ~~0.24~~ | ~~0.0293 0.0235~~ | ~~10.2 51.1~~ |
| | ~~for pH 7.9: 17.39~~ | ~~0.260 0.209~~ | ~~0.24~~ | ~~0.0624 0.0502~~ | ~~4.8 24.0~~ |

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

~~*) $PEC_{puddle}$ = (Application rate/10) * (1000*($w_{ret}$ + $K_{OC}$*$\varepsilon$) with $w_{ret}$ = 0.02 (pore water term) and $\varepsilon$ = 0.0015 (soil term)~~

The $PEC_{puddle}$ was calculated using following formula (from EFSA Guidance Document, 2009):

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

*where:*

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

The toxicity/exposure ratio (TER) is then calculated as follows:

$$TER_{LT} = \frac{Toxicity\ endpoint}{PEC_{puddle}\ x\ DWR}$$

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | Maize | | | | |
| **Active substance** | mesotrione | | | | |
| **Application rate (g/ha)** | 1 × 96 | | | | |
| **Reprod. toxicity (mg/kg bw/d)** | 0.3 | | | | |
| **TER criterion** | 5 | | | | |
| **Soil-relevant applic. rate (g/ha)** | **Koc (L/kg)** | **$PEC_{puddle}$ (mg/L)** | **DW uptake (L/kg bw/d)** | **Daily dose (mg/kg bw/d)** | **$TER_a$** / **$TER_{lt}$** |
| 96 | 14 (worst-case) | 0.23 | 0.24 | 0.056 | 5.34 |

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

Accordingly, the risk from the uptake of drinking water via puddles is indicated to be acceptable for mammals ~~at all pH values, pH 5.1 and 6.5, but not at pH 7.9.~~

~~It is noted that in a conservative approach the reproductive endpoint of 0.3 mg a.s./kg bw/d was used in the drinking water assessment. As already discussed above, it is considered justified to base the assessment on the endpoint of 2.0 mg a.s./kg bw/d. Based on this endpoint, an acceptable risk is indicated for pH 7.9 with a TER of 32.1.~~

~~In an alternative approach, a refinement based on FOCUS Step 3 runoff concentrations can be performed as well according to EFSA/2009/1438. The maximum $PEC_{sw}$ from the run-off scenarios was calculated as 0.003939 mg a.s./L. The refined assessment is presented in the table below.~~

**Table 9.3-19: Refined assessment of the risk for mammals due to exposure to mesotrione via contaminated drinking water in puddles at pH 7.9**

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | | **Maize** | | | |
| **Active substance** | | mesotrione | | | |
| **Application rate (g/ha)** | | 1 × 120 g a.s./ha | | | |
| **Reprod. toxicity (mg/kg bw/d)** | | 0.3 | | | |
| **TER criterion** | | 5 | | | |
| **Soil relevant application rate (g/ha)** | **Koc (L/kg)** | **FOCUS Step 3 runoff, worst-case $PEC_{sw}$ (mg/L)** | **DW uptake (L/kg bw/d)** | **Daily dose (mg/kg bw/d)** | **$TER_{lt}$** |
| 120 | for pH 7.9: 17.39 | 0.00394 | 0.24 | 0.00095 | 315.8 |

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

Accordingly, the risk to mammals from drinking water is indicated to be acceptable based on refined risk assessments for the use of SAE053H/01 in maize.

#### 9.3.2.4 Effects of secondary poisoning

The log $P_{ow}$ of mesotrione amounts to 0.11 and for nicosulfuron to maximum 0.61 (pH dependent) and thus both active substances do not exceed the trigger value of 3. Furthermore, no indication of bioaccumulation was found in the EFSA conclusions for both active substances. A risk assessment for effects due to secondary poisoning is not required.

Furthermore, all major degradation products in soil and water for both active substances have log $P_{OW}$ values below the relevant trigger of 3. Again, a risk assessment is not required.

**Risk assessment for earthworm-eating mammals via secondary poisoning**

Not required.

**Risk assessment for fish-eating mammals via secondary poisoning**

Not required.

#### 9.3.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

### 9.3.4 Overall conclusions

The risk from dietary exposure to mesotrione and nicosulfuron applied as SAE053H/01 in maize (shown ~~for both, the risk envelope: 1 x 120 g mesotrione/ha and 45 g nicosulfuron/ha as well as~~ the actual application rate: 1 x 96 g mesotrione/ha and 36 g nicosulfuron/ha) is indicated to be acceptable for mammals based on acute screening risk assessments for the single substance exposure and for the mixture exposure. The reproductive risk is indicated to be acceptable for nicosulfuron based on screening assessments and for mesotrione ~~and the mixture~~ based on higher tier assessment. The risk assessment from drinking water was not triggered to be investigated further for nicosulfuron and the acute risk for mesotrione and therefore the risk was considered low. For the reproductive risk of mesotrione from consumption of drinking water the refined assessment did indicate an acceptable risk for mammals. The risk from secondary poisoning and biomagnification in terrestrial food chains was not triggered and is therefore indicated to be low.

**Review Comments:**

In the screening step the $TER_A$ and $TER_{LT}$ values for nicosulfuron and the $TER_A$ mesotrione exceeds the trigger value set by Commission regulation (EU) 546/2011 for acceptability of effects. For mesotrione the $TER_{LT}$ values from the tier 1 reproductive risk assessment are below the trigger for all scenarios.

A higher tier risk assessment was based on the following refinement parameters: focal species, foliage residue dissipation ($DT_{50}$) and ecological data on PT value. Based on these refinements the quantitative higher tier risk assessments show that the dietary reproductive risks to mammals from the intended use of SAE053H/01 are acceptable for post-emergence (at 96 g a.s./ha) use in maize.

Evaluation of exposing to birds through the drinking water demonstrated the acceptable risk. Since the log $P_{ow}$ value of mesotrione, nicosulfuron and their relevant metabolites are all below the trigger of 3, the evaluation of the risk of secondary poisoning is not triggered.

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

No relevant data were reported during EU review of the active substances.

## 9.5 Effects on aquatic organisms (KCP 10.2)

### 9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with mesotrione and nicosulfuron and their relevant degradation products. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on aquatic organisms of SAE053H/01 were not evaluated as part of the EU assessment of mesotrione or nicosulfuron. 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 deviates from the results of the EU review process of mesotrione and ~~the supplementary dossier for the approval renewal of~~ nicosulfuron ~~(N2 document; 2016)~~. Justifications are provided below.

**Table 9.5-1: Endpoints and effect values relevant for the risk assessment for aquatic organisms – mesotrione and nicosulfuron with relevant degradation products**

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| **Mesotrione** | | | | |
| *Oncorhynchus mykiss* | mesotrione | 96 h, s | **$LC_{50}$ > 120 mg a.s./L $_{nom}$** | EFSA conclusion [a)] Kelso et al., 1994a, BL5492/B |
| *Lepomis macrochirus* | mesotrione | 96 h, s | $LC_{50}$ > 120 mg a.s./L $_{nom}$ | EFSA conclusion [a)] Kelso et al., 1994b, BL5491/B |
| *Pimephales promelas* | mesotrione | 36 d (ELS), f | **NOEC = 12.5 mg a.s./L** (physical symptoms) | EFSA conclusion [a)] Shillabeer & Kent, 1997, BL5925/B |
| *Daphnia magna* | mesotrione | 48 h, s | **$EC_{50}$ > 622 mg a.s./L $_{mm}$** | EFSA conclusion [a)] Gentle & Hamer, 1995, RJ1872B |
| *Daphnia magna* | mesotrione | 21 d, ss | **NOEC = 180 mg a.s./L $_{nom}$** (reproduction and length) | EFSA conclusion [a)] Morris et al., 1996, BL5832B |
| *Pseudokirchneriella subcapitata* | mesotrione | 120 h, s | **$E_rC_{50}$ = 13 mg a.s./L $_{nom}$**<br>$E_bC_{50}$ = 3.5 mg a.s./L $_{nom}$<br>$E_bC_{20}$ = 0.958 mg a.s./L $_{nom}$<br>$E_bC_{10}$ = 0.692 mg a.s./L $_{nom}$<br>$NOE_bC$ = 0.75 mg a.s./L $_{nom}$ | EFSA conclusion [a)] Kent et al., 1997, BL6113/B |
| *Lemna gibba* | mesotrione | 14 d, ss | Frond number:<br>$E_bC_{50}$ = 0.022 mg a.s./L $_{nom}$<br>$E_rC_{50}$ = 0.0599 mg a.s./L $_{nom}$ [h)]<br>Dry weight:<br>$E_bC_{50}$ = 0.0077 mg a.s./L $_{nom}$<br>$E_rC_{50}$ = 0.0257 mg a.s./L $_{nom}$ [h)]<br>$E_yC_{20}$ = 0.0022 mg a.s./L $_{nom}$<br>$E_yC_{10}$ = 0.0014 mg a.s./L $_{nom}$ | EFSA conclusion [a)] Smyth et al., 1997d, BL5849/B |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| | | | $NOE_bC$ = 0.002 mg a.s./L $_{nom}$ | |
| *Lemna gibba* | mesotrione | 7 d, ss | Frond number:<br>$E_rC_{50}$ = 0.0354 mg a.s./L $_{gmm}$<br>$E_yC_{50}$ = 0.00247 mg a.s./$L_{gmm}$<br>Dry weight:<br>$E_rC_{50}$ = 0.0113 mg a.s./L $_{gmm}$<br>$E_yC_{50}$ = 0.00321 mg a.s./$L_{gmm}$ | Bertrand, 2019, S19-03470 |
| *Spirodela polyrhiza* | mesotrione | 7 d, ss | Frond number:<br>$E_rC_{50}$ = 0.0120 mg a.s./L $_{gmm}$<br>$E_yC_{50}$ = 0.00416 mg a.s./$L_{gmm}$<br>Dry weight:<br>$E_rC_{50}$ = 0.0181 mg a.s./L $_{gmm}$<br>$E_yC_{50}$ = 0.00403 mg a.s./$L_{gmm}$ | Christmann, 2021a, 218-31 |
| *Wolffia arrhiza* | mesotrione | 7 d, ss | Frond number:<br>$E_rC_{50}$ = 0.0289 mg a.s./L $_{gmm}$<br>$E_yC_{50}$ = 0.00718 mg a.s./$L_{gmm}$<br>Dry weight:<br>**$E_rC_{50}$ = 0.00628 mg a.s./L $_{gmm}$**<br>$E_yC_{50}$ = 0.00283 mg a.s./$L_{gmm}$ | Christmann, 2021b, 218-32 |
| Geometric mean for aquatic macrophytes (n = 4) | mesotrione | - | **$E_rC_{50}$ = 0.0128 mg a.s./L (dry weight)**<br>$E_rC_{50}$ = 0.0255 mg a.s./L (frond number) | Applicant calculation, see Section 9.5.1.1 below. |
| **Degradation product of mesotrione: MNBA** | | | | |
| *Oncorhynchus mykiss* | MNBA | 96 h, s | **$LC_{50}$ > 120 mg/L $_{nom}$** | EFSA conclusion [a)]<br>Smyth et al., 1997a, BL6064/B |
| *Daphnia magna* | MNBA | 48 h, s | **$EC_{50}$ = 130 mg/L $_{nom}$** | EFSA conclusion [a)]<br>Kent & Shillaber, 1997, BL6108/B |
| *Pseudokirchneriella subcapitata* | MNBA | 72 h, s | $E_bC_{50}$ = 38 mg/L $_{nom}$<br>**$E_rC_{50}$ = 42 mg/L $_{nom}$**<br>$E_rC_{20}$ = 34.9 mg/L $_{nom}$<br>$E_rC_{10}$ = 33.4 mg/L $_{nom}$<br>$NOE_{b,\,r}C$ = 32 mg/L $_{nom}$ | EFSA conclusion [a)]<br>Smyth et al, 1997c, BL6066/B |
| *Lemna gibba* | MNBA | 7 d, ss | Frond no. and dry weight:<br>**$E_{r,\,y}C_{50,\,20,\,10}$ > 97 mg/L $_{mm}$**<br>Frond no.:<br>NOEC = 3.3 mg/L $_{mm}$ | EFSA conclusion [a)]<br>Liedtke, 2013c, D55592 |
| **Degradation product of mesotrione: AMBA** | | | | |
| *Oncorhynchus mykiss* | AMBA | 96 h, s | **$LC_{50}$ = 150 mg/L $_{nom}$** | EFSA conclusion [a)]<br>Magor & Gore, 1998a, BL6391/B |
| *Daphnia magna* | AMBA | 48 h, s | **$EC_{50}$ = 160 mg/L $_{nom}$** | EFSA conclusion [a)]<br>Magor & Gore, 1998b, BL6392/B |
| *Pseudokirchneriella* | AMBA | 72 h, s | $E_bC_{50}$ = 9.4 mg/L $_{nom}$ | EFSA conclusion [a)] |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *subcapitata* | | | **$E_rC_{50}$ = 14 mg/L nom**<br>$E_rC_{20}$ = 4.04 mg/L nom<br>$E_rC_{10}$ = 2.58 mg/L nom<br>$NOE_{b,r}C$ = 7.7 mg/L nom | Magor & Gore, 1998c, BL6354/B |
| *Lemna gibba* | AMBA | 7 d, ss | Frond no. and dry weight:<br>**$E_{r,y}C_{50,20}$ > 90 mg/L mm**<br>NOEC = 90 mg/L mm<br>Frond no.:<br>$E_yC_{10}$ = 24 mg/L mm | EFSA conclusion [a]<br>Liedtke, 2013b, D55614 |
| **Degradation product of mesotrione: SYN546974** | | | | |
| *Lemna gibba* | SYN546974 | 7 d, ss | Frond no. and dry weight:<br>**$E_rC_{50}$ > 95 mg/L mm**<br>Frond no.:<br>$E_yC_{50}$ = 93 mg/L mm<br>$E_yC_{20}$ = 21 mg/L mm<br>$E_yC_{10}$ = 9.9 mg/L mm<br>NOEC = 2.9 mg/L mm | EFSA conclusion [a]<br>Liedtke, 2013d, D77394 |
| **Nicosulfuron** | | | | |
| *Oncorhynchus mykiss* | nicosulfuron | 96 h, s | **$LC_{50}$ = 65.7 mg a.s./L nom** | EFSA conclusion [b]<br>Jenkins, 1991a, 91/ISK169,182/0012 |
| *Lepomis macrochirus* | nicosulfuron | 96 h, s | $LC_{50}$ > 100 mg a.s./L nom | DAR nicosulfuron [d]<br>Jenkins, 1991b, 91/ISK170/0013 |
| *Oncorhynchus mykiss* | nicosulfuron | 28 d (juvenile growth test), f | **NOEC = 10 mg a.s./L mm** | EFSA conclusion [b]<br>Bogers, 1994a, 117473 |
| ~~*Oncorhynchus mykiss*~~ | ~~nicosulfuron~~ | ~~90 d (ELS), f~~ | ~~NOEC = 24 mg a.s./L mm~~ | ~~Renewal dossier [c]<br>Author censored, 1999, DuPont 2880~~ |
| *Daphnia magna* | nicosulfuron | 48 h, s | **$EC_{50}$ = 90 mg a.s./L nom** | EFSA conclusion [b]<br>Jenkins, 1991c, 91/ISK171,181/0014 |
| *Daphnia magna* | nicosulfuron | 21 d, ss | **NOEC = 5.2 mg a.s./L nom** | EFSA conclusion [b]<br>Bogers, 1994b, 117484 |
| ~~*Pseudokirchneriella subcapitata*~~ | ~~nicosulfuron~~ | ~~72 h, s~~ | ~~$E_rC_{50}$ = 71.17 mg a.s./L nom~~<br>~~$E_rC_{20}$ = 50.32 mg a.s./L mm~~<br>~~$E_rC_{10}$ = 43.52 mg a.s./L mm~~<br>~~$NOE_rC$ = 7.5 mg a.s./L nom~~<br>~~$E_bC_{50}$ = 23.48 mg a.s./L nom~~<br>~~$NOE_bC$ < 7.5 mg a.s./L nom~~<br>~~$E_yC_{20}$ = 10.99 mg a.s./L mm~~<br>~~$E_yC_{10}$ = 7.48 mg a.s./L mm~~ | ~~Renewal dossier [c]<br>Sloman, 2004a, DuPont 13342~~ |
| *Scenedesmus subspicatus* | nicosulfuron | 96 h, s | $E_bC_{50}$ = 182 mg/L nom<br>NOEC = 100 mg/L nom | DAR nicosulfuron [d]<br>Wüthrich, 1992, 313830 |
| *Anabaena flos-aquae* | nicosulfuron | 72 h, s | **$E_rC_{50}$ = 8.4 mg a.s./L nom**<br>$E_rC_{10}$ = 4.5 mg a.s./L nom | EFSA conclusion [b]<br>Memmert, 1998a, |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| | | | $E_bC_{50}$ = 7.8 mg a.s./L nom<br>$E_bC_{10}$ = 4.6 mg a.s./L nom | 692278 |
| ~~*Anabaena flos-aquae*~~ | ~~nicosulfuron~~ | ~~96 h, s~~ | ~~$E_rC_{50}$ = 59.8 mg a.s./L nom~~<br>~~$NOE_rC$ = 60 mg a.s./L nom~~<br>~~$E_bC_{50}$ = 37.8 mg a.s./L nom~~<br>~~$NOE_bC$ = 30 mg a.s./L nom~~ | ~~Renewal dossier [c]~~<br>~~Sloman, 2004b, DuPont 13343~~ |
| ~~*Anabaena flos-aquae*~~ | ~~nicosulfuron~~ | ~~72 h, s~~ | ~~$E_rC_{50}$ > 100 mg a.s./L nom~~<br>~~$NOE_rC \geq$ 100 mg a.s./L nom~~<br>~~$E_yC_{50}$ > 100 mg a.s./L nom~~<br>~~$NOE_yC \geq$ 100 mg a.s./L nom~~ | ~~Renewal dossier [c]~~<br>~~Pupp & Wydra, 2008, 42721210~~ |
| *Lemna gibba* | nicosulfuron | 7 d, ss | Frond number:<br>**$E_rC_{50}$ = 0.0027 mg a.s./L mm**<br>$E_rC_{10}$ = 0.0008 mg a.s./L mm<br>$E_bC_{50}$ = 0.0017 mg a.s./L mm<br>$E_bC_{10}$ = 0.0005 mg a.s./L mm<br>Dry weight:<br>$E_bC_{50}$ > 0.034 mg a.s./L mm<br>$E_rC_{10}$ = 0.0009 mg a.s./L mm<br>Mortality rate: > 0.0275 mg a.s./L mm | EFSA conclusion [b]<br>Memmert, 1998c, 693854 |
| ~~*Lemna gibba*~~ | ~~nicosulfuron~~ | ~~14 d, s~~ | ~~Frond number (7 d):~~<br>~~$E_rC_{50}$ = 0.0051 mg a.s./L nom~~<br>~~$E_rC_{25}$ = 0.0020 mg a.s./L nom~~<br>~~$E_bC_{50}$ = 0.0032 mg a.s./L nom~~<br>~~$E_bC_{25}$ = 0.0012 mg a.s./L nom~~<br>~~$NOE_{r,b}C$ < 0.00032 mg a.s./L nom~~<br>~~Frond number (14 d):~~<br>~~$E_rC_{50}$ = 0.009 mg a.s./L nom~~<br>~~$E_rC_{25}$ = 0.0051 mg a.s./L nom~~<br>~~$E_bC_{50}$ = 0.0067 mg a.s./L nom~~<br>~~$E_bC_{25}$ = 0.0037 mg a.s./L nom~~<br>~~$NOE_{r,b}C$ = 0.0025 mg a.s./L nom~~<br>~~Dry weight (14 d):~~<br>~~$E_bC_{50}$ = 0.0073 mg a.s./L nom~~<br>~~$E_bC_{25}$ = 0.0039 mg a.s./L nom~~<br>~~$NOE_bC$ = 0.005 mg a.s./L nom~~ | ~~Renewal dossier [c]~~<br>~~Hoberg, 1992, AMR 2178-91~~ |
| ~~*Lemna gibba*~~ | ~~nicosulfuron~~ | ~~7 d, ss~~ | ~~Frond number:~~<br>~~$E_rC_{50}$ = 0.00187 mg a.s./L nom~~<br>~~$E_rC_{20}$ = 0.00103 mg a.s./L nom~~<br>~~$E_rC_{10}$ = 0.00073 mg a.s./L nom~~<br>~~$E_yC_{50}$ = 0.00105 mg a.s./L nom~~<br>~~$E_yC_{20}$ = 0.00066 mg a.s./L nom~~<br>~~$E_yC_{10}$ = 0.00052 mg a.s./L nom~~<br>~~$NOE_{r,y}C$ = 0.00037 mg a.s./L nom~~<br>~~Frond area:~~<br>~~**$E_rC_{50}$ = 0.00182 mg a.s./L nom**~~<br>~~$E_rC_{20}$ = 0.00075 mg a.s./L nom~~<br>~~$E_rC_{10}$ = 0.00047 mg a.s./L nom~~<br>~~$E_yC_{50}$ = 0.00091 mg a.s./L nom~~<br>~~$E_yC_{20}$ = 0.00053 mg a.s./L nom~~<br>~~$E_yC_{10}$ = 0.00040 mg a.s./L nom~~ | ~~Renewal dossier [c]~~<br>~~Weber, 2016, S08-00936~~ |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| | | | $NOE_{r,y}C$ = 0.00037 mg a.s./L nom | |
| *Lemna gibba* | nicosulfuron | 7 d, ss | Frond number:<br>$E_rC_{50}$ = 0.0025 mg a.s./L nm<br>$E_rC_{20}$ = 0.00070 mg a.s./L nm<br>$E_rC_{10}$ = 0.00036 mg a.s./L nm<br>$E_yC_{50}$ = 0.0011 mg a.s./L nm<br>$E_yC_{20}$ = 0.00042 mg a.s./L nm<br>$E_yC_{10}$ = 0.00025 mg a.s./L nm<br>Dry weight:<br>$E_rC_{50}$ > 0.028 mg a.s./L nm<br>$E_rC_{20}$ = 0.0011 mg a.s./L nm<br>$E_rC_{10}$ = 0.00016 mg a.s./L nm<br>$E_yC_{50}$ = 0.0023 mg a.s./L nm<br>$E_yC_{20}$ = 0.00017 mg a.s./L nm<br>$E_yC_{10}$ = 0.000043 mg a.s./L nm | Renewal dossier c)<br>Bätscher, 2008b, 90010591 |
| *Lemna gibba* | nicosulfuron | 7 d, s, variable exposure | Frond number:<br>12 h pulse<br>$E_rC_{50}$ = 0.088 mg a.s./L im<br>$E_rC_{20}$ = 0.0183 mg a.s./L im<br>$E_rC_{10}$ = 0.0074 mg a.s./L im<br>$E_yC_{50}$ = 0.020 mg a.s./L im<br>$E_yC_{20}$ = 0.0078 mg a.s./L im<br>$E_yC_{10}$ = 0.0045 mg a.s./L im<br>24 h pulse:<br>$E_rC_{50}$ = 0.056 mg a.s./L im<br>$E_rC_{20}$ = 0.0125 mg a.s./L im<br>$E_rC_{10}$ = 0.0058 mg a.s./L im<br>$E_yC_{50}$ = 0.011 mg a.s./L im<br>$E_yC_{20}$ = 0.0046 mg a.s./L im<br>$E_yC_{10}$ = 0.0025 mg a.s./L im<br>48 h pulse:<br>$E_rC_{50}$ = 0.015 mg a.s./L im<br>$E_rC_{20}$ = 0.0037 mg a.s./L im<br>$E_rC_{10}$ = 0.0017 mg a.s./L im<br>$E_yC_{50}$ = 0.0042 mg a.s./L im<br>$E_yC_{20}$ = 0.0019 mg a.s./L im<br>$E_yC_{10}$ = 0.0012 mg a.s./L im<br>96 h pulse:<br>$E_rC_{50}$ = 0.0077 mg a.s./L im<br>$E_rC_{20}$ = 0.0032 mg a.s./L im<br>$E_rC_{10}$ = 0.0019 mg a.s./L im<br>$E_yC_{50}$ = 0.0039 mg a.s./L im<br>$E_yC_{20}$ = 0.0022 mg a.s./L im<br>$E_yC_{10}$ = 0.0016 mg a.s./L im<br>Dry weight:<br>12 h pulse<br>$E_rC_{50}$ > 0.190 mg a.s./L im<br>$E_rC_{20}$ = 0.0372 mg a.s./L im<br>$E_rC_{10}$ = 0.0110 mg a.s./L im<br>$E_yC_{50}$ = 0.025 mg a.s./L im<br>$E_yC_{20}$ = 0.0087 mg a.s./L im<br>$E_yC_{10}$ = 0.0040 mg a.s./L im<br>24 h pulse: | Renewal dossier c)<br>Softcheck, 2011, DuPont-29702 |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| | | | ~~$E_rC_{50}$ > 0.150 mg a.s./L im~~<br>~~$E_rC_{20}$ = 0.0350 mg a.s./L im~~<br>~~$E_rC_{10}$ = 0.0076 mg a.s./L im~~<br>~~$E_yC_{50}$ = 0.015 mg a.s./L im~~<br>~~$E_yC_{20}$ = 0.0036 mg a.s./L im~~<br>~~$E_yC_{10}$ = 0.0014 mg a.s./L im~~<br>~~48 h pulse:~~<br>~~$E_rC_{50}$ > 0.160 mg a.s./L im~~<br>~~$E_rC_{20}$ = 0.0045 mg a.s./L im~~<br>~~$E_yC_{50}$ = 0.0053 mg a.s./L im~~<br>~~96 h pulse:~~<br>~~$E_rC_{50}$ > 0.120 mg a.s./L im~~<br>~~$E_rC_{20}$ = 0.0111 mg a.s./L im~~<br>~~$E_rC_{10}$ = 0.0016 mg a.s./L im~~<br>~~$E_yC_{50}$ = 0.0038 mg a.s./L im~~<br>~~$E_yC_{20}$ = 0.0011 mg a.s./L im~~ | |
| ~~*Lemna gibba*~~ | ~~nicosulfuron~~ | ~~2 x (24 h pulse + 6 d recovery)~~ | ~~Frond number:~~<br>~~1st 24 h pulse~~<br>~~$E_rC_{50}$ = 0.0088 mg a.s./L nom~~<br>~~$E_rC_{10}$ = 0.0018 mg a.s./L nom~~<br>~~$E_yC_{50}$ = 0.0071 mg a.s./L nom~~<br>~~$E_yC_{10}$ = 0.0016 mg a.s./L nom~~<br>~~$NOE_{r,y}C$ = 0.00125 mg a.s./L nom~~<br>~~1st recovery:~~<br>~~$E_rC_{50}$ > 0.01 mg a.s./L nom~~<br>~~$E_rC_{10}$ = 0.0024 mg a.s./L nom~~<br>~~$E_yC_{50}$ = 0.0052 mg a.s./L nom~~<br>~~$E_yC_{10}$ = 0.00086 mg a.s./L nom~~<br>~~$NOE_{r,y}C$ = 0.00625 mg a.s./L nom~~<br>~~2nd 24 h pulse~~<br>~~$E_rC_{50}$ = 0.0077 mg a.s./L nom~~<br>~~$E_rC_{10}$ = 0.0044 mg a.s./L nom~~<br>~~$E_yC_{50}$ = 0.0073 mg a.s./L nom~~<br>~~$E_yC_{10}$ = 0.0041 mg a.s./L nom~~<br>~~$NOE_{r,y}C$ = 0.0050 mg a.s./L nom~~<br>~~2nd recovery:~~<br>~~$E_rC_{50}$ > 0.01 mg a.s./L nom~~<br>~~$E_rC_{10}$ = 0.0045 mg a.s./L nom~~<br>~~$E_yC_{50}$ > 0.01 mg a.s./L nom~~<br>~~$E_yC_{10}$ = 0.0025 mg a.s./L nom~~<br>~~$NOE_{r,y}C$ = 0.0025 mg a.s./L nom~~<br>~~Dry weight:~~<br>~~1st recovery:~~<br>~~$E_rC_{50}$ > 0.01 mg a.s./L nom~~<br>~~$E_rC_{10}$ = 0.0034 mg a.s./L nom~~<br>~~$E_yC_{50}$ = 0.0074 mg a.s./L nom~~<br>~~$E_yC_{10}$ = 0.0011 mg a.s./L nom~~<br>~~$NOE_{r,y}C$ = 0.000625 mg a.s./L nom~~<br>~~2nd recovery:~~<br>~~$E_rC_{50}$ > 0.01 mg a.s./L nom~~<br>~~$E_rC_{10}$ = 0.0048 mg a.s./L nom~~<br>~~$E_yC_{50}$ > 0.01 mg a.s./L nom~~ | ~~Renewal dossier c)~~<br>~~Hoffmann & Deierling, 2010, 59911240~~ |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| | | | $E_yC_{10}$ = 0.0052 mg a.s./L nom<br>$NOE_{r,y}C$ = 0.0025 mg a.s./L nom | |
| *Lemna gibba* | nicosulfuron | 9 d, pulsed exposure test | NOEAEC = 0.015 mg a.s./L nom | Renewal dossier c)<br>Liedtke, 2012a, 90015014 |
| *Lemna gibba* | nicosulfuron | 24 h pulse, 14 d recovery | NOEAEC = 27 mg a.s./L nom | Renewal dossier c)<br>Memmert, 2006c, A45911 |
| *Myriophyllum spicatum* | nicosulfuron | 14 d | Shoot length:<br>$E_rC_{50}$ = 0.197 mg a.s./L nom<br>$E_rC_{20}$ = 0.0877 mg a.s./L nom<br>$E_rC_{10}$ = 0.0574 mg a.s./L nom<br>$E_yC_{50}$ = 0.125 mg a.s./L nom<br>$E_yC_{20}$ = 0.0543 mg a.s./L nom<br>$E_yC_{10}$ = 0.0351 mg a.s./L nom<br>NOEC = 0.0305 mg a.s./L nom<br>Fresh weight:<br>$E_rC_{50}$ = 0.346 mg a.s./L nom<br>$E_rC_{20}$ = 0.106 mg a.s./L nom<br>$E_rC_{10}$ = 0.0568 mg a.s./L nom<br>$E_yC_{50}$ = 0.167 mg a.s./L nom<br>$E_yC_{20}$ = 0.0584 mg a.s./L nom<br>$E_yC_{10}$ = 0.0337 mg a.s./L nom<br>NOEC = 0.0305 mg a.s./L nom<br>Dry weight:<br>$E_rC_{50}$ > 1 mg a.s./L nom<br>$E_rC_{20}$ = 0.172 mg a.s./L nom<br>$E_rC_{10}$ = 0.0587 mg a.s./L nom<br>$E_yC_{50}$ = 0.464 mg a.s./L nom<br>$E_yC_{20}$ = 0.0722 mg a.s./L nom<br>$E_yC_{10}$ = 0.0273 mg a.s./L nom<br>NOEC = 0.0305 mg a.s./L nom | Renewal dossier c)<br>Falk, 2016, S15-05639 |
| *Ranunculus lingua*<br>*Sparganium erectum*<br>*Glyceria aquatica*<br>*Myriophyllum prosperpinacoides*<br>*Elodea canadensis*<br>*Ceratophyllum demersum*<br>*Lemna minor* | nicosulfuron | Microcosm study | *R. lingua*:<br>NO(A)EC = 0.0125 mg a.s./L nom<br>*S. erectum*:<br>NOEC = 0.002 mg a.s./L nom<br>NOAEC = 0.005 mg a.s./L nom<br>*G. aquatica*:<br>NO(A)EC = 0.0125 mg a.s./L nom<br>*M. prosperpinacoides*:<br>NOEC = 0.0008 mg a.s./L nom<br>NOAEC = 0.0125 mg a.s./L nom<br>*E. canadensis*:<br>NOEC = 0.0008 mg a.s./L nom<br>NOAEC = 0.002 mg a.s./L nom<br>*C. demersum*:<br>NOEC = 0.0008 mg a.s./L nom<br>NOAEC = 0.0125 mg a.s./L nom<br>*L. minor*:<br>NOEC = 0.002 mg a.s./L nom<br>NOAEC = 0.0125 mg a.s./L nom | Renewal dossier c)<br>Burlingham et al., 2011, PH0011 |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| | | | ~~**NOAEC = 0.002 mg a.s./L nom**~~<br>~~MDD category: 1~~ | |
| **Degradation product of nicosulfuron: ASDM** | | | | |
| *Oncorhynchus mykiss* | ASDM | 96 h, s | **$LC_{50}$ > 996 mg/L nom** | DAR nicosulfuron [d]<br>Jenkins, 1993a, 93/ISK202/0627 |
| *Lepomis macrochirus* | ASDM | 96 h, s | $LC_{50}$ > 100 mg/L nom | EFSA conclusion [b]<br>Buchanan & Knight, 1997a, 15168 |
| *Daphnia magna* | ASDM | 48 h, s | **$EC_{50}$ > 954 mg/L mm** | EFSA conclusion [b]<br>Jenkins, 1993b, 93/ISK203/0628 |
| *Pseudokirchneriella subcapitata* | ASDM | 72 h, s | **$E_rC_{50}$ > 336 mg/L mm**<br>$E_rC_{20}$ = 68.34 mg/L mm<br>$E_rC_{10}$ = 23.95 mg/L mm<br>$E_bC_{50}$ = 54.0 mg/L mm<br>$E_yC_{50}$ = 44.96 mg/L mm<br>$E_yC_{20}$ = 15.84 mg/L mm<br>$E_yC_{10}$ = 9.18 mg/L mm | EFSA conclusion [b]<br>Jenkins, 1993c, 93/ISK206/0750 |
| ~~*Anabaena flos-aquae*~~ | ~~ASDM~~ | ~~120 h, s~~ | ~~$E_aC_{50}$ = 14 mg/L im~~ [e]<br>~~$NOE_aC$ = 3.6 mg/L im~~ [e]<br>~~$E_bC_{50}$ = 20 mg/L im~~<br>~~$NOE_bC$ = 1.8 mg/L im~~<br>~~**$E_rC_{50}$ = 50 mg/L im**~~<br>~~$NOE_rC$ = 1.8 mg/L im~~ | ~~Renewal dossier~~ [c]<br>~~Ward, Wyskiel & Boeri, 2004, DuPont-14027~~ |
| ~~*Lemna gibba*~~ | ~~ASDM~~ | ~~7 d, ss~~ | ~~Frond number:~~<br>~~$E_rC_{50}$ = 16 mg/L nom~~<br>~~$E_rC_{20}$ = 7.3 mg/L nom~~<br>~~$E_rC_{10}$ = 4.7 mg/L nom~~<br>~~$NOE_rC$ = 4.6 mg/L nom~~<br>~~$E_yC_{50}$ = 9.7 mg/L nom~~<br>~~$E_yC_{20}$ = 5.4 mg/L nom~~<br>~~$E_yC_{10}$ = 4.0 mg/L nom~~<br>~~$NOE_yC$ < 4.6 mg/L nom~~<br>~~Dry weight:~~<br>~~$E_rC_{50}$ > 100 mg/L nom~~<br>~~$E_rC_{20}$ = 15 mg/L nom~~<br>~~$E_rC_{10}$ = 5.2 mg/L nom~~<br>~~$NOE_rC$ = 4.6 mg/L nom~~<br>~~$E_yC_{50}$ = 23 mg/L nom~~<br>~~$E_yC_{20}$ = 5.0 mg/L nom~~<br>~~$E_yC_{10}$ = 2.2 mg/L nom~~<br>~~$NOE_yC$ < 4.6 mg/L nom~~ | ~~Renewal dossier~~ [c]<br>~~Höger, 2008, 90011711~~ |
| *Lemna gibba* | ASDM | 7 d, ss | Frond number:<br>**$E_rC_{50}$ > 100 mg/L nom**<br>NOEC ≥ 100 mg/L nom<br>Dry weight:<br>$E_rC_{50}$ > 100 mg/L nom | ~~DAR nicosulfuron~~ [d]<br>EFSA conclusion [b]<br>Memmert, 1998d, 693876 |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| | | | NOEC ≥ 100 mg/L nom | |
| **Degradation product of nicosulfuron: AUSN** | | | | |
| *Brachydanio rerio* | AUSN | 96 h, s | **$LC_{50}$ > 100 mg/L nom** | EFSA conclusion [b] Wüthrich, 1996b, 601031 |
| *Daphnia magna* | AUSN | 48 h, s | **$EC_{50}$ > 100 mg/L nom** | EFSA conclusion [b] Wüthrich, 1995a, 601053 |
| *Scenedesmus subspicatus* | AUSN | 72 h, s | **$E_{r,b}C_{50}$ > 100 mg/L nom**<br>$NOE_{r,b}C$ ≥ 100 mg/L nom | EFSA conclusion [b] Wüthrich, 1996f, 601108 |
| ~~*Lemna gibba*~~ | ~~AUSN~~ | ~~7 d, ss~~ | ~~Frond number:~~<br>~~$E_{r,y,b}C_{50}$ > 100 mg/L nom~~ [f]<br>~~$E_rC_{10}$ = 72.94 mg/L nom~~<br>~~$E_yC_{20}$ = 32.8 mg/L nom~~<br>~~$E_yC_{10}$ = 12.21 mg/L nom~~<br>~~$E_bC_{30}$ = 57.1 mg/L nom~~<br>~~Frond area:~~<br>~~$E_bC_{50}$ = 40.75 mg/L nom~~<br>~~$E_rC_{10}$ = 63.7 mg/L nom~~<br>~~$E_yC_{50}$ = 37.48 mg/L nom~~<br>~~NOEC = 11.11 mg/L nom~~ | ~~Renewal dossier [e]~~<br>~~Borrmann, 2010, S08-00826~~ |
| *Lemna gibba* | AUSN | 7 d, ss | Frond number:<br>**$E_rC_{50}$ > 100 mg/L nom**<br>NOEC ≥ 100 mg/L nom<br>Dry weight:<br>$E_rC_{50}$ > 100 mg/L nom<br>NOEC ≥ 100 mg/L nom | ~~DAR nicosulfuron [d]~~<br>EFSA conclusion [b]<br>Memmert, 1998f, 693898 |
| **Degradation product of nicosulfuron: MU-466** [g] | | | | |
| *Oncorhynchus mykiss* | MU-466 | 96 h, s | $LC_{50}$ > 100 mg/L nom | EFSA conclusion [b] Wüthrich, 1996a, 613080 |
| *Daphnia magna* | MU-466 | 48 h, s | $EC_{50}$ > 100 mg/L nom | EFSA conclusion [b] Wüthrich, 1996d, 613078 |
| *Scenedesmus subspicatus* | MU-466 | 72 h, s | $E_rC_{50}$ > 100 mg/L nom<br>$E_rC_{10}$ = 66.92 mg/L nom<br>$E_bC_{50}$ = 84.4 mg/L nom<br>$NOE_{r,b}C$ = 50 mg/L nom | EFSA conclusion [b] Grützner, 1996g, 613056 |
| ~~*Lemna gibba*~~ | ~~MU-466~~ | ~~7 d, ss~~ | ~~Frond number:~~<br>~~$E_{r,y}C_{50}$ > 100 mg/L nom~~<br>~~$E_rC_{20}$ > 100 mg/L nom~~<br>~~$E_rC_{10}$ = 80.3 mg/L nom~~<br>~~$E_yC_{20}$ = 65.8 mg/L nom~~<br>~~$E_yC_{10}$ = 3.57 mg/L nom~~<br>~~$NOE_{r,y}C$ = 31.3 mg/L nom~~<br>~~Dry weight:~~<br>~~$E_{r,y}C_{50,20}$ > 100 mg/L nom~~ | ~~Renewal dossier [e]~~<br>~~Obert-Rauser, 2016b, S15-05478~~ |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| | | | ~~$E_rC_{10}$ > 100 mg/L nom~~<br>~~$E_yC_{10}$ = 73.2 mg/L nom~~<br>~~$NOE_{r,y}C$ = 31.3 mg/L nom~~ | |
| **Degradation product of nicosulfuron: HMUD** | | | | |
| *Oncorhynchus mykiss* | HMUD | 96 h, s | **$LC_{50}$ > 100 mg/L nom** | EFSA conclusion [b]<br>Grützner, 1996a, 613912 |
| *Daphnia magna* | HMUD | 48 h, s | **$EC_{50}$ > 100 mg/L nom** | EFSA conclusion [b]<br>Grützner, 1996c, 613890 |
| ~~*Pseudokirchneriella subcapitata*~~ | ~~HMUD~~ | ~~72 h, s~~ | ~~$E_rC_{50}$ = 43.9 mg/L nom~~<br>~~$E_rC_{20}$ = 31.36 mg/L nom~~<br>~~$E_rC_{10}$ = 27.24 mg/L nom~~<br>~~$E_bC_{50}$ = 29.2 mg/L nom~~<br>~~$NOE_{r,b}C$ = 6.25 mg/L nom~~<br>~~$E_yC_{20}$ = 16.56 mg/L nom~~<br>~~$E_yC_{10}$ = 12.85 mg/L nom~~ | ~~Renewal dossier [c]~~<br>~~Sloman, 2004c,~~<br>~~DuPont 12205~~ |
| *Scenedesmus subspicatus* | HMUD | 72 h, s | **$E_{r,b}C_{50}$ > 100 mg/L nom**<br>NOEC = 45.5 mg/L nom | ~~DAR nicosulfuron [d]~~<br>EFSA conclusion [b]<br>Grützner, 1996f, 613901 |
| ~~*Lemna gibba*~~ | ~~HMUD~~ | ~~7 d, ss~~ | ~~Frond number:~~<br>~~$E_rC_{50}$ = 0.710 mg/L nom~~<br>~~$E_rC_{20}$ = 0.174 mg/L nom~~<br>~~$E_rC_{10}$ = 0.089 mg/L nom~~<br>~~$E_yC_{50}$ = 0.252 mg/L nom~~<br>~~$E_yC_{20}$ = 0.086 mg/L nom~~<br>~~$E_yC_{10}$ = 0.052 mg/L nom~~<br>~~$E_bC_{50}$ = 0.334 mg/L nom~~<br>~~Frond area~~<br>~~$E_rC_{50}$ = 0.514 mg/L nom~~<br>~~$E_rC_{20}$ = 0.169 mg/L nom~~<br>~~$E_rC_{10}$ = 0.098 mg/L nom~~<br>~~$E_yC_{50}$ = 0.191 mg/L nom~~<br>~~$E_yC_{20}$ = 0.075 mg/L nom~~<br>~~$E_yC_{10}$ = 0.046 mg/L nom~~<br>~~$E_bC_{50}$ = 0.244 mg/L nom~~ | ~~Renewal dossier [c]~~<br>~~Dengler, 2009, S08-00827~~ |
| *Lemna gibba* | HMUD | 7 d, ss | **$EC_{50}$ > 1.0 mg/L nom**<br>NOEC ≥ 1.0 mg/L nom | ~~DAR nicosulfuron [d]~~<br>EFSA conclusion [b]<br>Kitajima, 2004, ET0104 |
| **Degradation product of nicosulfuron: UCSN** | | | | |
| *Brachidanio rerio* | UCSN | 96 h, s | **$LC_{50}$ > 100 mg/L nom** | EFSA conclusion [b]<br>Grützner, 1996b, 601020 |
| *Daphnia magna* | UCSN | 48 h, s | **$EC_{50}$ > 100 mg/L nom** | EFSA conclusion [b]<br>Grützner, 1996d, 601042 |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Scenedesmus subspicatus* | UCSN | 72 h, s | **$E_rC_{50}$ > 100 mg/L** nom<br>$NOE_bC \geq 100$ mg/L nom | EFSA conclusion [b]<br>Grützner, 1996e, 601097 |
| *Lemna gibba* | UCSN | 7 d, ss | Frond number:<br>**$E_rC_{50}$ > 100 mg/L** nom<br>$NOEC \geq 100$ mg/L nom | EFSA conclusion [b]<br>Memmert, 1998e, 693911 |
| **Degradation product of nicosulfuron: ADMP** | | | | |
| ~~*Oncorhynchus mykiss*~~ | ~~ADMP~~ | ~~96 h, s~~ | ~~$LC_{50}$ > 97 mg/L nm~~ | ~~Renewal dossier [c]<br>Author censored, 2002b, DuPont-7358~~ |
| *Oncorhynchus mykiss* | ADMP | 96 h, s | **$LC_{50}$ > 100 mg/L** mm | EFSA conclusion [b]<br>Hertl, 1997a, 658034 |
| *Daphnia magna* | ADMP | 48 h, s | **$EC_{50}$ > 100 mg/L** nom | EFSA conclusion [b]<br>Hertl, 1997b, 658012 |
| ~~*Daphnia magna*~~ | ~~ADMP~~ | ~~48 h, s~~ | ~~$EC_{50}$ > 100 mg/L nom~~ | ~~Renewal dossier [c]<br>Hoke, 2002b, DuPont-7357~~ |
| ~~*Daphnia magna*~~ | ~~ADMP~~ | ~~21 d, ss~~ | ~~NOEC = 24.9 mg/L nom~~ | ~~Renewal dossier [c]<br>Samel, 2002, DuPont-9340~~ |
| *Scenedesmus subspicatus* | ADMP | 72 h, s | **$E_{r,y}C_{50,\ 20,\ 10}$ > 100 mg/L** nom<br>$NOE_{r,y}C \geq 100$ mg/L nom | EFSA conclusion [b]<br>Hertl, 1997c, 657990 |
| ~~*Lemna gibba*~~ | ~~ADMP~~ | ~~14 d, s~~ | ~~Frond number:<br>$E_rC_{50}$ > 100 mg/L nom<br>NOEC ≥ 100 mg/L nom~~ | ~~Renewal dossier [c]<br>Sloman, 2002b, DuPont-9339~~ |
| ~~**Degradation product of nicosulfuron: DUDN**~~ | | | | |
| ~~*Pseudokirchneriella subcapitata*~~ | ~~DUDN~~ | ~~72 h, s~~ | ~~$E_{r,b}C_{50}$ > 100 mg/L nom<br>$NOE_{r,b}C$ = 25 mg/L nom<br>$E_yC_{20}$ = 45.65 mg/L nom<br>$E_yC_{10}$ = 24.21 mg/L nom~~ | ~~Renewal dossier [c]<br>Sloman, 2004a, DuPont-13340~~ |
| ~~*Anabaena flos-aquae*~~ | ~~DUDN~~ | ~~96 h, s~~ | ~~$E_aC_{50}$ > 100 mg/L nom [e]<br>$NOE_aC \geq 100$ mg/L nom [e]<br>$E_{b,r}C_{50}$ > 100 mg/L nom<br>$NOE_{b,r}C \geq 100$ mg/L nom~~ | ~~Renewal dossier [c]<br>Ferrell et al., 2004, DuPont-14029~~ |
| ~~*Lemna gibba*~~ | ~~DUDN~~ | ~~7 d, ss~~ | ~~Frond number:<br>$E_{r,y}C_{50,\ 20}$ > 73 mg/L twm<br>$E_rC_{10}$ = 68 mg/L twm<br>$E_yC_{10}$ = 37 mg/L twm<br>Dry weight:<br>$E_{r,y}C_{50,\ 20,\ 10}$ > 73 mg/L twm~~ | ~~Renewal dossier [c]<br>Liedtke, 2013, 90015015~~ |
| ~~**Degradation product of nicosulfuron: ADHP**~~ | | | | |
| ~~*Pseudokirchneriella subcapitata*~~ | ~~ADHP~~ | ~~72 h, s~~ | ~~**$E_rC_{50}$ = 70.1 mg/L nom**<br>$E_rC_{20}$ = 34.3 mg/L nom<br>$E_rC_{10}$ = 21.3 mg/L nom<br>$E_yC_{50}$ = 35.3 mg/L nom<br>$E_yC_{20}$ = 14.5 mg/L nom~~ | ~~Renewal dossier [c]<br>Obert-Rauser, 2016a, S15-05481~~ |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| | | | ~~$E_yC_{10}$ = 8.04 mg/L nom~~<br>~~$NOE_{r,y}C$ = 9.77 mg/L nom~~ | |
| ~~*Lemna gibba*~~ | ~~ADHP~~ | ~~7 d, s~~ | ~~Frond number:~~<br>~~**$E_{r,y}C_{50,20,10}$ > 100 mg/L mm**~~<br>~~NOEC = 31.3 mg/L mm~~<br>~~Dry weight:~~<br>~~$E_{r,y}C_{50,20,10}$ > 100 mg/L mm~~<br>~~NOEC = 100 mg/L mm~~ | ~~Renewal dossier e)~~<br>~~Obert Rauser, 2016c, S15-05482~~ |
| **Higher-tier studies (micro- or mesocosm studies)** | | | | |
| None ~~From the microcosm study with nicosulfuron on aquatic plants it is possible to derive an effect class 2 concentration (2 µg a.s./L nominal or 2.02 µg a.s./L arithmetic mean measured in two replicates on day 0).~~<br>~~Therefore, in line with the decision scheme for the derivation of RAC values from appropriate micro-/mesocosm experiments on the basis of the ETO (ETO-RAC) presented in EFSA (2013) (chapter 9.3.5), an appropriate assessment factor (AF) should be selected based on the amount of uncertainty that is reduced by the study and the advice provided regarding the initially recommended assessment factors of 2 or 3.~~<br>~~The AFs presented above (2–3) are proposed for studies in which a sufficiently low MDD was obtained for adequate number of species (minimum 8 from the most sensitive taxonomic group). If this is not the case, the AF needs to be adjusted. In this case, eight species from the group monocotyledonous aquatic plants were present in the study. However, only for three of them sufficiently low MDD values were calculated. In total, 12 aquatic plant species (monocots and dicots) were present and sufficiently low MDD values were calculated for five of them. It is therefore concluded that this kind of uncertainty should be considered in the recommendation of a sufficiently protective assessment factor.~~<br>~~It is therefore considered that an assessment factor of 3 is applicable to be applied to the NOEC value of 2.02 µg a.s./L (measured value), giving an ETO-$RAC_{sw;ch}$ value of **0.673 µg a.s./L.**~~ | | | | |

[a] EFSA Journal 2016; 14(3):4419
[b] EFSA Scientific Report 2007, 120, 1 – 91
~~[e] Supplementary dossier for the approval renewal of nicosulfuron (N2 document; 2016)~~
[d] Draft Assessment Report Nicosulfuron, Volume 3, Annex B, B9, June 2006
~~[e] Endpoints for area under the growth curve~~
[f] Only $EC_{30}$ endpoints were calculated but the $EC_{50}$ values are estimated to be greater than the highest concentration tested at which effects were < 50%.
[g] No major metabolite in water according to EFSA Scientific Report 2007, 120, 1 – 91, although toxicity data available.
[h] These values were not part of the EU review on mesotrione, however recalculations were available from the ECHA RAC Report on mesotrione (2018)
Endpoints in **bold** were used for the risk assessment.
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, twm: based on time-weighted mean measured concentrations

**Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – SAE053H/01**

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Oncorhynchus mykiss* | SAE053H/01 | 96 h, s | **$LC_{50}$ = 2.15 mg product/L nom**<br>NOEC < 3.42 mg product/L nom | xxx, 2016, S16-03041 |
| *Daphnia magna* | SAE053H/01 | 48 h, s | **$EC_{50}$ = 4.64 mg product/L nom**<br>NOEC = 3.01 mg product/L nom | Zawadsky, 2016, S16-03042 |
| *Daphnia magna* | SAE053H/01 | 21 d, ss | **NOEC = 1.20 mg product/L nom**<br>(mortality and alive offspring per surviving adult)<br>NOEC = 0.0480 mg product/L **nom** | Lang née Zawadsky, 2016a, S16-03043 |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| | | | (alive offspring per adult from test start) | |
| *Pseudokirchneriella subcapitata* | SAE053H/01 | 72 h, s | **$E_rC_{50}$ = 5.46 mg product/L $_{nom}$**<br>$E_rC_{20}$ = 4.81 mg product/L $_{nom}$<br>$E_rC_{10}$ = 4.50 mg product/L $_{nom}$<br>$NOE_rC$ = 4.88 mg product/L $_{nom}$<br>$E_yC_{50}$ = 4.81 mg product/L $_{nom}$<br>$E_yC_{20}$ = 4.58 mg product/L $_{nom}$<br>$E_yC_{10}$ = 4.44 mg product/L $_{nom}$<br>$NOE_yC$ = 1.53 mg product/L $_{nom}$ | Falk, 2016a, S16-03039 |
| *Navicula pelliculosa* | SAE053H/01 | 72 h, s | **$E_rC_{50}$ = 64.9 mg product/L $_{nom}$**<br>$E_rC_{20}$ = 32.0 mg product/L $_{nom}$<br>$E_rC_{10}$ = 22.2 mg product/L $_{nom}$<br>$E_yC_{50}$ = 34.3 mg product/L $_{nom}$<br>$E_yC_{20}$ = 19.7 mg product/L $_{nom}$<br>$E_yC_{10}$ = 14.7 mg product/L $_{nom}$<br>$NOE_{r,y}C$ = 12.5 mg product/L $_{nom}$ | Falk, 2016b, S16-03040 |
| *Lemna gibba* | SAE053H/01 | 7 d, ss | Frond number:<br>**$E_rC_{50}$ = 0.058 mg product/L $_{nom}$**<br>$E_rC_{20}$ = 0.029 mg product/L $_{nom}$<br>$E_rC_{10}$ = 0.020 mg product/L $_{nom}$<br>$E_yC_{50}$ = 0.030 mg product/L $_{nom}$<br>$E_yC_{20}$ = 0.018 mg product/L $_{nom}$<br>$E_yC_{10}$ = 0.014 mg product/L $_{nom}$<br>$NOE_{r,y}C$ = 0.025 mg product/L $_{nom}$<br>Dry weight:<br>$E_rC_{50}$ > 0.100 mg product/L $_{nom}$<br>$E_rC_{20}$ = 0.027 mg product/L $_{nom}$<br>$E_rC_{10}$ = 0.016 mg product/L $_{nom}$<br>$E_yC_{50}$ = 0.031 mg product/L $_{nom}$<br>$E_yC_{20}$ = 0.015 mg product/L $_{nom}$<br>$E_yC_{10}$ = 0.010 mg product/L $_{nom}$<br>$NOE_{r,y}C$ = 0.025 mg product/L $_{nom}$ | Lang née Zawadsky, 2016b, S16-03044 |
| *Myriophyllum spicatum* | SAE053H/01 | 14 d, s, water/sediment system | Shoot length:<br>$E_rC_{50}$ = 0.634 mg product/L $_{nom}$<br>$E_rC_{20}$ = 0.122 mg product/L $_{nom}$<br>$E_rC_{10}$ = 0.0518 mg product/L $_{nom}$<br>$E_yC_{50}$ = 0.232 mg product/L $_{nom}$<br>$E_yC_{20}$ = 0.0598 mg product/L $_{nom}$<br>$E_yC_{10}$ = 0.0294 mg product/L $_{nom}$<br>$NOE_{r,y}C$ = 0.0305 mg product/L $_{nom}$<br>Fresh weight:<br>$E_rC_{50}$ > 1.00 mg product/L $_{nom}$<br>$E_rC_{20}$ = 0.110 mg product/L $_{nom}$<br>$E_rC_{10}$ = 0.0390 mg product/L $_{nom}$<br>$E_yC_{50}$ = 0.248 mg product/L $_{nom}$<br>$E_yC_{20}$ = 0.0440 mg product/L $_{nom}$<br>$E_yC_{10}$ = 0.0178 mg product/L $_{nom}$<br>$NOE_{r,y}C$ = 0.0305 mg product/L $_{nom}$<br>Dry weight:<br>$E_rC_{50}$ = 0.334 mg product/L $_{nom}$ | Gonsior, 2016, S16-03045 |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| | | | $E_rC_{20}$ = 0.0681 mg product/L $_{nom}$<br>$E_rC_{10}$ = 0.0296 mg product/L $_{nom}$<br>$E_yC_{50}$ = 0.179 mg product/L $_{nom}$<br>$E_yC_{20}$ = 0.0419 mg product/L $_{nom}$<br>$E_yC_{10}$ = 0.0196 mg product/L $_{nom}$<br>$NOE_{r,y}C$ = 0.0305 mg product/L $_{nom}$ | |
| **Higher-tier studies (micro- or mesocosm studies)** | | | | |
| None. | | | | |

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations; bold endpoints were used for the risk assessment

#### 9.5.1.1 Justification for new endpoints

The lower tier risk assessments for mesotrione and nicosulfuron are presented in line with EU agreed endpoints, except for primary producers, for which in line with recent guidance (EFSA Technical Report 2016) the growth-rate-based endpoints have been used.
For aquatic macrophytes, additional studies with mesotrione were performed by the applicant on *Lemna gibba* (Bertrand, 2019, KCP 10.2.1/07), *Spirodela polyrhiza* (Christmann, 2021a KCP 10.2.1/08) and *Wolffia arrhiza* (Christmann, 2021b, KCP 10.2.1/09). Studies are included in Appendix 1 and summarised in Appendix 2 of this document.
The worst-case Tier 1 endpoint for mesotrione for aquatic macrophytes was derived from the study on *W. arrhiza* with an $E_rC_{50}$ = 6.28 µg a.s./L and used for Tier 1 risk assessment of aquatic macrophytes. In addition, the available data on aquatic macrophytes for mesotrione was used to derive a geometric mean endpoint as presented in the table below.

**Table 9.5-3: Aquatic macrophytes: geometric mean approach for mesotrione**

| Species | Frond number $E_rC_{50}$ [µg a.s./L] | Dry weight $E_rC_{50}$ [µg a.s./L] | Source |
|---|---|---|---|
| *Lemna gibba* | 59.9 | 25.7 | Smyth et al. 1997d; recalculations from ECHA RAC (2018) |
| | 35.4 | 11.3 | Bertrand 2019 |
| *Lemna gibba* (mean value) [a)] | **47.7** | **18.5** | Applicant calculation |
| *Spirodela polyrhiza* | **12.0** | **18.1** | Christmann 2021a |
| *Wolffia arrhiza* | **28.9** | **6.28** | Christmann 2021b |
| **Geometric mean (n = 4)** [b)] | **25.5** | **12.8** | Applicant calculation |

[a)] The mean value was used where more than one study was available for the same species.
[b)] Only species endpoints in bold were considered for the geometric mean calculation.

The geometric mean of **12.8 µg a.s./L** for the more sensitive parameter dry weight was used for the Tier 2A risk assessment for mesotrione.

Lower tier risk assessments for mesotrione are presented basically in line with EU agreed endpoints. However, with reference to recent EFSA guidance (2013), risk assessments for primary producers are conducted on basis of growth rate based toxicity estimates (i.e. $E_rC_{50}$). As no $E_rC_{50}$ data was available for *Lemna gibba* from the EU agreed study by Smyth et al. (1997), a new study was performed to establish an $E_rC_{50}$ for frond number and dry weight based on the most recent guidelines. The results of this study were compared to the available data from the EU study and since the comparison of those endpoints, that are available for both studies, showed that the new study by Bertrand (2019, KCP 10.2.1/07) presents the worst-case (see table below), the endpoints of the new study have been used for the risk assessment. A study summary is provided in Appendix 2 below.

**Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mesotrione for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of SAE053H/01 in maize (1.5 L product/ha)**

| Endpoint [µg a.s./L] | Frond number | | Dry weight | |
|---|---|---|---|---|
| | Smyth et al. (1997) | Bertrand (2019) | Smyth et al. (1997) | Bertrand (2019) |
| $E_bC_{50}$ | 22 | - | 7.7 | - |
| $E_yC_{50}$ | - | 2.47 | - | 3.21 |
| $E_yC_{20}$ | 7.9 [a)] | 0.224 | 2.2 [a)] | 0.912 |
| $E_yC_{10}$ | 5.6 [a)] | - | 1.4 [a)] | 0.440 |
| $E_rC_{50}$ | - | 35.4 | - | 11.3 |
| $E_rC_{20}$ | 15 [a)] | 1.28 | 4.7 [a)] | 2.10 |
| $E_rC_{10}$ | 6.8 [a)] | 0.227 | 2.0 [a)] | 0.784 |

[a)] Re-evaluation by Liedtke (2013a) from EFSA Journal 2016; 14(3):4419

For the active substance nicosulfuron as well as relevant degradation products in water, reference is made to the on-going re-evaluation process in the EU partly providing endpoints deviating from the current list of endpoints as provided in the EFSA Scientific Report (2007). Reference is made to the N2 document from the current EU evaluation as well as the respective M-CA document (Section 8). Further relevant data are provided and used for risk assessments as listed in **Błąd! Nie można odnaleźć źródła odwołania.**. For detailed study summaries, reference is made to the EU documents. A letter of access is available for the new data.

Assessments based on data for the actual formulated product are presented for exposure via drift as relevant route of entry.

### 9.5.2 Risk assessment

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

The relevant global maximum FOCUS Step 1, 2 and 3 $PEC_{SW}$ for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the tables below. Initial risk assessments are also presented for the formulated product data related to $PEC_{SW}$ resulting from drift entry at the default distance. Further consideration of potential mixture toxicity of the active substances is presented as well. Risk assessments are shown for the risk envelope of 1.5 L product/ha actual application rate of 1.2 L

product/ha.

**Risk assessment based on individual substance data**

**Mesotrione**

**Table 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mesotrione for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of SAE053H/01 in maize (~~1.5~~ 1.2 L product/ha)**

| **Group** | | **Fish acute** | **Fish chronic** | **Inverteb. acute** | **Inverteb. chronic** | **Algae** | **Macrophytes** | |
|---|---|---|---|---|---|---|---|---|
| **Test species** | | ***O. mykiss*** | ***P. promelas*** | ***D. magna*** | ***D. magna*** | ***P. subcapitata*** | ~~***L. gibba***~~ ***W. arrhiza*** **Tier 1** | **Geomean (n = 4), Tier 2A** |
| **Endpoint (µg/L)** | | $LC_{50}$ > 120'000 | NOEC 12'500 | $EC_{50}$ > 622'000 | NOEC 180'000 | $E_rC_{50}$ 13'000 | $E_rC_{50}$ ~~11.3~~ 6.28 | $E_rC_{50}$ 12.8 |
| **AF** | | 100 | 10 | 100 | 10 | 10 | 10 | 10 |
| **RAC (µg/L)** | | > 1200 | 1250 | > 6220 | 18'000 | 1300 | ~~1.13~~ 0.628 | 1.28 |
| **FOCUS Scenario** | **PEC $_{gl-max}$ (µg/L)** | | | | | | | |
| **Step 1 based on worst-case $PEC_{SW}$ considering different soil pH values** | | | | | | | | |
| | ~~40.20~~ 32.16 [al] | ~~< 0.0335~~ < 0.027 | ~~0.0322~~ 0.026 | ~~< 0.0065~~ < 0.0052 | ~~0.0022~~ 0.0018 | ~~0.031~~ 0.025 | ~~**35.58**~~ **49.78** | **25.13** |
| **Step 2 based on worst-case $PEC_{SW}$ considering different soil pH values** | | | | | | | | |
| N-Europe | ~~5.26~~ 4.21 [n] | - | - | - | - | - | ~~**4.655**~~ **6.704** | **3.289** |
| S-Europe | ~~9.88~~ 7.90 [n] | - | - | - | - | - | ~~**8.743**~~ **12.58** | **6.172** |
| **Step 3 based on worst-case $PEC_{SW}$ considering different soil pH values** | | | | | | | | |
| D3/ditch | ~~0.630~~ 0.504 [s] | - | - | - | - | - | ~~0.558~~ 0.803 | 0.394 |
| D4/pond | ~~0.069~~ 0.055 [ac] | - | - | - | - | - | ~~0.061~~ 0.088 | 0.043 |
| D4/stream | ~~0.542~~ 0.434 [ac] | - | - | - | - | - | ~~0.480~~ 0.691 | 0.339 |
| D5/pond | ~~0.054~~ 0.037 [ac] | - | - | - | - | - | ~~0.048~~ 0.059 | 0.029 |
| D5/stream | ~~0.587~~ 0.459 [ac] | - | - | - | - | - | ~~0.519~~ 0.731 | 0.359 |
| ~~D6/ditch~~ | ~~0.627~~ ~~0.507~~ [ac] | - | - | - | - | - | ~~0.555~~ ~~0.802~~ | ~~0.396~~ |
| R1/pond | ~~0.229~~ [n] 0.074 [ac] | - | - | - | - | - | ~~0.203~~ 0.118 | 0.058 |

| Group | | Fish acute | Fish chronic | Inverteb. acute | Inverteb. chronic | Algae | Macrophytes | |
|---|---|---|---|---|---|---|---|---|
| R1/stream | ~~3.657~~ [n] 1.560 [ac] | - | - | - | - | - | **~~3.236~~ 2.484** | **1.219** |
| R2/stream | ~~0.931~~ [ac] 2.200 [n] | - | - | - | - | - | ~~0.824~~ **3.503** | **1.719** |
| R3/stream | ~~4.738~~ 3.780 [n] | - | - | - | - | - | **~~4.193~~ 6.019** | **2.953** |
| R4/stream | ~~4.724~~ 3.760 [n] | - | - | - | - | - | **~~4.181~~ 5.987** | **2.938** |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold; n.a. not applicable; - not relevant/required
[s] $PEC_{SW}$ independent from soil type ; [ac] worst-case $PEC_{SW}$ from acidic soil type; [n] worst-case $PEC_{SW}$ from neutral soil type; [al] worst-case $PEC_{SW}$ from alkaline soil

For the intended use in maize, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for aquatic macrophytes as characterised by an $E_rC_{50}$ of ~~11.3~~ 6.28 µg a.s./L for ~~*Lemna gibba*~~ *Wolffia arrhiza* in connection with an assessment factor of 10 for Tier 1 or an $E_rC_{50}$ of 12.8 µg a.s./L for the geometric mean of several species in connection with an assessment factor of 10 for Tier 2A) in several FOCUS Steps 1-3 scenarios. Therefore, further PEC/RAC ratios were calculated for the affected scenarios based on FOCUS Step 4 $PEC_{SW}$ considering reduced exposure of surface water bodies.

**Table 9.5-6: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for mesotrione based on FOCUS Step 4 calculations with mitigation of run-off for the use of SAE053H/01 in maize (~~1.5~~ 1.2 L product/ha)**

| **Intended use** | | **Maize** | | | | |
|---|---|---|---|---|---|---|
| **Active substance** | | mesotrione | | | | |
| **Application rate (g/ha)** | | 1 × ~~120~~ 96 | | | | |
| **Vegetated buffer strip (m)** | Step 3 (default distance) | - | 10 | 20 | 5 (VFSmod) | 10 (VFSmod) |
| **No-spray buffer (m)** | | 5 | 10 | 20 | 5 (VFSmod) | 10 (VFSmod) |
| R1/stream | ~~3.657~~ [n] 1.560 [ac] | 1.560 [ac] | ~~1.662~~ [n] 0.705 [ac] | ~~0.870~~ [n] 0.369 [ac] | 0.144 [s] | 0.077 [s] |
| R2/stream | 2.200 [n] | 2.200 [n] | 0.972 [n] | 0.503 [n] | 0.197 [s] | 0.104 [s] |
| R3/stream | ~~4.738~~ [n] 3.780 [n] | 3.780 [n] | ~~2.139~~ [n] 1.710 [n] | ~~1.119~~ [n] 0.894 [n] | 0.206 [s] | 0.109 [s] |
| R4/stream | ~~4.724~~ [n] 3.760 [n] | 3.760 [n] | ~~2.138~~ [n] 1.710 [n] | ~~1.118~~ [n] 0.895 [n] | 0.147 [s] | 0.078 [s] |
| **RAC (µg/L)** | | **Aquatic macrophytes - Worst-case Tier 1** | | | | |
| ~~1.13~~ 0.628 | | **PEC/RAC ratio** | | | | |
| **Vegetated filter strip (m)** | Step 3 (default distance) | - | 10 | 20 | 5 (VFSmod) | 10 (VFSmod) |
| **No-spray buffer (m)** | | 5 | 10 | 20 | 5 (VFSmod) | 10 (VFSmod) |
| R1/stream | **~~3.236~~ 2.484** | **2.484** | **~~1.471~~ 1.123** | ~~0.770~~ 0.588 | 0.229 | 0.123 |

| | | | | | | |
|---|---|---|---|---|---|---|
| R2/stream | **3.503** | **3.503** | **1.548** | 0.801 | 0.314 | 0.166 |
| R3/stream | **~~4.193~~ 6.019** | **6.019** | **~~1.893~~ 2.723** | **~~0.990~~ 1.424** | 0.328 | 0.174 |
| R4/stream | **~~4.181~~ 5.987** | **5.987** | **~~1.892~~ 2.723** | **~~0.989~~ 1.425** | 0.234 | 0.124 |
| **RAC (µg/L)** 1.28 | | **Aquatic macrophytes - Geometric mean Tier 2A PEC/RAC ratio** | | | | |
| **Vegetated filter strip (m)** | Step 3 (default distance) | - | 10 | 20 | 5 (VFSmod) | 10 (VFSmod) |
| **No-spray buffer (m)** | | 5 | 10 | 20 | 5 (VFSmod) | 10 (VFSmod) |
| R1/stream | **1.219** | **1.219** | 0.551 | 0.288 | 0.113 | 0.060 |
| R2/stream | **1.719** | **1.719** | 0.759 | 0.393 | 0.154 | 0.081 |
| R3/stream | **2.953** | **2.953** | **1.336** | 0.698 | 0.161 | 0.085 |
| R4/stream | **2.938** | **2.938** | **1.336** | 0.699 | 0.115 | 0.061 |

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; VFSmod: Vegetated Filter Strip modelling, further explanations to this modelling approach are available from Document B8. PEC/RAC ratios above the relevant trigger of 1 are shown in bold
[s] $PEC_{SW}$ independent from soil type, [n] worst-case $PEC_{SW}$ from neutral soil type, [ac] worst-case $PEC_{SW}$ from acidic soil type

**Conclusion on the active substance mesotrione:**

**An acceptable risk for all aquatic organism groups is indicated under the consideration of FOCUS Step 4 $PEC_{SW}$ including a 20 m vegetated filter strip based on Tier 2A effect data or including a 5 m vegetated filter strip (VFSmod).**

**Degradation products of mesotrione**

**Table 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for MNBA for each organism group based on FOCUS Steps 1 calculations for the use of SAE053H/01 in maize (~~1.5~~ 1.2 L product/ha)**

| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** | **Macrophytes** |
|---|---|---|---|---|---|
| **Test species** | | ***O. mykiss*** | ***D. magna*** | ***P. subcapitata*** | ***L. gibba*** |
| **Endpoint** **(µg/L)** | | $LC_{50}$ > 120’000 | $EC_{50}$ 130’000 | $E_rC_{50}$ 42’000 | $E_rC_{50}$ > 97’000 |
| **AF** | | 100 | 100 | 10 | 10 |
| **RAC (µg/L)** | | > 1200 | 1300 | 4200 | > 9700 |
| **FOCUS Scenario** | **PEC $_{gl\text{-}max}$ (µg/L)** | | | | |
| **Step 1** | | | | | |
| | ~~18.79~~ 15.03 | ~~< 0.0157~~ < 0.013 | ~~0.0145~~ 0.012 | ~~0.0045~~ 0.0036 | ~~< 0.0019~~ < 0.0015 |

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

**Table 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AMBA for each organism group based on FOCUS Steps 1 calculations for the use of SAE053H/01 in maize (~~1.5~~ 1.2 L product/ha)**

| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** | **Macrophytes** |
|---|---|---|---|---|---|
| **Test species** | | ***O. mykiss*** | ***D. magna*** | ***P. subcapitata*** | ***L. gibba*** |
| **Endpoint** (µg/L) | | $LC_{50}$ 150'000 | $EC_{50}$ 160'000 | $E_rC_{50}$ 14'000 | $E_rC_{50}$ > 90'000 |
| **AF** | | 100 | 100 | 10 | 10 |
| **RAC (µg/L)** | | 1500 | 1600 | 1400 | > 9000 |
| **FOCUS Scenario** | **PEC $_{gl\text{-}max}$ (µg/L)** | | | | |
| **Step 1 based on worst-case $PEC_{SW}$ considering different soil pH values** | | | | | |
| | ~~8.62~~ 6.90 [al] | ~~0.0057~~ 0.0046 | ~~0.0054~~ 0.0043 | ~~0.0062~~ 0.0049 | ~~< 0.00096~~ < 0.0008 |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
[al] worst-case $PEC_{SW}$ from alkaline soil type

**Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for SYN546974 for the most sensitive species of aquatic macrophytes based on FOCUS Steps 1 calculations for the use of SAE053H/01 in maize (~~1.5~~ 1.2 L product/ha)**

| **Group** | | **Macrophytes** |
|---|---|---|
| **Test species** | | ***L. gibba*** |
| **Endpoint** (µg/L) | | $E_rC_{50}$ > 95'000 |
| **AF** | | 10 |
| **RAC (µg/L)** | | > 9500 |
| **FOCUS Scenario** | **PEC $_{gl\text{-}max}$ (µg/L)** | |
| **Step 1** | | |
| | ~~0.94~~ 0.75 | ~~< 0.000099~~ < 0.00008 |

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

**Conclusion on the degradation products of the active substance mesotrione:**

**An acceptable risk for aquatic organisms is presented for relevant degradation products of the active substance mesotrione based on standard testing and FOCUS Step 1 modelling.**

**Nicosulfuron**

~~The risk assessment presented for the exposure of aquatic macrophytes to the active substance nicosulfuron deviates from the EU agreed peer review and instead follows approaches based on partially new data from the supplementary dossier for the approval renewal of nicosulfuron (M-CP 10 document; 2016). To avoid unnecessary bloating of this submission, the approaches presented in the M-CP 10 document are only~~

~~summarized. Please refer to the relevant document for the detailed evaluations and discussions.~~

~~Initially, t~~The aquatic risk assessment is based on the available Tier 1 data for nicosulfuron as presented in the table below.

**Table 9.5-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for nicosulfuron for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of SAE053H/01 in maize (~~1.5~~ 1.2 L product/ha)**

| **Group** | | **Fish acute** | **Fish chronic** | **Inverteb. acute** | **Inverteb. chronic** | **Algae** | **Macrophytes** |
|---|---|---|---|---|---|---|---|
| **Test species** | | ***O. mykiss*** | ***O. mykiss*** | ***D. magna*** | ***D. magna*** | ***A. flos-aquae*** | ***L. gibba*** |
| **Endpoint (µg/L)** | | $LC_{50}$ 65'700 | NOEC 10'000 | $EC_{50}$ 90'000 | NOEC 5200 | $E_rC_{50}$ 8400 | $E_rC_{50}$ ~~1.82~~ 2.70 |
| **AF** | | 100 | 10 | 100 | 10 | 10 | 10 |
| **RAC (µg/L)** | | 657 | 1000 | 900 | 520 | 840 | ~~0.182~~ 0.270 |
| **FOCUS Scenario** | **PEC $_{gl\text{-}max}$ (µg/L)** | | | | | | |
| **Step 1** | | | | | | | |
| | ~~14.93~~ 12.01 | ~~0.023~~ 0.018 | ~~0.015~~ 0.012 | ~~0.017~~ 0.013 | ~~0.029~~ 0.023 | ~~0.018~~ 0.014 | **~~82.03~~ 44.48** |
| **Step 2** | | | | | | | |
| N-Europe | ~~2.16~~ 1.78 | - | - | - | - | - | **~~11.87~~ 6.59** |
| S-Europe | ~~3.93~~ 3.26 | - | - | - | - | - | **~~21.59~~ 12.07** |
| **Step 3** | | | | | | | |
| D3/ditch | ~~0.238~~ 0.195 | - | - | - | - | - | **~~1.308~~** 0.722 |
| D4/pond | ~~0.016~~ 0.019 | - | - | - | - | - | ~~0.088~~ 0.070 |
| D4/stream | ~~0.205~~ 0.166 | - | - | - | - | - | **~~1.126~~** 0.615 |
| D5/pond | ~~0.024~~ 0.014 | - | - | - | - | - | ~~0.132~~ 0.052 |
| D5/stream | ~~0.216~~ 0.171 | - | - | - | - | - | **~~1.187~~** 0.633 |
| D6/ditch | ~~0.235~~ 0.190 | - | - | - | - | - | **~~1.291~~** 0.704 |
| R1/pond | ~~0.100~~ 0.015 | - | - | - | - | - | ~~0.549~~ 0.056 |
| R1/stream | ~~1.504~~ 0.407 | - | - | - | - | - | **~~8.264~~ 1.507** |
| R2/stream | ~~0.252~~ 1.140 | - | - | - | - | - | **~~1.385~~ 4.222** |
| R3/stream | ~~1.812~~ 1.470 | - | - | - | - | - | **~~9.956~~ 5.444** |
| R4/stream | ~~1.871~~ 1.530 | - | - | - | - | - | **~~10.28~~ 5.667** |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold; n.a. not applicable; - not relevant/required

For the intended use in maize, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for aquatic macrophytes as characterised by an $E_rC_{50}$ for *Lemna gibba* of 1.82 µg a.s./L in connection with an assessment factor of 10) in several FOCUS Steps 1-3 scenarios. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 $PEC_{SW}$ considering reduced exposure of surface water bodies. ~~Furthermore, the refined risk assessment mentioned above is~~

~~presented subsequently.~~

**Table 9.5-11: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for nicosulfuron based on FOCUS Step 4 calculations with mitigation of run-off for the use of SAE053H/01 in maize (1.2 L product/ha)**

| **Intended use** | | **Maize** | | | | |
|---|---|---|---|---|---|---|
| **Active substance** | | nicosulfuron | | | | |
| **Application rate (g/ha)** | | 1 × 36 | | | | |
| **Vegetated filter strip (m)** | Step 3 (default distance) | - | 10 | 20 | 5 (VFSmod) | 10 (VFSmod) |
| **No-spray buffer (m)** | | 5 | 10 | 20 | 5 (VFSmod) | 10 (VFSmod) |
| R1/stream | 0.407 | 0.407 | 0.167 | 0.084 | 0.054 | 0.029 |
| R2/stream | 1.140 | 1.140 | 0.504 | 0.261 | 0.074 | 0.039 |
| R3/stream | 1.470 | 1.470 | 0.666 | 0.349 | 0.077 | 0.041 |
| R4/stream | 1.530 | 1.530 | 0.694 | 0.364 | 0.055 | 0.029 |
| **RAC (µg/L)** | | **Aquatic macrophytes** | | | | |
| 0.270 | | **PEC/RAC ratio** | | | | |
| **Vegetated filter strip (m)** | Step 3 (default distance) | - | 10 | 20 | 5 (VFSmod) | 10 (VFSmod) |
| **No-spray buffer (m)** | | 5 | 10 | 20 | 5 (VFSmod) | 10 (VFSmod) |
| R1/stream | **1.507** | **1.507** | 0.619 | 0.311 | 0.200 | 0.107 |
| R2/stream | **4.222** | **4.222** | **1.867** | 0.967 | 0.274 | 0.144 |
| R3/stream | **5.444** | **5.444** | **2.467** | **1.293** | 0.285 | 0.152 |
| R4/stream | **5.667** | **5.667** | **2.570** | **1.281** | 0.204 | 0.107 |

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; VFSmod: Vegetated Filter Strip modelling, further explanations to this modelling approach are available from Document B8. PEC/RAC ratios above the relevant trigger of 1 are shown in bold

**Conclusion on the active substance nicosulfuron:**

**In conclusion, the risk for exposure to nicosulfuron is indicated to be acceptable when considering FOCUS Step 4 $PEC_{SW}$ and 5 m vegetated filter strip (VFSmod).**

~~**Higher tier risk assessment for aquatic macrophytes – Nicosulfuron**~~

~~*Intra-species geometric mean approach and reduced safety factor (5) due to high sensitivity of Lemna to sulfonylureas*~~

~~Numerous studies are available which investigate the effects of nicosulfuron (as technical active substance; four studies) and formulations containing nicosulfuron (12 studies) on the aquatic plant *Lemna gibba*. As the range of endpoints obtained from these studies is very narrow ($E_rC_{50}$ for frond number/area: 1.82 – 5.1 µg a.s./L; $E_yC_{50}$ for frond number/area: 0.91 – 2.78 µg a.s./L), is it considered justified to calculate the geometric mean of these endpoints for growth rate and yield. Consequently, a geomean $E_rC_{50}$ of 2.94 µg~~

a.s./L and a geomean $E_yC_{50}$ of 1.72 µg a.s./L was obtained. However, in line with recent EFSA guidance, risk assessments for aquatic macrophytes are presented based on $E_rC_{50}$ estimates as growth rate was considered to be the most relevant endpoint.

No inter-species geometric mean was calculated as available laboratory endpoints for *Myriophyllum* are approximately two orders of magnitude higher compared to *Lemna*. The specific sensitivity of *Lemna* to nicosulfuron was furthermore shown in a microcosm outdoor study, where species other than *Lemna gibba* were not affected at concentrations similar to those used to derive the $RAC_{sw;ch}$ at Tier 1.

The specific sensitivity of *Lemna* to sulfonylureas is additionally supported by data on other sulfonylureas such as flupyrsulfuron-methyl salts (EFSA conclusion, 2014)[14] and metsulfuron-methyl (EFSA conclusion, 2015)[15] where again *Lemna* was the most sensitive species and it was accepted at EU level that *Lemna* clearly covers the risk for other aquatic macrophytes. As a consequence, the experts during the EU assessments agreed to reduce the safety factor from 10 to 5. This is therefore considered justified also in the case at hand.

*Microcosm study with aquatic plants*

A microcosm study including 12 aquatic plant species (mono- and dicotyledons) was performed by Burlingham (2011) using a dose-response design with five nominal concentrations between 0.4 and 12.5 µg a.s./L and two replicates for each treatment and four replicates for the control. Four weeks after the initial application of nicosulfuron to the water surface (approximately 1.6 m²), 80% of the water was replaced by fresh water to mimic modelled losses. Monitoring of the microcosm was performed for additional four weeks after the replacement to investigate the potential for recovery.

During the preparation of the supplementary dossier for the renewal of nicosulfuron, the Minimum Detectable Differences (MDDs) for the microcosm were determined as this was not part of the original study. The overall NOEC was determined as 2 µg a.s./L and the overall NOEAEC as 5 µg a.s./L based on nominal concentrations. The RAC was derived based on an assessment factor of 3, which was discussed in detail in the renewal dossier and considered justified mainly due to the higher exposure compared to the worst-case FOCUS stream scenarios. The final RAC was determined to be 0.673 µg a.s./L based on the mean measured NOEC of 2.02 µg a.s./L.

The risk assessment taking into account the geometric mean, the reduced safety factor and the microcosm data is presented in the table below.

[14] EFSA (2014) Conclusion on the peer review of the pesticide risk assessment of the active substance flupyrsulfuron (variant evaluated flupyrsulfuron-methyl-sodium). EFSA Journal 2014;12(11):3881
[15] EFSA (2015) Conclusion regarding the peer review of the pesticide risk assessment of the active substance metsulfuron-methyl. EFSA Journal 2015;13(1):3936

**Table 9.5-12: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for nicosulfuron for aquatic macrophytes based on FOCUS Steps 3 and 4 calculations for the use of SAE053H/01 in maize (1.5 L product/ha) and different refinement options**

| **Group** | | **Aquatic macrophytes** | | | |
|---|---|---|---|---|---|
| **Test species** | | ***L. gibba*** | ***L. gibba*** | ***L. gibba*** | **Microcosm, 12 species** |
| **Endpoint** | | $E_rC_{50}$ geomean | $E_rC_{50}$ | $E_rC_{50}$ geomean | NOEC |
| **(µg/L)** | | 2.94 | 1.82 | 2.94 | 2.02 |
| **AF** | | 10 | 5 | 5 | 3 |
| **RAC (µg/L)** | | 0.294 | 0.364 | 0.588 | 0.673 |
| **FOCUS Scenario** | **PEC $_{gl\ max}$ (µg/L)** | | | | |
| **Step 3** | | | | | |
| D3/ditch | 0.238 | 0.810 | 0.654 | 0.405 | 0.354 |
| D4/pond | 0.016 | 0.054 | 0.044 | 0.027 | 0.024 |
| D4/stream | 0.205 | 0.697 | 0.563 | 0.349 | 0.305 |
| D5/pond | 0.024 | 0.082 | 0.066 | 0.041 | 0.036 |
| D5/stream | 0.216 | 0.735 | 0.593 | 0.367 | 0.321 |
| D6/ditch | 0.235 | 0.799 | 0.646 | 0.400 | 0.349 |
| R1/pond | 0.100 | 0.340 | 0.275 | 0.170 | 0.149 |
| R1/stream | 1.504 | **5.116** | **4.132** | **2.558** | **2.235** |
| R2/stream | 0.252 | 0.857 | 0.692 | 0.429 | 0.374 |
| R3/stream | 1.812 | **6.163** | **4.978** | **3.082** | **2.692** |
| R4/stream | 1.871 | **6.364** | **5.140** | **3.182** | **2.780** |
| **Step 4 – 10 m vegetated filter strip** | | | | | |
| R1/stream | 0.683 | **2.323** | **1.876** | **1.162** | **1.015** |
| R3/stream | 0.819 | **2.786** | **2.250** | **1.393** | **1.217** |
| R4/stream | 0.847 | **2.881** | **2.327** | **1.440** | **1.258** |
| **Step 4 – 20 m vegetated filter strip** | | | | | |
| R1/stream | 0.358 | **1.218** | 0.984 | 0.609 | 0.532 |
| R3/stream | 0.429 | **1.459** | **1.179** | 0.730 | 0.637 |
| R4/stream | 0.443 | **1.507** | **1.217** | 0.753 | 0.658 |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold; n.a. not applicable; – not relevant/required

Accordingly, an acceptable risk is indicated for aquatic macrophytes when considering a 20 m vegetated buffer distance and based on the $E_rC_{50}$ geomean with an assessment factor of 5 or based on the microcosm data with an assessment factor of 3.

*Time-weighted average $PEC_{SW}$*

In the supplementary dossier for the renewal of nicosulfuron (2016) it was examined in detail whether the conditions for the applicability of twa PECs are fulfilled. The main points considered were:

a) Maintenance of test concentrations in those studies were the $RAC_{sw; ch}$ was derived from
b) Latency of effects was not observed in any short-term and long-term exposure study including also long non-exposure periods
c) Linear reciprocity was demonstrated for nicosulfuron effects on *Lemna*
d) Other sulfonylureas did also show linear reciprocity for aquatic plants and the use of twa PECs was agreed on at EU level for the substances metsulfuron-methyl (EFSA, 2015) and prosulfuron (EFSA, 2014)[16]

For the detailed evaluation, reference is made to the renewal dossier (M-CP-10 document, 2016). Time-weighted $PEC_{SW}$ values for 1, 2, 4 and 7 days were used for the refined risk assessment of macrophytes in combination with the worst-case endpoint for *Lemna gibba* and the geometric mean endpoint for *L. gibba*, both with assessment factors of 10 and 5. As the risk was already indicated to be low for the drainage scenarios and the R1 pond and R2 stream scenario based on the initial $PEC_{SW}$ values, these scenarios are not included in the following assessment.

[16] EFSA (2015) Conclusion regarding the peer review of the pesticide risk assessment of the active substance prosulfuron. EFSA Journal 2014;12(9):3815

**Table 9.5-13: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for nicosulfuron for each organism group based on time-weighted average FOCUS Steps 3 and 4 calculations for the use of SAE053H/01 in maize (1.5 L product/ha)**

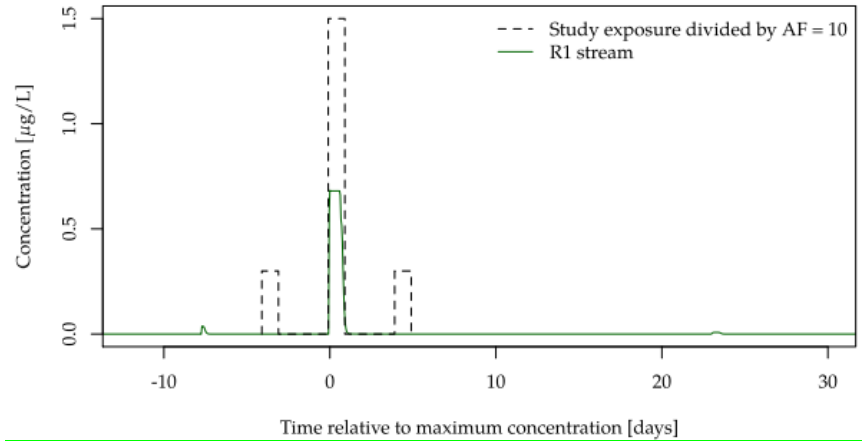
| Group | | Aquatic macrophytes | | | | | Aquatic macrophytes | | | | | Aquatic macrophytes | | | | | Aquatic macrophytes | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Test species | | *L. gibba* | | | | | *L. gibba* | | | | | *L. gibba* | | | | | *L. gibba* | | | |
| Endpoint | | $E_rC_{50}$ | $E_rC_{50}$ | $E_rC_{50}$ geo-mean | $E_rC_{50}$ geo-mean | | $E_rC_{50}$ | $E_rC_{50}$ | $E_rC_{50}$ geo-mean | $E_rC_{50}$ geo-mean | | $E_rC_{50}$ | $E_rC_{50}$ | $E_rC_{50}$ geo-mean | $E_rC_{50}$ geo-mean | | $E_rC_{50}$ | $E_rC_{50}$ | $E_rC_{50}$ geo-mean | $E_rC_{50}$ geo-mean |
| (µg/L) | | 1.82 | 1.82 | 2.94 | 2.94 | | 1.82 | 1.82 | 2.94 | 2.94 | | 1.82 | 1.82 | 2.94 | 2.94 | | 1.82 | 1.82 | 2.94 | 2.94 |
| AF | | 10 | 5 | 10 | 5 | | 10 | 5 | 10 | 5 | | 10 | 5 | 10 | 5 | | 10 | 5 | 10 | 5 |
| RAC (µg/L) | | 0.182 | 0.364 | 0.294 | 0.588 | | 0.182 | 0.364 | 0.294 | 0.588 | | 0.182 | 0.364 | 0.294 | 0.588 | | 0.182 | 0.364 | 0.294 | 0.588 |
| FOCUS Scenario | 1-d PEC $_{twa}$ (µg/L) | | | | | 2-d PEC $_{twa}$ (µg/L) | | | | | 4-d PEC $_{twa}$ (µg/L) | | | | | 7-d PEC $_{twa}$ (µg/L) | | | | |
| Step 3 | | | | | | | | | | | | | | | | | | | | |
| R1/stream | 1.238 | **6.802** | **3.401** | **4.211** | **2.105** | 0.623 | **3.423** | **1.712** | **2.119** | **1.060** | 0.312 | **1.714** | 0.857 | **1.061** | 0.531 | 0.178 | 0.978 | 0.489 | 0.605 | 0.303 |
| R3/stream | 1.054 | **5.791** | **2.896** | **3.585** | **1.793** | 0.530 | **2.912** | **1.456** | **1.803** | 0.901 | 0.265 | **1.456** | 0.728 | 0.901 | 0.451 | 0.176 | 0.967 | 0.484 | 0.599 | 0.299 |
| R4/stream | 1.341 | **7.368** | **3.684** | **4.561** | **2.281** | 0.671 | **3.687** | **1.843** | **2.282** | **1.141** | 0.336 | **1.846** | 0.923 | **1.143** | 0.571 | 0.217 | **1.192** | 0.596 | 0.738 | 0.369 |
| Step 4 – 10 m vegetated filter strip | | | | | | | | | | | | | | | | | | | | |
| R1/stream | 0.562 | **3.088** | **1.544** | **1.912** | 0.956 | 0.283 | **1.555** | 0.777 | 0.963 | 0.481 | 0.142 | 0.780 | - | 0.483 | - | 0.081 | 0.445 | - | - | - |
| R3/stream | 0.477 | **2.621** | **1.310** | **1.622** | 0.811 | 0.240 | **1.319** | 0.659 | 0.816 | 0.408 | 0.120 | 0.659 | - | 0.408 | - | 0.079 | 0.434 | - | - | - |

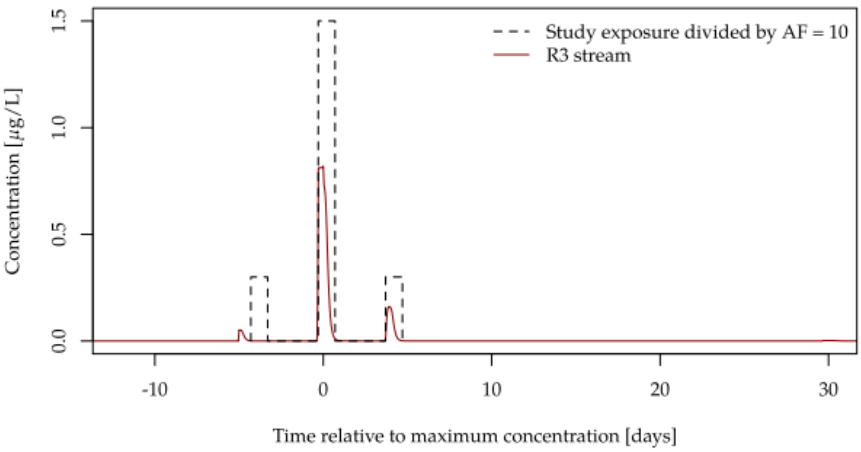
| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R4/stream | 0.607 | **3.335** | **1.668** | **2.065** | **1.032** | 0.303 | **1.665** | 0.832 | **1.031** | 0.515 | 0.152 | 0.835 | - | 0.517 | - | 0.098 | 0.538 | - | - | - |
| **Step 4 – 20 m vegetated filter strip** | | | | | | | | | | | | | | | | | | | | |
| R1/stream | 0.295 | **1.621** | 0.810 | **1.003** | 0.502 | 0.148 | 0.813 | - | 0.503 | - | 0.074 | - | - | - | - | 0.042 | - | - | - | - |
| R3/stream | 0.249 | **1.368** | 0.684 | 0.847 | 0.423 | 0.125 | 0.687 | - | 0.425 | - | 0.063 | - | - | - | - | 0.041 | - | - | - | - |
| R4/stream | 0.317 | **1.742** | 0.871 | **1.078** | 0.539 | 0.159 | 0.874 | - | 0.541 | - | 0.079 | - | - | - | - | 0.051 | - | - | - | - |

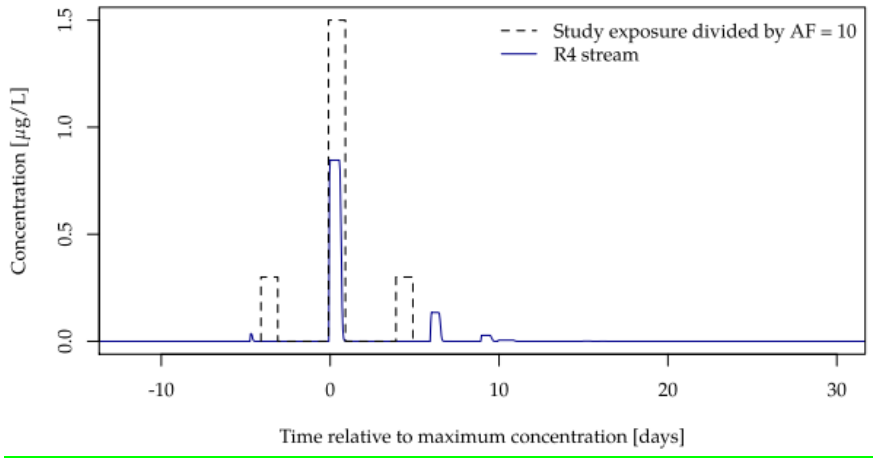
AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold; n.a. not applicable; - not relevant/required

~~*Pulsed exposure studies*~~

~~Stream scenarios are characterised by short-lived pulses of exposure via drift, run-off and drainage, which are partially quickly diluted by downstream currents. Exposure analyses of the FOCUS stream scenarios R1, R3 and R4 were performed in detail by Ranke (2017).~~
~~The available pulsed exposure studies were examined and compared to the FOCUS profiles to check whether effects and exposure can be linked. The study by Liedtke (2012a) investigated three 24 h pulse concentrations (concentration profiles: 0.6 – 3.0 – 0.6; 1.5 – 7.5 – 1.5 and 3.0 – 15 – 3.0 µg a.s./L) which were separated by 72 h of non-exposure. After the last exposure, three weeks of non-exposure were monitored. The exposure in the study was compared to the predicted environmental concentrations based on FOCUS Step 4 with 10 meter vegetated filter strip. The graphical comparison is shown below.~~






~~**Figure 1: Comparison of simulated (dotted line) and tested (colored line) exposure using an assessment factor of 10 for the three FOCUS stream scenarios R1 (green), R3 (red) and R4 (blue).**~~
~~Accordingly, the tested exposure by Liedtke (2012a) covers the simulated FOCUS Step 4 exposure in the stream scenarios R1, R3 and R4 considering a 10 m vegetated buffer distance.~~

~~**Conclusion on the active substance nicosulfuron:**~~

~~**In conclusion, the risk for exposure to nicosulfuron is indicated to be acceptable when considering FOCUS Step 4 $PEC_{sw}$ and a 10 m vegetated buffer strip based on pulsed exposure studies.**~~

**Degradation products of nicosulfuron**

**Table 9.5-14: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for ASDM for each organism group based on FOCUS Steps 1 calculations for the use of SAE053H/01 in maize (~~1.5~~ 1.2 L product/ha)**

| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** | **Macrophytes** |
|---|---|---|---|---|---|
| **Test species** | | ***O. mykiss*** | ***D. magna*** | ~~***A. flos-aquae***~~ ***P. subcapitata*** | ***L. gibba*** |
| **Endpoint (µg/L)** | | $LC_{50}$ > 996’000 | $EC_{50}$ > 954’000 | $E_rC_{50}$ ~~50’000~~ > 336’000 | $E_rC_{50}$ 16’000 |
| **AF** | | 100 | 100 | 10 | 10 |
| **RAC (µg/L)** | | > 9960 | > 9540 | ~~5000~~ > 33’600 | 1600 |
| **FOCUS Scenario** | **PEC $_{gl-max}$ (µg/L)** | | | | |
| **Step 1** | | | | | |
| | ~~11.20~~ 15.08 | ~~< 0.001~~ < 0.002 | ~~< 0.001~~ < 0.002 | ~~0.002~~ < 0.001 | ~~0.007~~ 0.009 |

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

**Table 9.5-15: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AUSN for each organism group based on FOCUS Steps 1 calculations for the use of SAE053H/01 in maize (~~1.5~~ 1.2 L product/ha)**

| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** | **Macrophytes** |
|---|---|---|---|---|---|
| **Test species** | | ***B. rerio*** | ***D. magna*** | ***S. subspicatus*** | ***L. gibba*** |
| **Endpoint (µg/L)** | | $LC_{50}$ > 100'000 | $EC_{50}$ > 100'000 | $E_rC_{50}$ > 100'000 | $E_rC_{50}$ > 100'000 |
| **AF** | | 100 | 100 | 10 | 10 |
| **RAC (µg/L)** | | > 1000 | > 1000 | > 10'000 | > 10'000 |
| **FOCUS Scenario** | **PEC $_{gl\text{-}max}$ (µg/L)** | | | | |
| **Step 1** | | | | | |
| | ~~7.14~~ 3.45 | ~~< 0.007~~ < 0.003 | ~~< 0.007~~ < 0.003 | ~~< 0.0007~~ < 0.0003 | ~~< 0.0007~~ < 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

~~**Table 9.5-16: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for MU-466 for each organism group based on FOCUS Steps 1 calculations for the use of SAE053H/01 in maize (1.5 L product/ha)**~~

| ~~**Group**~~ | | ~~**Fish acute**~~ | ~~**Inverteb. acute**~~ | ~~**Algae**~~ | ~~**Macrophytes**~~ |
|---|---|---|---|---|---|
| ~~**Test species**~~ | | ~~***O. mykiss***~~ | ~~***D. magna***~~ | ~~***S. subspicatus***~~ | ~~***L. gibba***~~ |
| ~~**Endpoint (µg/L)**~~ | | ~~$LC_{50}$ > 100'000~~ | ~~$EC_{50}$ > 100'000~~ | ~~$E_rC_{50}$ > 100'000~~ | ~~$E_rC_{50}$ > 100'000~~ |
| ~~**AF**~~ | | ~~100~~ | ~~100~~ | ~~10~~ | ~~10~~ |
| ~~**RAC (µg/L)**~~ | | ~~> 1000~~ | ~~> 1000~~ | ~~> 10'000~~ | ~~> 10'000~~ |
| ~~**FOCUS Scenario**~~ | ~~**PEC $_{gl\text{-}max}$ (µg/L)**~~ | | | | |
| ~~**Step 1**~~ | | | | | |
| ~~-~~ | ~~0.16~~ | ~~< 0.0002~~ | ~~< 0.0002~~ | ~~< 0.00002~~ | ~~< 0.00002~~ |

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

**Table 9.5-17: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for HMUD for each organism group based on FOCUS Steps 1 calculations for the use of SAE053H/01 in maize (~~1.5~~ 1.2 L product/ha)**

| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** | **Macrophytes** |
|---|---|---|---|---|---|
| **Test species** | | ***O. mykiss*** | ***D. magna*** | ~~***P. subcapitata***~~ ***S. subspicatus*** | ***L. gibba*** |
| **Endpoint (µg/L)** | | $LC_{50}$ > 100'000 | $EC_{50}$ > 100'000 | $E_rC_{50}$ ~~43'900~~ > 100'000 | $E_rC_{50}$ ~~514~~ > 1000 |
| **AF** | | 100 | 100 | 10 | 10 |
| **RAC (µg/L)** | | > 1000 | > 1000 | ~~4390~~ > 10'000 | ~~51.4~~ > 100 |
| **FOCUS Scenario** | **PEC $_{gl\text{-}max}$ (µg/L)** | | | | |
| **Step 1** | | | | | |
| | ~~9.03~~ 3.96 | ~~< 0.009~~ < 0.003 | ~~< 0.009~~ < 0.003 | ~~0.002~~ < 0.0003 | ~~0.176~~ < 0.396 |

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

**Table 9.5-18: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for UCSN for each organism group based on FOCUS Steps 1 calculations for the use of SAE053H/01 in maize (~~1.5~~ 1.2 L product/ha)**

| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** | **Macrophytes** |
|---|---|---|---|---|---|
| **Test species** | | ***B. rerio*** | ***D. magna*** | ***S. subspicatus*** | ***L. gibba*** |
| **Endpoint (µg/L)** | | $LC_{50}$ > 100'000 | $EC_{50}$ > 100'000 | $E_rC_{50}$ > 100'000 | $E_rC_{50}$ > 100'000 |
| **AF** | | 100 | 100 | 10 | 10 |
| **RAC (µg/L)** | | > 1000 | > 1000 | > 10'000 | > 10'000 |
| **FOCUS Scenario** | **PEC $_{gl\text{-}max}$ (µg/L)** | | | | |
| **Step 1** | | | | | |
| | ~~2.54~~ 1.63 | ~~< 0.0025~~ < 0.002 | ~~< 0.0025~~ < 0.002 | ~~< 0.0003~~ < 0.0002 | ~~< 0.0003~~ < 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

**Table 9.5-19: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for ADMP for each organism group based on FOCUS Steps 1 calculations for the use of SAE053H/01 in maize (~~1.5~~ 1.2 L product/ha)**

| **Group** | | **Fish acute** | **Inverteb. acute** | **~~Inverteb. chronic~~** | **Algae** | **~~Macrophytes~~** |
|---|---|---|---|---|---|---|
| **Test species** | | ***O. mykiss*** | ***D. magna*** | ***~~D. magna~~*** | ***S. subspicatus*** | ***~~L. gibba~~*** |
| **Endpoint (µg/L)** | | $LC_{50}$ > 100’000 | $EC_{50}$ > 100’000 | ~~NOEC~~ ~~24’900~~ | $E_rC_{50}$ > 100’000 | ~~$E_rC_{50}$~~ ~~> 100’000~~ |
| **AF** | | 100 | 100 | ~~100~~ | 10 | ~~10~~ |
| **RAC (µg/L)** | | > 1000 | > 1000 | ~~2490~~ | > 10’000 | ~~> 10’000~~ |
| **FOCUS Scenario** | **PEC $_{gl\text{-}max}$ (µg/L)** | | | | | |
| **Step 1** | | | | | | |
| | ~~3.79~~ 3.20 | ~~< 0.0038~~ < 0.0032 | ~~< 0.0038~~ < 0.0032 | ~~0.0015~~ | ~~< 0.0004~~ < 0.0003 | ~~< 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

~~**Table 9.5-20: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for DUDN for each organism group based on FOCUS Steps 1 calculations for the use of SAE053H/01 in maize (1.5 L product/ha)**~~

| ~~**Group**~~ | | ~~**Algae**~~ | ~~**Macrophytes**~~ |
|---|---|---|---|
| ~~**Test species**~~ | | ~~***P. subcapitata***~~ | ~~***L. gibba***~~ |
| ~~**Endpoint (µg/L)**~~ | | ~~$E_rC_{50}$ > 100’000~~ | ~~$E_rC_{50}$ > 73’000~~ |
| ~~**AF**~~ | | ~~10~~ | ~~10~~ |
| ~~**RAC (µg/L)**~~ | | ~~> 10’000~~ | ~~> 7300~~ |
| ~~**FOCUS Scenario**~~ | ~~**PEC $_{gl\text{-}max}$ (µg/L)**~~ | | |
| ~~**Step 1**~~ | | | |
| ~~-~~ | ~~3.37~~ | ~~< 0.0003~~ | ~~< 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~~

~~**Table 9.5-21: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for ADHP for each organism group based on FOCUS Steps 1 calculations for the use of SAE053H/01 in maize (1.5 L product/ha)**~~

| ~~**Group**~~ | | ~~**Algae**~~ | ~~**Macrophytes**~~ |
|---|---|---|---|
| ~~**Test species**~~ | | ~~***P. subcapitata***~~ | ~~***L. gibba***~~ |
| ~~**Endpoint**~~<br>~~**(µg/L)**~~ | | ~~$E_rC_{50}$~~<br>~~70'100~~ | ~~$E_rC_{50}$~~<br>~~> 100'000~~ |
| ~~**AF**~~ | | ~~10~~ | ~~10~~ |
| ~~**RAC (µg/L)**~~ | | ~~7010~~ | ~~> 10'000~~ |
| ~~**FOCUS Scenario**~~ | ~~**PEC $_{gl\text{-}max}$ (µg/L)**~~ | | |
| ~~**Step 1**~~ | | | |
| ~~-~~ | ~~0.68~~ | ~~0.0001~~ | ~~< 0.00007~~ |

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

**Conclusion on the degradation products of the active substance nicosulfuron:**

**An acceptable risk for aquatic organisms is presented for relevant degradation products of the active substance nicosulfuron based on standard testing and FOCUS Step 1 modelling.**

**Risk assessment based on product data**

Risk assessment based on product data (spray drift entry), is presented hereafter:

**Table 9.5-22: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for SAE053H/01 for each organism group based on FOCUS drift entry for the use in maize (~~1.5~~ 1.2 L product/ha)**

| **Group** | | **Fish acute** | **Inverteb. acute** | **Inverteb. chronic** | **Algae** | **Macrophytes** | |
|---|---|---|---|---|---|---|---|
| **Test species** | | ***O. mykiss*** | ***D. magna*** | ***D. magna*** | ***P. subcapitata*** | ***L. gibba*** | ***M. spicatum*** |
| **Endpoint** (µg/L) | | $LC_{50}$ 2150 | $LC_{50}$ 4640 | NOEC 1200 | $E_rC_{50}$ 5460 | $E_rC_{50}$ 58 | $E_rC_{50}$ 634 |
| **AF** | | 100 | 100 | 10 | 10 | 10 | 10 |
| **RAC (µg/L)** | | 21.5 | 46.4 | 120 | 546 | 5.8 | 63.4 |
| **FOCUS Scenario** | **PEC gl-max (µg/L)** | | | | | | |
| **Drift** | | | | | | | |
| default distance | ~~7.91~~ ~~6.06~~ | ~~0.368~~ ~~0.284~~ | ~~0.168~~ ~~0.131~~ | ~~0.065~~ ~~0.051~~ | ~~0.014~~ ~~0.011~~ | ~~1.347~~ ~~1.045~~ | ~~0.123~~ ~~0.096~~ |
| | 7.555 | 0.35 | 0.16 | 0.06 | 0.01 | **1.3** | 0.12 |
| ~~3~~ 5 m buffer | ~~4.00~~ ~~3.10~~ | - | - | - | - | ~~0.696~~ ~~0.534~~ | - |
| | 2.048 | | | | | 0.35 | |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold; - not relevant/required

**<u>Conclusion on the product SAE053H/01:</u>**

For the intended use in maize, calculated PEC/RAC ratios did indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for aquatic macrophytes as characterised by an $E_rC_{50}$ for *Lemna gibba* of 58 µg a.s./L in connection with an assessment factor of 10) when considering a ~~3~~ 5 m buffer zone, which is already included in the ~~10 m buffer zone~~ risk mitigation measures required for both active substances.

**Potential mixture toxicity**

As SAE053H/01 is a combination product containing two active substances, mixture toxicity considerations are required.

If taking into consideration the assessments for drift entry of the product as demonstrated above, a ~~3~~ 5 m drift buffer distance is indicated to be necessary.

Further mixture toxicity considerations based on active substance data under consideration of all entry pathways are presented under consideration of the aquatic EFSA guidance document (2013).

A ´toxicity per fraction´ assessment is performed providing information on the relative contribution of the active substances to the overall toxicity of the mixture based on the fractions of active substances as in the formulated product by assuming concentration addition (CA). For detailed explanation of the calculations reference is made to the EFSA birds and mammals guidance (2009). A surrogate endpoint for CA is calculated using the following equation.

$$EC_{X\ mix-CA} = \left( \sum_{i=1}^{n} \frac{p_i}{EC_{Xi}} \right)^{-1}$$

With:

| | |
|---|---|
| $EC_{X\ mix-CA}$ | surrogate endpoint for additive mixture toxicity |
| 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 ($\Sigma\ p_i = 1$) |
| $EC_{Xi}$ | concentration of component I provoking X % effect (or $NOEC_i$) |

Fractions in the mixture are calculated according to the following equation with the sum of fractions being 1.

$$p_1 = c_1/c_1 + \cdots + c_n$$

Based on active substance concentrations of 120 g mesotrione/L and 45 g nicosulfuron/L, fractions ($p_i$) of 0.73 and 0.27, respectively are calculated for the product composition.

The surrogate endpoint is related to the measured $EC_X$ or NOEC ($EC_{X\ PPP}$) from the product studies, where available, building the Model Deviation Ratio (MDR).

$$MDR = \frac{EC_{X\ mix-CA}}{EC_{X\ PPP}}$$

With an MDR in the range of 0.2 to 5 the predicted endpoint for CA is interpreted as to be in line with the measured toxicity. Values below 0.2 indicate a potential antagonism (i.e. CA overestimates mixture toxicity), whereas values greater than 5 might indicate a potential synergism (i.e. CA potentially underestimates mixture toxicity).

In the following table, the acute and chronic mixture toxicity assessments and MDR calculations are presented for all relevant aquatic organisms. In addition, the ratios of surrogate toxicity estimates are shown.

**Table 9.5-23: Toxicity per fraction assessment and MDR calculation for additive mixture toxicity for aquatic organisms**

| Organism | Time scale | Test substance | Toxicity endpoint [µg/L] | Toxicity per fraction for PPP/ **Surrogate endpoint $EC_{X\ mix\text{-}CA}$ PPP [µg/L]** | Contribution to overall toxicity [%] | MDR |
|---|---|---|---|---|---|---|
| Fish | acute | mesotrione | > 120‘000 | 165‘100 | **59.3** | 397 |
| | | nicosulfuron | 65‘700 | 240‘500 | **40.7** | |
| | | SAE053H/01 | 2150 / **247** [a)] | **97‘900** | n.a. | |
| | chronic | mesotrione | 12‘500 | 17‘200 | **68.0** | n.a. |
| | | nicosulfuron | 10‘000 | 36‘600 | **32.0** | |
| | | SAE053H/01 | n.a. | **11‘700** | n.a. | |
| Aquatic invertebrates | acute | mesotrione | > 622‘000 | 855‘700 | **27.8** | 447 |
| | | nicosulfuron | 90‘000 | 329‘500 | **72.2** | |
| | | SAE053H/01 | 4640 / **532** [a)] | **237‘900** | n.a. | |
| | chronic | mesotrione | 180‘000 | 247‘600 | 7.1 | 128 |
| | | nicosulfuron | 5200 | 19‘000 | **92.9** | |
| | | SAE053H/01 | 1200 / **138** [a)] | **17‘700** | n.a. | |
| Algae, *P. subcapitata* | chronic | mesotrione | 13’000 | 17’900 | ~~**93.6**~~ **97.4** | ~~26.7~~ 27.8 |
| | | nicosulfuron | ~~71’170~~ 182’000 [b)] | ~~260’600~~ 666’300 | ~~6.4~~ 2.6 | |
| | | SAE053H/01 | 5460 / **626** [a)] | ~~**16’700**~~ **17’400** | n.a. | |
| Aquatic macrophytes, *Lemna gibba* | chronic | mesotrione | 11.3 | 15.55 | ~~**30.0**~~ **38.9** | ~~0.70~~ 0.91 |
| | | nicosulfuron | ~~1.82~~ 2.7 | ~~6.66~~ 9.89 | ~~**70.0**~~ **61.1** | |
| | | SAE053H/01 | 58 / **6.65** [a)] | ~~**4.66**~~ **6.04** | n.a. | |

n.a. not available/applicable; PPP Plant Protection Product; MDR: Model Deviation Ratio
[a)] Product endpoint corrected for active substance content (sum: 112.4 g/L) and product density (0.98 g/mL)
[b)] Value for *Scenedesmus subspicatus* since no data for nicosulfuron on *Pseudokirchneriella subcapitata* is available. Since both algae belong to the green single cell algae, this is considered applicable.

The assessment indicates that assuming Concentration Addition (CA) and underlying the product composition, toxicity is driven by both active substances in case of fish (acute and chronic), invertebrates (acute) as well as aquatic macrophytes. For chronic invertebrates, the toxicity is mainly driven by nicosulfuron and for algae mesotrione contributes most to the overall toxicity.

The Model Deviation Ratios (MDR) with a value between 0.2 and 5 in case of aquatic macrophytes indicates that predicted mixture toxicity based on CA is in line with the observed toxicity of the product (even suggesting slight antagonistic action). In case of all other organism groups, however, a value of > 5 indicates an increased toxicity of the active substances when in formulation (i.e. potential synergism).

Based on the mixture composition at $PEC_{mix}$ (FOCUS Step 2, Step 3 and Step 4 were considered), the MDRs are comparable to the ones at product composition for the single organisms groups, i.e. between 0.2 and 5 for aquatic macrophytes and above 5 for the remaining groups.

Consequently, the mixture toxicity for all organisms groups having an MDR > 5 is shown based on product endpoints since the ratio of calculated surrogate toxicity estimates is in the range of 0.8 to 1.2 indicating that the product toxicity endpoints are applicable for the ratio of active substances as predicted in the field. The product endpoints (corrected for active substance content and density) are therefore compared to $PEC_{mix}$ for FOCUS Step 2, 3 and 4 (only worst-case scenarios) in the table below. The FOCUS scenario R2 stream was not considered, although belonging to the worst-case scenarios, as it is not relevant for any of the countries in the GAP table.

**Table 9.5-24: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for SAE053H/01 for each organism group based on FOCUS Step 2, 3 and 4 for the use in maize (~~1.5~~ 1.2 L product/ha)**

| **Group** | | **Fish acute** | **Inverteb. acute** | **Inverteb. chronic** | **Algae** |
|---|---|---|---|---|---|
| **Test species** | | ***O. mykiss*** | ***D. magna*** | ***D. magna*** | ***P. subcapitata*** |
| **Endpoint** | | $LC_{50}$ | $LC_{50}$ | NOEC | $E_rC_{50}$ |
| **(µg/L)** | | 247 | 532 | 138 | 626 |
| **AF** | | 100 | 100 | 10 | 10 |
| **RAC (µg/L)** | | 2.47 | 5.32 | 13.8 | 62.6 |
| **FOCUS Scenario** | **PEC $_{gl\text{-}max}$ (µg/L)** | | | | |
| **Step 2 – $PEC_{mix}$ for worst-case scenario** | | | | | |
| S-Europe | ~~9.88~~ 7.90 (meso) + ~~3.93~~ 3.26 (nico) = ~~13.81~~ 11.16 | ~~**5.591**~~ **4.518** | ~~**2.596**~~ **2.098** | ~~**1.001**~~ 0.809 | ~~0.221~~ 0.185 |
| **Step 3 – $PEC_{mix}$ for worst-case scenarios** | | | | | |
| R1/stream | ~~3.657~~ 1.560 (meso) + ~~1.504~~ 0.407 (nico) = ~~5.161~~ 1.967 | ~~**2.089**~~ 0.796 | ~~0.970~~ 0.370 | ~~0.374~~ - | - |
| R3/stream | ~~4.738~~ 3.780 (meso) + ~~1.812~~ 1.470 (nico) = ~~6.55~~ 5.250 | ~~**2.652**~~ **2.126** | ~~**1.231**~~ 0.987 | ~~0.475~~ - | - |
| R4/stream | ~~4.724~~ 3.760 (meso) + ~~1.871~~ 1.530 (nico) = ~~6.60~~ 5.290 | ~~**2.672**~~ **2.142** | ~~**1.241**~~ 0.994 | ~~0.478~~ - | - |
| **Step 4 – $PEC_{mix}$ for worst-case scenarios including 10 m buffer strip** | | | | | |
| R1/stream | ~~1.662~~ 0.705 (meso) + ~~0.683~~ 0.167 (nico) = ~~2.345~~ 0.872 | ~~0.949~~ 0.353 | - | - | - |
| R3/stream | ~~2.139~~ 1.710 (meso) + ~~0.819~~ 0.666 (nico) = ~~2.958~~ 2.376 | ~~**1.198**~~ 0.962 | ~~0.556~~ - | - | - |
| R4/stream | ~~2.138~~ 1.710 (meso) + ~~0.847~~ 0.694 (nico) = ~~2.985~~ 2.404 | ~~**1.209**~~ 0.973 | ~~0.561~~ - | - | - |
| ~~**Step 4 – $PEC_{mix}$ for worst-case scenarios including 20 m buffer strip**~~ | | | | | |
| ~~R1/stream~~ | ~~0.870 (meso) + 0.358 (nico) = 1.228~~ | - | - | - | - |

| Group | | Fish acute | Inverteb. acute | Inverteb. chronic | Algae |
|---|---|---|---|---|---|
| R3/stream | 1.119 (meso) + 0.429 (nico) = 1.548 | 0.627 | - | - | - |
| R4/stream | 1.118 (meso) + 0.443 (nico) = 1.561 | 0.632 | - | - | - |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold; - not relevant/required

**Accordingly, the mixture toxicity assessment for all organism groups except macrophytes shows an acceptable risk based on FOCUS Step 4 $PEC_{mix}$ when applying a 20 10 m buffer strip.**

For the mixture toxicity for aquatic macrophytes, the MDR of 0.70 0.91 had shown that no higher toxicity is indicated from the product and therefore, risk assessments can be shown assuming concentration addition. The Risk Quotient approach ($RQ_{mix}$) is employed using the following equation which also allows for the integration of higher tier data and/or differing assessment factors.

$$RQ_{mix} = \sum_{n} \frac{PEC_{SW}}{RAC}$$

For mesotrione, no higher tier data is available for aquatic macrophytes, therefore the worst-case toxicity estimate for *Lemna* is taken into account together with the peak exposure concentration from FOCUS Step 4 modelling. For nicosulfuron, various risk refinements are available, however, the pulsed exposure testing with *Lemna* is considered the most relevant with regard to realism. Therefore, an RAC is derived from this test under consideration of the standard assessment factor of 10 and compared to the peak exposure concentration from FOCUS Step 4 modelling. Only the worst-case scenarios R1, R2, R3 and R4 stream are assessed since they are considered to cover the risk from the remaining scenarios. The assessment is shown below for both, the risk envelope of 1.5 L product/ha as well as the actual application rate of 1.2 L product/ha.

**Table 9.5-25: $RQ_{mix}$ for 1 x 1.5 L product/ha based on FOCUS Step 4 $PEC_{SW}$ (10 and 20 m vegetated buffer distance)**

| FOCUS scenario | Mesotrione | | Nicosulfuron | | $RQ_{mix}$ | $RQ_{mix}$ acceptability criterion |
|---|---|---|---|---|---|---|
| | $PEC_{SW}$ [µg a.s./L] | RAC [µg a.s./L] | $PEC_{SW}$ [µg a.s./L] | RAC [µg a.s./L] | | |
| **Step 4 – 10 m vegetated buffer** | | | | | | |
| R1/stream | 1.662 | 1.13 | 0.683 | 1.5 | **1.10** | ≤ 1 |
| R3/stream | 2.139 | 1.13 | 0.819 | 1.5 | **1.38** | |
| R4/stream | 2.138 | 1.13 | 0.847 | 1.5 | **1.40** | |
| **Step 4 – 20 m vegetated buffer** | | | | | | |
| R1/stream | 0.870 | 1.13 | 0.358 | 1.5 | **1.01** | ≤ 1 |
| R3/stream | 1.119 | 1.13 | 0.429 | 1.5 | **1.23** | |
| R4/stream | 1.118 | 1.13 | 0.443 | 1.5 | **1.23** | |

**Table 9.5-26: $RQ_{mix}$ for 1 x 1.2 L product/ha based on FOCUS Step 4 $PEC_{SW}$ (20 m vegetated buffer distance)**

| FOCUS scenario | Mesotrione | | Nicosulfuron | | $RQ_{mix}$ | $RQ_{mix}$ acceptability criterion |
|---|---|---|---|---|---|---|
| | $PEC_{SW}$ [µg a.s./L] | RAC [µg a.s./L] | $PEC_{SW}$ [µg a.s./L] | RAC [µg a.s./L] | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| ~~**Step 4 – 20 m vegetated buffer**~~ | | | | | | |
| ~~R1/stream~~ | ~~0.694~~ | ~~1.13~~ | ~~0.286~~ | ~~1.5~~ | ~~0.80~~ | ~~≤ 1~~ |
| ~~R3/stream~~ | ~~0.894~~ | ~~1.13~~ | ~~0.343~~ | ~~1.5~~ | ~~**1.02**~~ | |
| ~~R4/stream~~ | ~~0.894~~ | ~~1.13~~ | ~~0.354~~ | ~~1.5~~ | ~~**1.03**~~ | |

For both substances, the worst-case RAC from Tier 1 testing is considered and for mesotrione additionally the RAC from Tier 2A is considered for the $RQ_{mix}$ approach. The assessment is shown for all FOCUS scenarios that are relevant for at least one country of the GAP table (i.e. all EU-relevant scenarios for maize except D6 and R2) and is based on the actual application rate of 1.2 L product/ha. For mesotrione, the worst-case $PEC_{SW}$ considering the different pH was considered.

Different mitigation options have been considered for the calculations with standard FOCUS Step 4 mitigation measures including 10 or 20 m vegetated filter strip and, in case of failure, also considering FOCUS Step 4 mitigation measures of 5 m vegetated filter strip calculated with VFSmod for the failing scenarios.

**Table 9.5-27: $RQ_{mix}$ for 1 x 1.2 L product/ha based on FOCUS Step 4 $PEC_{SW}$ (different mitigation options)**

| FOCUS scenario | Mesotrione | | Nicosulfuron | | $RQ_{mix}$ | $RQ_{mix}$ acceptability criterion |
|---|---|---|---|---|---|---|
| | $PEC_{SW}$ [µg a.s./L] | RAC [µg a.s./L] | $PEC_{SW}$ [µg a.s./L] | RAC [µg a.s./L] | | |
| **Step 4 – 10 m vegetated filter strip** | | | | | | |
| D3/ditch | 0.088 | 0.628 (Tier 1) | 0.039 | 0.270 | 0.285 | ≤ 1 |
| | | 1.28 (Tier 2A) | | | 0.213 | |
| D4/pond | 0.055 | 0.628 (Tier 1) | 0.019 | 0.270 | 0.158 | |
| | | 1.28 (Tier 2A) | | | 0.113 | |
| D4/stream | 0.099 | 0.628 (Tier 1) | 0.040 | 0.270 | 0.306 | |
| | | 1.28 (Tier 2A) | | | 0.225 | |
| D5/pond | 0.037 | 0.628 (Tier 1) | 0.012 | 0.270 | 0.103 | |
| | | 1.28 (Tier 2A) | | | 0.073 | |
| D5/stream | 0.109 | 0.628 (Tier 1) | 0.040 | 0.270 | 0.322 | |
| | | 1.28 (Tier 2A) | | | 0.233 | |
| R1/pond | 0.032 | 0.628 (Tier 1) | 0.007 | 0.270 | 0.077 | |
| | | 1.28 (Tier 2A) | | | 0.051 | |
| R1/stream | 0.705 | 0.628 (Tier 1) | 0.167 | 0.270 | **1.741** | |
| | | 1.28 (Tier 2A) | | | **1.169** | |
| R3/stream | 1.710 | 0.628 (Tier 1) | 0.666 | 0.270 | **5.190** | |
| | | 1.28 (Tier 2A) | | | **3.803** | |
| R4/stream | 1.710 | 0.628 (Tier 1) | 0.694 | 0.270 | **5.293** | |
| | | 1.28 (Tier 2A) | | | **3.906** | |
| **Step 4 – 20 m vegetated filter strip** | | | | | | |
| R1/stream | 0.369 | 0.628 (Tier 1) | 0.084 | 0.270 | 0.899 | ≤ 1 |
| | | 1.28 (Tier 2A) | | | 0.599 | |
| R3/stream | 0.894 | 0.628 (Tier 1) | 0.349 | 0.270 | **2.716** | |
| | | 1.28 (Tier 2A) | | | **1.991** | |
| R4/stream | 0.895 | 0.628 (Tier 1) | 0.364 | 0.270 | **2.773** | |
| | | 1.28 (Tier 2A) | | | **2.047** | |
| **Step 4 – 5 m VFSmod** | | | | | | |
| R3/stream | 0.206 | 0.628 (Tier 1) | 0.077 | 0.270 | 0.613 | ≤ 1 |
| | | 1.28 (Tier 2A) | | | 0.446 | |
| R4/stream | 0.147 | 0.628 (Tier 1) | 0.055 | 0.270 | 0.438 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | 1.28 (Tier 2A) | | | 0.319 | |

As can be seen from the table above, the mixture toxicity assessment for the most sensitive organism aquatic macrophytes is acceptable for FOCUS scenarios D3 (ditch), D4 (pond and stream), D5 (pond and stream) and R1 (pond) based on FOCUS Step 4 $PEC_{SW}$ when considering a 10 m vegetated filter strip while for FOCUS scenario R1 (stream) the risk is indicated to be acceptable considering a 20 m vegetated filter strip. For FOCUS scenarios R3 and R4 stream, an acceptable risk is indicated based on VFSmod including a 5 m VFS (RA cover the R1 scenario).

~~As can be seen from the tables above, for the risk envelope of 1.5 L product/ha the mixture toxicity assessment for macrophytes fails for all relevant FOCUS scenarios. For the actual application rate, however, the R1 stream scenario is passed and the R3 stream and R4 stream scenarios are very close to the required trigger value. When rounding to only one significant number, the assessment would actually be passed. It must be noted that the mixture toxicity assessment based on concentration addition as shown above is very conservative, since the actual product SAE053H/01 was already demonstrated to have a lower toxicity to macrophytes compared to the predicted toxicity based on concentration addition of the active substances (reference is made to the MDR of 0.70 as shown in Table 9.5-21 above). In consequence, the slight exceedance of the trigger is considered to still demonstrate an acceptable risk for aquatic macrophytes when exposed to SAE053H/01 at the actual application rate of 1.2 L product/ha.~~

**Conclusion on mixture toxicity:**

The risk from the mixture of mesotrione and nicosulfuron applied as SAE053H/01 is indicated to be acceptable for the actual application rate of 1.2 L product/ha based on FOCUS Step 4 $PEC_{SW}$ when considering a 20 m vegetated buffer strip for FOCUS scenarios D3, D4, D5 and R1. ~~and taking into account higher tier effect data~~. For those countries considering FOCUS scenarios R3 and R4 as relevant, an acceptable risk is indicated based on FOCUS Step 4 considering 5 m VFS calculated using VFSmod. In the table below, an overview on the required FOCUS scenarios for each country included in the GAP is shown together with an information on which mitigation measures are required for the specific country.

**Table 9.5-28: Relevant FOCUS scenarios for CEU countries included in the GAP and required risk mitigation measures.**

| CEU Country | FOCUS scenarios | | | | | | | | National modelling | Comment[b)] |
|---|---|---|---|---|---|---|---|---|---|---|
| | D3 | D4 | D5 | D6 | R1 | R2 | R3 | R4 | | |
| Austria (AT) | | X | | | X | | X | | | Passes with 5 m VFSmod |
| Belgium (BE) | X | X | | | X | | | | | Passes with Step 4, 20 m |
| Czech Republik (CZ) | | X | | | X | | | | | Passes with Step 4, 20 m |
| Germany (DE) | | | | | X [a)] | | | | X | refer to national addendum |
| Hungary (HU) | X | | X | | X | | X | X | | Passes with 5 m VFSmod |
| Ireland (IE) | | | | | | | | | X | refer to national addendum (UK) |
| The Netherlands (NL) | | | | | | | | | X | refer to national addendum |
| Poland (PL) | X | X | | | X | | | | | Passes with Step 4, 20 m or 5 m VFSmod |
| Romania (RO) | | | X | | X | | | | | Passes with Step 4, 20 m or 5 m VFSmod |
| Slovakia (SK) | | X | X | | X | | | | | Passes with Step 4, 20 m or 5 m VFSmod |
| Slovenia (SI) | | | | | | | | | X | refer to national addendum |
| United Kingdom (UK) | | | | | | | | | X | refer to national addendum |

FOCUS scenario / national modelling not relevant for this country
FOCUS scenario is passed without VFSmod
FOCUS scenario is passed using VFSmod

[a)] The higher assessment factor for primary producers of 30 was considered for the calculations, reference is made to the German National Addendum.
b) buffer zones are required only for R scenarios

## 9.5.3 Overall conclusions

~~The risk from exposure to mesotrione and nicosulfuron applied as SAE053H/01 in maize at the actual application rate of 1 x 1.2 L product/ha is indicated to be acceptable for the individual active substances and for the mixture based on higher tier data and FOCUS Step 4 calculations with 20 m vegetated buffer zone. The risk from metabolites of mesotrione and nicosulfuron is indicated to be acceptable based on Tier 1 data and FOCUS Step 1 calculations.~~

The risk from the active substances mesotrione and nicosulfuron as well as the mixture is indicated to be acceptable based on Tier 2A data and FOCUS Step 4 calculations when considering risk mitigation options. An overview on the country-specific requirements is given above. For those countries for which specific national modelling was considered, reference is made to the corresponding national addenda (i.e. Germany, The Netherlands, Slovenia and United Kingdom).

The risk from the product via spray drift exposure is indicated to be acceptable when applying a ~~3~~ 5 m buffer zone. The risk from metabolites of mesotrione and nicosulfuron is indicated to be acceptable based on Tier 1 data and FOCUS Step 1 calculations.

**Review Comments:**

The relevant predicted environmental concentrations in water ($PEC_{sw}$) for risk assessments covering the proposed use pattern are taken from Part B Section 8 (Environmental Fate). The risk assessment was based on the worst case PEC values and the results of laboratory toxicity testing.

SAE053H/01 pose no unacceptable risk to aquatic organisms according to the label with appropriate buffer zone.

The acceptability of risk mitigation measures used in refined risk assessment for aquatic plants should be checked on national level (width of buffer zones, VFSmod).

## 9.6 Effects on bees (KCP 10.3.1)

### 9.6.1 Toxicity data

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

Effects on bees of SAE053H/01 were not evaluated as part of the EU assessments of mesotrione or nicosulfuron. 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 ~~deviates from~~ is in line with the results of the EU review process of mesotrione and ~~the supplementary dossier for the approval renewal of~~ nicosulfuron ~~(N2 document; 2016). Justifications are provided below.~~

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

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| **Mesotrione** | | | | |
| *Apis mellifera* | mesotrione | Oral<br>48 h<br>Acute | **$LD_{50}$ > 11 µg a.s./bee** | EFSA conclusion [a)]<br>Jackson & Gough, 1995, RJ1959B |
| | | Contact<br>48 h<br>Acute | **$LD_{50}$ > 100 µg a.s./bee** | |
| **Nicosulfuron** | | | | |
| ~~*Apis mellifera*~~ | ~~nicosulfuron~~ | ~~Oral<br>48 h<br>Acute~~ | ~~$LD_{50}$ > 22.4 µg a.s./bee~~ | ~~Renewal dossier [c)]<br>Kling, 2015a, S15-04040~~ |
| | | ~~Contact<br>48 h<br>Acute~~ | ~~$LD_{50}$ > 50 µg a.s./bee~~ | |
| *Apis mellifera* | nicosulfuron | Oral<br>48 h<br>Acute | $LC_{50}$ > 1000 mg a.s./L (in diet) [e)] | EFSA conclusion [b)]<br>Morris, 1991, 1411-90-209-04-21F-03 |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Apis mellifera* | SL-950 4% SC | Oral<br>48 h<br>Acute | $LD_{50}$ > 131 µg product/bee; i.e. **5.24 µg a.s./bee** [f] | EFSA conclusion [b]<br>Petto, 1994, 480400 |
| *Apis mellifera* | nicosulfuron | Contact<br>48 h<br>Acute | **$LD_{50}$ = 76 µg a.s./bee** | EFSA conclusion [b]<br>Winter et al., 1991, 272-102 |
| ~~*Bombus terrestris*~~ | ~~nicosulfuron~~ | ~~Oral<br>96 h<br>Acute~~ | ~~$LD_{50}$ > 35.96 µg a.s./bee~~ | ~~Renewal dossier [c]<br>Kling, 2015b, S15-04040~~ |
| | | ~~Contact<br>96 h<br>Acute~~ | ~~$LD_{50}$ > 50 µg a.s./bee~~ | |
| ~~*Apis mellifera*~~ | ~~nicosulfuron~~ | ~~Oral<br>10 d<br>Chronic~~ | ~~$LDD_{50}$ > 11.43 µg a.s./bee/day (i.e. 280 mg a.s./kg diet)~~ | ~~Renewal dossier [c]<br>Schmitt, 2014, DuPont 39664~~ |
| ~~*Apis mellifera* larvae~~ | ~~nicosulfuron~~ | ~~Oral<br>72 h<br>bee brood development (single feeding)~~ | ~~NOED = 20 µg a.s./larva~~ | ~~Renewal dossier [c]<br>Klank, 2014, S14-00341~~ |
| SAE053H/01 | | | | |
| *Apis mellifera* | SAE053H/01 | Oral<br>48 h<br>Acute | **$LD_{50}$ > 655.01 µg product/bee** | Molitor, 2016a, S16-02516 |
| *Apis mellifera* | SAE053H/01 | Contact<br>48 h<br>Acute | **$LD_{50}$ > 1000 µg product/bee** | Molitor, 2016a, S16-02516 |
| *Apis mellifera* | SAE053H/01 | Oral<br>10 d<br>Chronic | **$LDD_{50}$ > 138.21 µg product/bee/day**<br>(i.e. > 4000 mg product/kg diet)<br>**$LDD_{50}$ > 11.51 µg mesotrione/bee/day and > 4.33 µg nicosulfuron/bee/day** [g]<br>NOEDD ≥ 138.21 µg product/bee/day (i.e. ≥ 4000 mg product/kg diet) | Molitor, 2016b, S16-02518 |
| *Apis mellifera* larvae | SAE053H/01 | Oral<br>8 d<br>Repeated exposure | **NOED = 554 µg product/larva**<br>(i.e. 3600 mg product/kg diet)<br>**NOED = 46.15 µg mesotrione/larva and 17.34 µg nicosulfuron/larva** [g]<br>$LD_{50}$ = 859 µg product/larva (i.e. 5580 mg product/kg diet) | Vergé & Wagner, 2016, S16-02503 |
| **Higher-tier studies (tunnel test, field studies)** | | | | |
| None. | | | | |

[a] EFSA Journal 2016; 14(3):4419
[b] EFSA Scientific Report 2007, 120, 1 – 91
~~c) Supplementary dossier for the approval renewal of nicosulfuron (N2 document; 2016)~~

~~[d] Draft Assessment Report Nicosulfuron, Volume 3, Annex B, B9, June 2006~~
[e] Study details did not allow calculation of oral $LD_{50}$ in µg a.s./bee.
[f] Since no data on oral toxicity of the active substance is available, the information from the solo-formulation from the EU review was used to derive a surrogate endpoint.
[g] Recalculated based on active ingredient contents of 8.33% (w/w) for mesotrione and 3.13% (w/w) for nicosulfuron.
Endpoints in **bold** were used for the risk assessment.

#### 9.6.1.1 Justification for new endpoints

Risk assessments are provided based on overall worst-case toxicity endpoints of mesotrione and nicosulfuron and product endpoints for the actual formulation SAE053H/01.

It is noted that the study with SAE053H/01 on bee larvae was only conducted over 8 days instead of 22 days which is the current standard. This is due to the fact that at the time of study conduct (2016), the 8-day repeated exposure study was the most current approach and no guideline on the 22-day repeated exposure study was available. A repetition of the study over 22 days is not considered required for the following reasons:

1. The current study over 8 days does not indicate any effects compared to the control up to and including the second highest dose tested (i.e. 554 µg product/larva or 3600 mg product/kg diet).
2. No toxicity to bee larvae emergence is expected based on the results from the other studies on SAE053H/01, which are all very high limit endpoints.
3. Maize at the intended BBCH stages 12 - 18 is not relevant for collection of pollen or nectar; only weeds could potentially be available for bees to collect pollen and nectar. However, due to the mode of action of SAE053H/01 as an herbicide, weeds are also not considered to be present over a longer time period, i.e. chronic exposure of SAE053H/01 to larvae is unlikely.

In conclusion, the currently available data on bees is considered sufficient to conclude on a safe use for bees of SAE053H/01 at the intended application at BBCH 12 - 18 in maize.

### 9.6.2 Risk assessment

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

Risk assessments are presented for the risk envelope of 1.5 L product/ha in early post-emergence maize (BBCH 12 – 19) covering the actual application rate of 1.2 L product/ha. It is noted that the crop at these early growth stages is not attractive for bees; however, in a conservative approach a risk assessment is still performed.

### 9.6.2.1 Hazard quotients for bees

**Table 9.6-2: First-tier assessment of the risk for bees due to the use of SAE053H/01 in maize (1.5 L product/ha)**

| | | | |
|---|---|---|---|
| **Intended use**<br>**Active substance**<br>**Application rate (g/ha)** | **Maize**<br>mesotrione<br>1 x 120 g a.s./ha | | |
| **Test design** | **$LD_{50}$ (lab.) (µg a.s./bee)** | **Single application rate (g a.s./ha)** | **$Q_{HO}$, $Q_{HC}$ criterion: $Q_H \leq 50$** |
| Oral toxicity | > 11 | 120 | < 10.9 |
| Contact toxicity | > 100 | | < 1.20 |
| **Active substance**<br>**Application rate (g/ha)** | nicosulfuron<br>1 x 45 g a.s./ha | | |
| **Test design** | **$LD_{50}$ (lab.) (µg a.s./bee)** | **Single application rate (g a.s./ha)** | **$Q_{HO}$, $Q_{HC}$ criterion: $Q_H \leq 50$** |
| Oral toxicity | ~~> 22.4~~ 5.24 | 45 | ~~< 2.01~~ 8.59 |
| Contact toxicity | ~~> 50~~ 76 | | ~~< 0.90~~ 0.59 |
| **Product**<br>**Application rate (g/ha)** | SAE053H/01<br>1 x 1470 g product/ha [a)] | | |
| **Test design** | **$LD_{50}$ (lab.) (µg product/bee)** | **Single application rate (g product/ha)** | **$Q_{HO}$, $Q_{HC}$ criterion: $Q_H \leq 50$** |
| Oral toxicity | > 655.01 | 1470 | < 2.24 |
| Contact toxicity | > 1000 | | < 1.47 |

$Q_{HO}$, $Q_{HC}$: Hazard quotients for oral and contact exposure. $Q_H$ values shown in bold breach the relevant trigger.
[a)] Calculated based on the product density of 0.98 g/cm³ and maximum single application rate of 1.5 L product/ha

As outlined in the table above, both the Hazard Quotients for oral ($Q_{HO}$) and contact exposure ($Q_{HC}$) are well below the trigger of 50 for all active substances and the product. Therefore, an acceptable low risk to bees is expected from the application of SAE053H/01 in maize.

It is noted that no chronic effects on adults or juvenile stages of bees are expected for the following reasons:

The exposure to honeybees can be caused by the application of plant protection products through direct overspray, by contact with residues on plants or by oral intake of treated food items (nectar or pollen) whilst bees are foraging on food. These sources are highly unlikely in case of the application SAE053H/01 because the early application timing (BBCH 12 – 19) is distinctly before the inflorescence emergence/flowering which is at principal growth stage 5/6 (BBCH Monograph, 2001[17]). Furthermore, maize is only attractive for bees to forage on pollen but not for nectar. In conclusion, intense foraging on the crop for pollen and nectar can be excluded.

In general, weeds in fields might be attractive to bees. However SAE053H/01 is an herbicide which affects the growing of weeds at early growth stages before flowering. Therefore, no adverse effects on populations and communities are considered to be expected in consideration of the GAP use proposed for SAE053H/01.

[17] BBCH Monograph (2001). Growth stages of mono- and dicotyledonous plants. 2. Edition, 2001, (ed. U. Meier), Federal Biological Research Centre for Agriculture and Forestry.

Furthermore, the results of the chronic feeding studies to adult bees and bee larvae from SAE053H/01 do not give rise to a specific concern.

In conclusion, it is reasonable to conclude that the acute and chronic risk for bees can be considered as acceptable, both from the toxicity and the exposure point of view.

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

Not required.

### 9.6.3 Effects on bumble bees

No data available and ~~are~~ considered necessary. ~~Acute contact data are available for the active substance nicosulfuron from the on-going AIR process for the active substance. The limit dose endpoints of > 35.96 and > 50 µg a.s./bee for oral and contact toxicity, respectively, do not give indications for an increased toxicity of nicosulfuron to bumble bees as compared to honey bees (> 22.4 and > 50 µg a.s./bee).~~

### 9.6.4 Effects on solitary bees

No data available and considered necessary.

### 9.6.5 Overall conclusions

The risk from oral and contact exposure to mesotrione and nicosulfuron applied as SAE053H/01 in maize (risk envelope: 1 x 1.5 L product/ha, i.e. 120 g a.s./h mesotrione and 45 g a.s./ha nicosulfuron) is indicated to be acceptable for bees based on active substance and product data.

**Review Comments:**

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 submitted risk assessment, based on laboratory studies, has been accepted. It can therefore be concluded that there will be negligible risk associated with the exposure of bees to SAE053H/01.

## 9.7 Effects on arthropods other than bees (KCP 10.3.2)

### 9.7.1 Toxicity data

Studies on the toxicity to non-target arthropods have been carried out with several formulations containing mesotrione or nicosulfuron during the EU reviews of the active substances. Full details of these studies are provided in the respective EU DAR and related documents but are not considered relevant for the actual product SAE053H/01.

Effects on non-target arthropods of SAE053H/01 were not evaluated as part of the EU assessment of

mesotrione or nicosulfuron. 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 deviates from the results of the EU review process of mesotrione and ~~the supplementary dossier for the approval renewal of~~ nicosulfuron ~~(N2 document; 2016).~~ Justifications are provided below.

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

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Typhlodromus pyri* (protonymphs) | SAE053H/01 | Laboratory test glass plates (2D) | **$L/ER_{50}$ > 1000 mL product/ha** | Walter, 2016b, S16-01608 |
| *Aphidius rhopalosiphi* (adults) | SAE053H/01 | Laboratory test glass plates (2D) | $LR_{50}$ = 871.2 mL product/ha **$ER_{50}$ > 250 mL product/ha** | Walter, 2016a, S16-01607 |
| *Aphidius rhopalosiphi* (adults) | SAE053H/01 | Extended laboratory test with aged residues maize plants (3D) | **$L/ER_{50}$ > 1850 mL product/ha** | Röhlig, 2017a, 17 48 NAR 0001 |
| *Aleochara bilineata* (adults) | SAE053H/01 | Extended laboratory test LUFA soil (2D) | **$ER_{50}$ > 1500 mL product/ha** | Röhlig, 2017b, 17 48 NKE 0002 |
| **Field or semi-field tests** | | | | |
| None. | | | | |

Endpoints in **bold** were used for the risk assessment.

#### 9.7.1.1 Justification for new endpoints

Standard laboratory studies on the formulation SAE053H/01 are available for *Typhlodromus pyri* and *Aphidius rhopalosiphi*. Therefore, risk assessments are based on these data as they are most relevant. As a risk was indicated at Tier 1 for *A. rhopalosiphi*, an extended laboratory test was provided. Furthermore, an additional species, *Aleochara bilineata*, was tested as demanded by the current data requirements. The soil-dwelling arthropod *Aleochara bilineata* was chosen as test species due to the early application timing of SAE053H/01 (BBCH 12 – 19), as it is assumed that a significant proportion of the test item will reach the soil.

### 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.

The risk assessment is shown for the risk envelope of 1.5 L product/ha, covering the actual application rate of 1.2 L product/ha.

### 9.7.2.1 Risk assessment for in-field exposure

**Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of SAE053H/01 in maize (1.5 L product/ha)**

| | | | | |
|---|---|---|---|---|
| **Intended use** | **Maize** | | | |
| **Product** | SAE053H/01 | | | |
| **Application rate (g/ha)** | 1 x 1.5 L product/ha, i.e. 120 g a.s./ha mesotrione and 45 g a.s./ha nicosulfuron | | | |
| **MAF** | 1.00 | | | |
| **Test species Tier I** | **L/ER$_{50}$ (lab.) (mL product/ha)** | | **PER$_{in\text{-}field}$ (mL product/ha)** | **HQ$_{in\text{-}field}$ criterion: HQ ≤ 2** | |
| *Aphidius rhopalosiphi* | > 250 | 871.2 | 1500 | < 6 | 1.72 |
| *Typhlodromus pyri* | > 1000 | | 1500 | < 1.5 | |
| **Test species Higher-tier** | **Rate with ≤ 50 % effect* (mL product/ha)** | | **PER$_{in\text{-}field}$ (mL product/ha)** | **PER$_{in\text{-}field}$ below rate with ≤ 50 % effect?** | |
| *Aphidius rhopalosiphi* | > 1850 | | 1500 | Yes | |
| *Aleochara bilineata* | > 1500 | | 1500 | Yes | |

MAF: Multiple application factor; PER: Predicted environmental rate; 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.

Accordingly, the in-field risk to non-target arthropods is indicated to be acceptable for *Typhlodromus pyri* based on a Tier 1 study and for *Aphidius rhopalosiphi* and *Aleochara bilineata* based on Tier 2 studies.

### 9.7.2.2 Risk assessment for off-field exposure

**Table 9.7-3: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of SAE053H/01 in maize (1.5 L product/ha)**

| | | | | | | |
|---|---|---|---|---|---|---|
| **Intended use** | | **Maize** | | | | |
| **Product** | | SAE053H/01 | | | | |
| **Application rate (g/ha)** | | 1 x 1.5 L product/ha, i.e. 120 g a.s./ha mesotrione and 45 g a.s./ha nicosulfuron | | | | |
| **MAF** | | 1.00 | | | | |
| **vdf** | | 10 | | | | |
| **Test species Tier I** | **L/ER$_{50}$ (lab.) (mL product/ha)** | **Drift percentile (%)** | **Drift rate (mL product/ha)** | **PER$_{off\text{-}field}$ (mL product/ha)** | **CF** | **HQ$_{off\text{-}field}$ criterion: HQ ≤ 2** |
| *Aphidius rhopalosiphi* | > 250 | 2.77 | 41.55 | 4.155 | 10 | 0.166 |
| *Typhlodromus pyri* | > 1000 | 2.77 | 41.55 | 4.155 | 10 | 0.042 |
| ~~**Test species Higher-tier**~~ | ~~**Rate with ≤ 50 % effect* (mL product/ha)**~~ | ~~**Drift percentile (%)**~~ | ~~**Drift rate (mL product/ha)**~~ | ~~**PER$_{off\text{-}field}$ (mL product/ha)**~~ | ~~**CF**~~ | ~~**corr. PER$_{off\text{-}field}$ below rate with ≤ 50 % effect?**~~ |
| ~~*Aphidius rhopalosiphi*~~ | ~~> 1850~~ | ~~2.77~~ | ~~41.55~~ | ~~41.55~~ | ~~5~~ | ~~Yes~~ |
| ~~*Aleochara bilineata*~~ | ~~> 1500~~ | ~~2.77~~ | ~~41.55~~ | ~~41.55~~ | ~~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.

Accordingly, the off-field risk to non-target arthropods is indicated to be acceptable for both standard test species based on Tier 1 data and for *Aleochara bilineata* based on Tier 2 data.

### 9.7.2.3 Additional higher-tier risk assessment

Not required.

### 9.7.2.4 Risk mitigation measures

No risk mitigation needed.

## 9.7.3 Overall conclusions

The in-field and off-field risk from exposure to mesotrione and nicosulfuron applied as SAE053H/01 in maize (risk envelope: 1 x 1.5 L product/ha, i.e. 120 g a.s./ha mesotrione and 45 g a.s./ha nicosulfuron) is indicated to be acceptable for non-target arthropods other than bees based on Tier 2 data.

**Review Comments:**

Based on the results of the conducted risk assessment it can be concluded that low risk for non-target arthropods is expected from the use of SAE053H/01 according to the proposed use pattern. No unacceptable effects on non-target arthropods are expected in in-field and off-field habitats.

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

### 9.8.1 Toxicity data

Studies on the toxicity to soil meso- and macrofauna have been carried out with mesotrione and nicosulfuron and relevant soil degradation products. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of SAE053H/01 were not evaluated as part of the EU assessment of mesotrione or nicosulfuron. 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 deviates from the results of the EU review process of mesotrione and ~~the supplementary dossier for the approval renewal of~~ nicosulfuron ~~(N2 document; 2016)~~. Justifications are provided below.

**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 |
|---|---|---|---|---|
| **Mesotrione** | | | | |
| *Eisenia fetida* | mesotrione | Mixed into substrate<br>14 d, acute<br>10 % peat content | $LC_{50}$ > 2000 mg a.s./kg dw | EFSA conclusion [a]<br>Bembridge & Jackson, 1996, RJ2225B |
| *Eisenia fetida* | MNBA | Mixed into substrate<br>14 d, acute<br>10 % peat content | $LC_{50}$ > 1000 mg/kg dw | EFSA conclusion [a]<br>Travis & Gough, 1999, RJ2871B |
| *Eisenia fetida* | MNBA | Mixed into substrate<br>56 d, chronic<br>5 % peat content | **NOEC ≥ 1050 mg/kg dw**<br>$EC_{10, 20}$ > 1050 mg/kg dw | EFSA conclusion [a]<br>Friedrich, 2013a, 13 10 48 086 S |
| *Eisenia fetida* | AMBA | Mixed into substrate<br>56 d, chronic<br>5 % peat content | **NOEC ≥ 1050 mg/kg dw**<br>$EC_{10, 20}$ > 1050 mg/kg dw | EFSA conclusion [a]<br>Friedrich, 2013b, 13 10 48 111 S |
| **Nicosulfuron** | | | | |
| ~~*Eisenia fetida*~~ | ~~nicosulfuron~~ | ~~Mixed into substrate<br>56 d, chronic<br>10 % peat content~~ | ~~**NOEC ≥ 100 mg a.s./kg dw**~~ | ~~Renewal dossier [b]<br>Gehrig, 2010, DuPont-29704~~ |
| *Eisenia fetida* | ASDM | Mixed into substrate<br>56 d, chronic<br>10 % peat content | **NOEC ≥ 0.350 mg/kg dw** | EFSA conclusion [b]<br>Lührs, 2006a, 31611022 |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Eisenia fetida* | UCSN | Mixed into substrate<br>56 d, chronic<br>10 % peat content | **NOEC ≥ 0.050 mg/kg dw** | EFSA conclusion [b)]<br>Lührs, 2006a, 31611022 |
| *Eisenia fetida* | AUSN | Mixed into substrate<br>56 d, chronic<br>10 % peat content | **NOEC ≥ 0.100 mg/kg dw** | EFSA conclusion [b)]<br>Lührs, 2006a, 31611022 |
| *Folsomia candida* | ASDM | Mixed into substrate<br>28 d, chronic<br>10 % peat content | **NOEC ≥ 0.350 mg/kg dw** | EFSA conclusion [b]<br>Lührs, 2006b, 31612016 erroneously mentioned as „AUSN“ in EFSA conclusion but correct in Addendum 3 to DAR (May 2007) |
| *Folsomia candida* | UCSN | Mixed into substrate<br>28 d, chronic<br>10 % peat content | **NOEC ≥ 0.050 mg/kg dw** | EFSA conclusion [b]<br>Lührs, 2006b, 31612016 |
| *Folsomia candida* | AUSN | Mixed into substrate<br>28 d, chronic<br>10 % peat content | **NOEC ≥ 0.100 mg/kg dw** | EFSA conclusion [b]<br>Lührs, 2006b, 31612016 |
| ~~*Eisenia fetida*~~ | ~~ASDM~~ | ~~Mixed into substrate<br>56 d, chronic<br>10 % peat content~~ | ~~**NOEC ≥ 1000 mg/kg dw**~~ | ~~Renewal dossier [b)]<br>Lührs, 2003a, DuPont-12116~~ |
| ~~*Eisenia fetida*~~ | ~~AUSN~~ | ~~Mixed into substrate<br>56 d, chronic<br>10 % peat content~~ | ~~**NOEC ≥ 1000 mg/kg dw**~~ | ~~Renewal dossier [b)]<br>Lührs, 2004a, DuPont-12346~~ |
| ~~*Eisenia fetida*~~ | ~~HMUD~~ | ~~Mixed into substrate<br>56 d, chronic<br>10 % peat content~~ | ~~**NOEC ≥ 1000 mg/kg dw**~~ | ~~Renewal dossier [b)]<br>Wagenhoff, 2016a, S15-04103~~ |
| ~~*Eisenia fetida*~~ | ~~UCSN~~ | ~~Mixed into substrate<br>56 d, chronic<br>10 % peat content~~ | ~~**NOEC ≥ 1000 mg/kg dw**~~ | ~~Renewal dossier [b)]<br>Lührs, 2004b, DuPont-14030~~ |
| ~~*Eisenia fetida*~~ | ~~ADMP~~ | ~~Mixed into substrate<br>56 d, chronic<br>5 % peat content~~ | ~~**NOEC ≥ 100 mg/kg dw**~~ | ~~Renewal dossier [b)]<br>Jeyalakshmi, 2010, DuPont 30060~~ |
| ~~*Folsomia candida*~~ | ~~nicosulfuron~~ | ~~Mixed into substrate<br>28 d, chronic<br>5 % peat content~~ | ~~**NOEC = 556 mg a.s./kg dw**~~ | ~~Renewal dossier [b)]<br>Höhn, 2010, DuPont-29701~~ |
| ~~*Folsomia candida*~~ | ~~ASDM~~ | ~~Mixed into substrate<br>28 d, chronic<br>10 % peat content~~ | ~~**NOEC ≥ 100 mg/kg dw**~~ | ~~Renewal dossier [b)]<br>Hughes, 2005, DuPont-14468~~ |
| ~~*Folsomia candida*~~ | ~~AUSN~~ | ~~Mixed into substrate<br>28 d, chronic<br>10 % peat content~~ | ~~**NOEC ≥ 100 mg/kg dw**~~ | ~~Renewal dossier [b)]<br>Lührs, 2004c, DuPont-15769~~ |
| ~~*Folsomia candida*~~ | ~~HMUD~~ | ~~Mixed into substrate<br>28 d, chronic<br>5 % peat content~~ | ~~**NOEC ≥ 1000 mg/kg dw**~~ | ~~Renewal dossier [b)]<br>Wagenhoff, 2016b, S15-04106~~ |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| ~~*Folsomia candida*~~ | ~~UCSN~~ | ~~Mixed into substrate 28 d, chronic 10 % peat content~~ | ~~**NOEC ≥ 100 mg/kg dw**~~ | ~~Renewal dossier [b] Lührs, 2004d, DuPont-15766~~ |
| ~~*Folsomia candida*~~ | ~~ADMP~~ | ~~Mixed into substrate 28 d, chronic 5 % peat content~~ | ~~**NOEC = 50 mg/kg dw**~~ | ~~Renewal dossier [b] Lührs, 2010, DuPont-30446~~ |
| ~~*Hypoaspis aculeifer*~~ | ~~nicosulfuron~~ | ~~Mixed into substrate 14 d, chronic 5 % peat content~~ | ~~**NOEC = 29.63 mg a.s./kg dw**~~ | ~~Renewal dossier [b] Klug, 2010, DuPont-29700~~ |
| ~~*Hypoaspis aculeifer*~~ | ~~ASDM~~ | ~~Mixed into substrate 14 d, chronic 5 % peat content~~ | ~~**NOEC ≥ 1000 mg/kg dw**~~ | ~~Renewal dossier [b] Wagenhoff, 2016, S15-04104~~ |
| ~~*Hypoaspis aculeifer*~~ | ~~AUSN~~ | ~~Mixed into substrate 14 d, chronic 5 % peat content~~ | ~~**NOEC ≥ 100 mg/kg dw**~~ | ~~Renewal dossier [b] Lührs, 2014a, DuPont-39330~~ |
| ~~*Hypoaspis aculeifer*~~ | ~~HMUD~~ | ~~Mixed into substrate 14 d, chronic 5 % peat content~~ | ~~**NOEC ≥ 1000 mg/kg dw**~~ | ~~Renewal dossier [b] Wagenhoff, 2016, S15-04104~~ |
| ~~*Hypoaspis aculeifer*~~ | ~~UCSN~~ | ~~Mixed into substrate 14 d, chronic 5 % peat content~~ | ~~**NOEC ≥ 100 mg/kg dw**~~ | ~~Renewal dossier [b] Lührs, 2014b, DuPont-30054~~ |
| ~~*Hypoaspis aculeifer*~~ | ~~ADMP~~ | ~~Mixed into substrate 14 d, chronic 5 % peat content~~ | ~~**NOEC ≥ 100 mg/kg dw**~~ | ~~Renewal dossier [b] Lührs, 2010, DuPont-30054~~ |
| **SAE053H/01** | | | | |
| *Eisenia fetida* | SAE053H/01 | Mixed into substrate 56 d, chronic 10 % peat content | **NOEC ≥ 100 mg product/kg dw NOEC ≥ 8.33 mg mesotrione/kg dw and ≥ 3.13 mg nicosulfuron/kg dw [c]** | Wagenhoff, 2016a, S16-01484 |
| *Folsomia candida* | SAE053H/01 | Mixed into substrate 28 d, chronic 5 % peat content | **NOEC = 125 mg product/kg dw NOEC = 10.41 mg mesotrione/kg dw and 3.91 mg nicosulfuron/kg dw [c]** | Häuser, 2016, S16-01485 |
| *Hypoaspis aculeifer* | SAE053H/01 | Mixed into substrate 14 d, chronic 5 % peat content | **NOEC = 80.0 mg product/kg dw NOEC = 6.66 mg mesotrione/kg dw and 2.50 mg nicosulfuron/kg dw [c]** | Wagenhoff, 2016b, S16-01486 |
| **Field studies** | | | | |
| None. | | | | |
| **Litter bag test** | | | | |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| None. | | | | |

* Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002.
[a)] EFSA Journal 2016; 14(3):4419
[b)] ~~Supplementary dossier for the approval renewal of nicosulfuron (N2 document; 2016)~~ EFSA Scientific Report (2007) 120, 1-91.
[c)] Based on analysed contents of active substances of 8.33% (w/w) for mesotrione and 3.13% (w/w) for nicosulfuron.
Endpoints in **bold** were used for the risk assessment.

#### 9.8.1.1 Justification for new endpoints

Risk assessments are provided based on overall worst-case toxicity endpoints of mesotrione and nicosulfuron and product endpoints for the actual formulation SAE053H/01 for available data on *Eisenia fetida*, *Folsomia candida* and *Hypoaspis aculeifer*. In accordance with the EU review of mesotrione and ~~the supplementary dossier for the approval renewal of~~ nicosulfuron ~~(N2 document; 2016)~~, available data on degradation products of mesotrione and nicosulfuron were used for the risk assessment.
Since for earthworms, *Folsomia* and *Hypoaspis*, chronic data on the active substance mesotrione is not available from the EU review, the corresponding product data was used by re-calculating the endpoint to mesotrione equivalents. The same approach was used for nicosulfuron since no chronic studies on either earthworms, *Folsomia* or *Hypoaspis* are available from the EU review.
These re-calculated endpoints were also used to assess the chronic risk from soil metabolites in those cases, where no chronic studies were available from the EU reviews. For this purpose the toxicity of the metabolites was assumed to be ten times higher compared to the corresponding parent substance.

### 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).

The risk assessment is shown for the ~~risk envelope of 1.5 L product/ha, covering the~~ actual application rate of 1.2 L product/ha.

#### 9.8.2.1 First-tier risk assessment

The relevant $PEC_{soil}$ for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate). According to the assessment of environmental-fate data, multi-annual accumulation in soil does not need to be considered for the active substances mesotrione and nicosulfuron as well as for the degradation products MNBA, AMBA, HMUD and UCSN. Multi-annual accumulation was considered for the degradation products ADMP, ASDM and AUSN.

**Table 9.8-2: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of SAE053H/01 in maize (~~1.5~~ 1.2 L product/ha)**

| **Intended use** | **Maize** | | |
|---|---|---|---|
| **Chronic effects on earthworms** | | | |
| **Product/ active substance/ degradation product** | **NOEC (mg/kg dw)** | **$PEC_{soil}$ (mg/kg dw)** | **$TER_{lt}$ (criterion TER ≥ 5)** |
| SAE053H/01 | ≥ 100 | ~~1.47~~ 1.18 | ≥ ~~68.03~~ 84.8 |
| Mesotrione | ≥ 8.33 [a)] | 0.096 | ≥ 86.8 |
| MNBA (d.p. of mesotrione) | ≥ 1050 | ~~0.050~~ 0.040 | ~~≥ 21'000~~ 26'250 |
| AMBA (d.p. of mesotrione) | ≥ 1050 | ~~0.007~~ 0.006 | ~~≥ 150'000~~ 175'000 |
| Nicosulfuron | ~~≥ 100 ≥~~ 3.13 [b)] | ~~0.045~~ 0.040 | ≥ ~~2222~~ 78.3 |
| ASDM (d.p. of nicosulfuron) | ~~≥ 1000~~ ≥ 0.350 | ~~0.0259~~ 0.014 | ≥ ~~38'610~~ 25.0 |
| AUSN (d.p. of nicosulfuron) | ~~≥ 1000~~ ≥ 0.100 | ~~0.0226~~ 0.008 | ≥ ~~44'248~~ 12.5 |
| HMUD (d.p. of nicosulfuron) | ~~≥ 1000~~ ≥ 0.313 [c)] | ~~0.0156~~ 0.006 | ≥ ~~64'103~~ 52.3 |
| UCSN (d.p. of nicosulfuron) | ~~≥ 1000~~ ≥ 0.050 | ~~0.0054~~ 0.003 | ≥ ~~185'185~~ 16.7 |
| ADMP (d.p. of nicosulfuron) | ~~≥ 100~~ ≥ 0.313 [c)] | ~~0.0126~~ 0.001 | ≥ ~~7937~~ 313 |
| **Chronic effects on other soil macro- and mesofauna** | | | |
| **Product/ active substance/ degradation product** | **NOEC (mg/kg dw)** | **$PEC_{soil}$ (mg/kg dw)** | **$TER_{lt}$ (criterion TER ≥ 5)** |
| ***Folsomia candida*** | | | |
| SAE053H/01 | 125 | ~~1.47~~ 1.18 | ~~85.03~~ 105.9 |
| Mesotrione | 10.41 [a)] | 0.096 | 108.4 |
| MNBA (d.p. of mesotrione) | 1.041 [d)] | 0.040 | 26.0 |
| AMBA (d.p. of mesotrione) | 1.041 [d)] | 0.006 | 173.5 |
| Nicosulfuron | ~~556~~ 3.91 [b)] | ~~0.045~~ 0.040 | ~~12'356~~ 97.8 |
| ASDM (d.p. of nicosulfuron) | ~~≥ 100~~ 0.350 | ~~0.0259~~ 0.014 | ~~≥ 3861~~ 25 |
| AUSN (d.p. of nicosulfuron) | ~~≥ 100~~ ≥ 0.100 | ~~0.0226~~ 0.008 | ~~≥ 4425~~ 12.5 |
| HMUD (d.p. of nicosulfuron) | ~~≥ 1000~~ 0.391 | ~~0.0156~~ 0.006 | ~~≥ 64'103~~ 65.2 |
| UCSN (d.p. of nicosulfuron) | ~~≥ 100~~ ≥ 0.050 | ~~0.0054~~ 0.003 | ~~≥ 18'519~~ 16.7 |
| ADMP (d.p. of nicosulfuron) | ~~50~~ 0.391 | ~~0.0126~~ 0.001 | ~~3968~~ 391 |
| ***Hypoaspis aculeifer*** | | | |
| SAE053H/01 | 80.0 | ~~1.47~~ 1.18 | ~~54.42~~ 67.8 |
| Mesotrione | 6.66 [a)] | 0.096 | 69.4 |
| MNBA (d.p. of mesotrione) | 0.666 [d)] | 0.040 | 16.7 |
| AMBA (d.p. of mesotrione) | 0.666 [d)] | 0.006 | 111 |
| Nicosulfuron | ~~29.63~~ 2.50 [b)] | ~~0.045~~ 0.040 | ~~658~~ 62.5 |
| ASDM (d.p. of nicosulfuron) | ~~≥ 1000~~ 0.250 [c)] | ~~0.0259~~ 0.014 | ~~≥ 38'610~~ 17.9 |

| **Intended use** | **Maize** | | |
|---|---|---|---|
| **Chronic effects on earthworms** | | | |
| **Product/ active substance/ degradation product** | **NOEC (mg/kg dw)** | **$PEC_{soil}$ (mg/kg dw)** | **$TER_{lt}$ (criterion TER ≥ 5)** |
| AUSN (d.p. of nicosulfuron) | ~~≥ 100~~ 0.250 [c)] | ~~0.0226~~ 0.008 | ~~≥ 4425~~ 31.3 |
| HMUD (d.p. of nicosulfuron) | ~~≥ 1000~~ 0.250 [c)] | ~~0.0156~~ 0.006 | ~~≥ 64'103~~ 41.7 |
| UCSN (d.p. of nicosulfuron) | ~~≥ 100~~ 0.250 [c)] | ~~0.0054~~ 0.003 | ~~≥ 18'519~~ 83.3 |
| ADMP (d.p. of nicosulfuron) | ~~≥ 100~~ 0.250 [c)] | ~~0.0126~~ 0.001 | ~~≥ 7937~~ 250 |

d.p. = degradation product
TER values shown in bold fall below the relevant trigger.
[a)] Based on product endpoint and the analysed active substance content of 8.33% (w/w) mesotrione.
[b)] Based on product endpoint and the analysed active substance content of 3.13% (w/w) nicosulfuron.
[c)] Based on product endpoint, analysed nicosulfuron content of 3.13% (w/w) and ten times higher toxicity compared to parent.
[d)] Based on product endpoint, analysed mesotrione content of 8.33% (w/w) and ten times higher toxicity compared to parent.

An acceptable risk for the product, the two active substances and all relevant degradation products is indicated even when assuming conservative assumptions of ten times higher toxicity for degradation products.

#### 9.8.2.2 Higher-tier risk assessment

Not required.

### 9.8.3 Overall conclusions

The risk from exposure to mesotrione and nicosulfuron applied as SAE053H/01 in maize (~~risk envelope: 1 x 1.5 L product/ha, i.e. 120 g a.s./ha mesotrione and 45 g a.s./ha nicosulfuron~~ 1 x 1.2 L product/ha, i.e. 96 g mesotrione/ha and 36 g nicosulfuron/ha) is indicated to be acceptable for earthworms and the soil macro- and mesofauna. The risk from the product itself and from relevant soil degradation products is indicated to be acceptable as well.

**Review Comments:**

All TER values for SAE053H/01, the active substances and relevant metabolites for chronic exposure of earthworms and other non-target soil organisms (meso- and macrofauna) are considerably higher than the Commission Regulation (EU) 546/2011 trigger value of 5. This indicates that SAE053H/01 poses no unacceptable risk to earthworms and other non-target soil organisms (meso- and macrofauna) when applied according to the proposed use pattern.

## 9.9 Effects on soil microbial activity (KCP 10.5)

### 9.9.1 Toxicity data

Studies on effects on soil microorganisms have been carried out with nicosulfuron and relevant soil degradation products. Full details of these studies are provided in the respective EU DARs and related documents.

Effects on soil microorganisms of SAE053H/01 were not evaluated as part of the EU assessment of mesotrione or nicosulfuron. 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 deviates from the results of the EU review process of mesotrione and ~~the supplementary dossier for the approval renewal of~~ nicosulfuron ~~(N2 document; 2016)~~. Justifications are provided below.

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

| Endpoint | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| **Mesotrione** | | | | |
| N-mineralisation | MNBA | 28 d, aerobic soil type | **NOAEC ≥ 1.13 mg/kg soil dw**; (Inhibition: -4.8%) | EFSA conclusion [b] Schulz, 2013b, 12 10 48 045 C/N |
| | AMBA | 28 d, aerobic soil type | **NOAEC ≥ 1.13 mg/kg soil dw**; (Inhibition: -7.6%) | |
| **Nicosulfuron** | | | | |
| ~~N-mineralisation~~ | ~~nicosulfuron~~ | ~~28 d, aerobic soil type~~ | ~~NOAEC ≥ 4.88 mg a.s./kg soil dw; (Inhibition: 24.22%)~~ | ~~Renewal dossier [c] Hammesfahr, 2014, Du-Pont 39331~~ |
| N-mineralisation | nicosulfuron | 28 d, aerobic soil type | **NOAEC ≥ 0.8 mg a.s./kg soil dw;** (Inhibition: -11.0% in sand, -2.5 in sandy silt loam) | EFSA conclusion [b] Müller-Kallert, 1992, 301195 |
| N-mineralisation | ASDM | 28 d, aerobic soil type | **NOAEC ≥ 0.191 mg/kg soil dw** | EFSA conclusion [b] Völkel, 2003, 848319 |
| N-mineralisation | UCSN | 28 d, aerobic soil type | **NOAEC ≥ 0.034 mg/kg soil dw** | EFSA conclusion [b] Völkel, 2003, 848319 |
| N-mineralisation | AUSN | 28 d, aerobic soil type | **NOAEC ≥ 0.082 mg/kg soil dw** | EFSA conclusion [b] Völkel, 2003, 848319 |
| ~~N-mineralisation~~ | ~~ASDM~~ | ~~28 d, aerobic soil type~~ | ~~**NOAEC ≥ 0.448 mg/kg soil dw;** (Inhibition: -10.0%)~~ | ~~Renewal dossier [c] Kölzer, 2003, DuPont-12101~~ |
| ~~N-mineralisation~~ | ~~ADMP~~ | ~~28 d, aerobic soil type~~ | ~~**NOAEC ≥ 0.151 mg/kg soil dw;** (Inhibition: -8.51%)~~ | ~~Renewal dossier [c] Kölzer, 2002, DuPont-9199~~ |
| ~~N-mineralisation~~ | ~~AUSN~~ | ~~28 d, aerobic soil type~~ | ~~**NOAEC ≥ 0.44 mg/kg soil dw**~~ | ~~Renewal dossier [c] Reis, 2004b, DuPont-~~ |

| Endpoint | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| | | | | ~~12755~~ |
| ~~N-mineralisation~~ | ~~UCSN~~ | ~~28 d, aerobic soil type~~ | ~~**NOAEC ≥ 0.41 mg/kg soil dw**; (Inhibition: 1.8%)~~ | ~~Renewal dossier [c] Reis, 2004c, DuPont-14025~~ |
| ~~N-mineralisation~~ | ~~HMUD~~ | ~~28 d, aerobic soil type~~ | ~~**NOAEC ≥ 1.0 mg/kg soil dw**; (Inhibition: 1.52%)~~ | ~~Renewal dossier [c] Wagenhoff, 2016, S15-04102~~ |
| SAE053H/01 | | | | |
| N-mineralisation | SAE053H/01 | 42 d, aerobic soil type | **NOAEC ≥ 9.80 mg product/kg soil dw** (Inhibition: 5.61%) **NOAEC ≥ 0.816 mg mesotrione/kg soil dw** and ≥ 0.307 mg nicosulfuron/kg soil dw [d] | Duffner, 2016, S16-01487 |

NOAEC/R: No Observed Adverse Effect Concentration/Rate; i.e. ≤ 25% effect at ≤ 100 days as compared to untreated controls
Endpoints in **bold** were used for the risk assessment.
[a] EFSA Journal 2016; 14(3):4419
[b] EFSA Scientific Report 2007, 120, 1 – 91
~~[c] Supplementary dossier for the approval renewal of nicosulfuron (N2 document; 2016)~~
[d] Based on analysed contents of active substances of 8.33% (w/w) for mesotrione and 3.13% (w/w) for nicosulfuron.

#### 9.9.1.1 Justification for new endpoints

Risk assessments are provided based on overall worst-case toxicity endpoints of mesotrione and nicosulfuron and product endpoints for the actual formulation SAE053H/01 for available data on nitrogen transformation. In accordance with the EU review of mesotrione and ~~the supplementary dossier for the approval renewal of~~ nicosulfuron ~~(N2 document; 2016)~~, available data on degradation products of mesotrione and nicosulfuron were used for the risk assessment.
Since for mesotrione, data on N-mineralisation is not available from the EU review, the corresponding product data was used by re-calculating the endpoint to mesotrione equivalents.
In case data for soil metabolite on N-mineralisation was lacking from the EU review, a ten times higher toxicity compared to the corresponding parent substance was assumed in a worst-case approach.

### 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 risk assessment is shown for the ~~risk envelope of 1.5 L product/ha, covering the~~ actual application rate of 1.2 L product/ha.

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).

**Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of SAE053H/01 in maize (~~1.5~~ 1.2 L product/ha)**

| **Intended use** | **Maize** | | |
|---|---|---|---|
| **N-mineralisation** | | | |
| **Product/ active substance/ degradation product** | **Max. conc. with effects ≤ 25 % (mg/kg dw)** | **$PEC_{soil}$ [a] (mg/kg dw)** | **Risk acceptable?** |
| SAE053H/01 | ≥ 9.80 (at 42 d) | ~~1.47~~ 1.18 | yes (margin of safety ≥ ~~6.7~~ 8.3) |
| Mesotrione | ≥ 0.816 (at 42 d) [b] | 0.096 | yes (margin of safety ≥ 8.5) |
| MNBA (d.p. of mesotrione) | ≥ 1.13 (at 28 d) | ~~0.050~~ 0.040 | yes (margin of safety ≥ ~~23~~ 28) |
| AMBA (d.p. of mesotrione) | ≥ 1.13 (at 28 d) | ~~0.007~~ 0.006 | yes (margin of safety ≥ ~~161~~ 188) |
| Nicosulfuron | ~~≥ 4.88 (at 28 d)~~ ≥ 0.8 (at 28 d) | ~~0.045~~ 0.040 | yes (margin of safety ≥ ~~108~~ 20) |
| ASDM (d.p. of nicosulfuron) | ~~≥ 0.488 (at 28 d)~~ ≥ 0.191 (at 28 d) | ~~0.0259~~ 0.014 | yes (margin of safety ≥ ~~19~~ 14) |
| AUSN (d.p. of nicosulfuron) | ~~≥ 0.44 (at 28 d)~~ ≥ 0.082 (at 28 d) | ~~0.0226~~ 0.008 | yes (margin of safety ≥ ~~19~~ 10) |
| HMUD (d.p. of nicosulfuron) | ~~≥ 1.0 (at 28 d)~~ ≥ 0.08 (at 28 d) [c] | ~~0.0156~~ 0.006 | yes (margin of safety ≥ ~~64~~ 13) |
| UCSN (d.p. of nicosulfuron) | ~~≥ 0.41 (at 28 d)~~ ≥ 0.034 (at 28 d) | ~~0.0054~~ 0.003 | yes (margin of safety ≥ ~~76~~ 11) |
| ADMP (d.p. of nicosulfuron) | ~~≥ 0.151 (at 28 d)~~ ≥ 0.08 (at 28 d) [c] | ~~0.0126~~ 0.001 | yes (margin of safety ≥ ~~12~~ 80) |

d.p. = degradation product
[a] Highest predicted concentration for the worst-case use
[b] Based on product endpoint and the analysed active substance content of 8.33% (w/w) mesotrione.
[c] Based on nicosulfuron endpoint and ten times higher toxicity compared to parent.

An acceptable risk for the product, the two active substances and all relevant degradation products is indicated even when assuming conservative assumptions of ten times higher toxicity for degradation products.

## 9.9.3 Overall conclusions

The risk from exposure to mesotrione and nicosulfuron applied as SAE053H/01 in maize (~~risk envelope: 1 x 1.5 L product/ha, i.e. 120 g a.s./ha mesotrione and 45 g a.s./ha nicosulfuron~~ 1 x 1.2 L product/ha, i.e. 96 g mesotrione/ha and 36 g nicosulfuron/ha) is indicated to be acceptable for the soil microflora. The risk from the product itself and from relevant soil degradation products is indicated to be acceptable as well.

**Review Comments:**

For the formulation SAE053H/01, the active substances as well as for the relevant metabolites, the maximum concentration with effects < 25% (SANCO/10329/2002 trigger) are all above the maximum $PEC_{soil}$ values. Therefore, it is concluded that the use of SAE053H/01 will not pose an unacceptable risk to non-target soil micro-organisms, if applied according to good agricultural practice.

## 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 formulations containing mesotrione or nicosulfuron during the first inclusion of the active substances. Full details of these studies are provided in the respective EU DAR and related documents but are not considered relevant for the actual product SAE053H/01.

Effects on non-target terrestrial plants of SAE053H/01 were not evaluated as part of the EU assessment of mesotrione or nicosulfuron. New data submitted with this application are listed in Appendix 1 summarised in Appendix 2.

~~The selection of studies and endpoints for the risk assessment deviates from the results of the EU review process of mesotrione and the supplementary dossier for the approval renewal of nicosulfuron (N2 document, 2015). Justifications are provided below.~~

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

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Lactuca sativa* d<br>*Brassica oleracea* d [2)]<br>*Glycine max* d<br>*Beta vulgaris* d<br>*Cucumis sativus* d<br>*Brassica rapa* d<br>*Avena sativa* m<br>*Lolium perenne* m<br>*Zea mays* m<br>*Allium cepa* m | SAE053H/01 | 21 d Seedling emergence | $ER_{50}$ emergence > 93.75 mL product/ha<br>[2)] **$ER_{50}$ plant dry weight = 57.7 mL product/ha** | Gröning, 2017a, S16-02421 |
| *Lactuca sativa* d [1)]<br>*Brassica oleracea* d<br>*Glycine max* d<br>*Beta vulgaris* d<br>*Cucumis sativus* d<br>*Brassica rapa* d<br>*Avena sativa* m<br>*Lolium perenne* m<br>*Zea mays* m<br>*Allium cepa* m | SAE053H/01 | 21 d Vegetative vigour | [1)] **$ER_{50}$ plant dry weight = 8.47 mL product/ha** | Gröning, 2017b, S16-02422 |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Lactuca sativa* [1] $_{d}$<br>*Brassica oleracea* [2] $_{d}$<br>*Glycine max* [3] $_{d}$<br>*Cucumis sativus* [4] $_{d}$<br>*Brassica rapa* [5] $_{d}$<br>*Avena sativa* [6] $_{m}$<br>*Lolium perenne* [7] $_{m}$<br>*Allium cepa* [8] $_{m}$ | SAE053H/01 | 21 d Vegetative vigour | **$HC_5$ = 0.00589 L product/ha** | - |

m: monocotyledonous; d: dicotyledonous
Endpoints in **bold** were used for the risk assessment.

#### 9.10.1.1 Justification for new endpoints

Studies on the vegetative vigour and seedling emergence and growth of non-target plants were conducted with the actual formulated product SAE053H/01. Therefore, risk assessments are based on these data as they are the most relevant.

For vegetative vigour data, a Species Sensitivity Distribution (SSD) assessment based on the available $ER_{50}$ estimates was performed by calculating normal distribution of the data sets and plotting 'Fraction affected' against 'log10 Toxicity data' using ETX 2.0 software.

It is considered justified to use mono- and dicotyledonous data as an overlap of sensitivity was observed and furthermore the intended use of SAE053H/01 targets both, dicotyledonous broadleaved weeds and monocotyledonous grasses.

The following table presents the individual median effect rates ($ER_{50}$) for the sensitive parameter shoot dry weight for the product SAE053H/01 for the more critical vegetative vigour endpoint.

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

| Plant species | Plant group | $ER_{50}$ [L product/ha] |
|---|---|---|
| Onion (*Allium cepa*) | Monocotyledons | 0.8376 |
| Oat (*Avena sativa*) | | 0.6467 |
| Ryegrass (*Lolium perenne*) | | 0.2101 |
| Maize (*Zea mays*) | | > 1.500 [a)] |
| White cabbage (*Brassica oleracea*) | Dicotyledons | 0.0322 |
| Soybean (*Glycine max*) | | 0.2272 |
| Sugar beet (*Beta vulgaris*) | | > 0.00938 [a)] |
| Lettuce (*Lactuca sativa*) | | **0.00847** |
| Turnip (*Brassica rapa*) | | 0.02913 |
| Cucumber (*Cucumis sativus*) | | 0.04374 |

[a)] Limit rate endpoint excluded from the SSD
Endpoint in bold represents the worst-case endpoint.

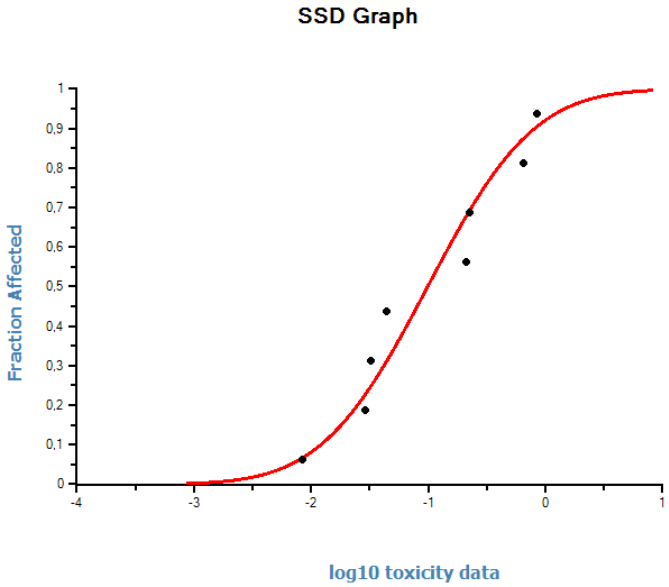
The result of the SSD is presented in the following table and graph.

**Table 9.10-3: SSD over $ER_{50}$ from the relevant vegetative vigour data for SAE053H/01**

| Parameter | $ER_{50}$ shoot dry weight (n = 8) [L product/ha] |
|---|---|
| Goodness of fit of toxicity data (normal distribution) | |
| Anderson-Darling test for normality | Accepted [a)] |
| Kolmogorov-Smirnov test for normality | Accepted [a)] |
| Cramer von Mises test for normality | Accepted [a)] |
| Median $HC_5$ | **0.00589** |
| 95% confidence limits | 0.00053 – 0.0205 |

[a)] Acceptable normal distribution at 1%/lowest significance level

The data fulfills the criterion for normal distribution even at the lowest significance level and in accordance with all tests for normality.




**Graph 9.10-1: SSD over $ER_{50}$ from the relevant vegetative vigour data for SAE053H/01**

It is noted that the median $HC_5$ estimate of 0.00589 L product/ha is below the endpoint for the most sensitive species of the tested plants (i.e. lettuce with an $ER_{50}$ of 0.00847 L product/ha) supporting the conclusion that the $HC_5$ is sufficiently protective for the community of terrestrial non-target plants.

Accordingly, the exposure estimates are directly compared to the endpoint from the SSD (i.e. assuming an assessment factor of 1).

## 9.10.2 Risk assessment

### 9.10.2.1 Tier-1 risk assessment (based on 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.

The risk assessment is shown for the risk envelope of 1.5 L product/ha, covering the actual application rate of 1.2 L product/ha.

**Table 9.10-4: Assessment of the risk for non-target plants due to the use of SAE053H/01 in maize (1.5 L product/ha)**

| | |
|---|---|
| **Intended use** | **Maize** |
| **Active substance/product** | SAE053H/01 |
| **Application rate (g/ha)** | 1 x 1.5 L product/ha, i.e. 120 g a.s./ha mesotrione and 45 g a.s./ha nicosulfuron |
| **MAF** | 1.00 |

| **Test species** | **Test type** | **$ER_{50}$ (L product/ha)** | **Drift percentile (%)** | **$PER_{off\text{-}field}$ (L product/ha)** | **TER criterion: TER ≥ 5** |
|---|---|---|---|---|---|
| *Brassica oleracea* | Seedling emergence | 0.0577 | 2.77 | 0.04155 | **1.389** |
| **Test species** | **Test type** | **$ER_{50}$ (L product/ha)** | **Drift percentile (%)** | **$PER_{off\text{-}field}$ (L product/ha)** | **TER criterion: TER ≥ 5 or ≥ 1** |
| *Lactuca sativa* | Vegetative vigour | 0.00847 | 2.77 | 0.04155 | **0.204** |
| $HC_5$ (n = 8) | Vegetative vigour | 0.00589 | 2.77 | 0.04155 | **0.142** |

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

Accordingly, a potential risk is indicated for the use of SAE053H/01 in maize at 1.5 L product/ha based on the deterministic and probabilistic. The use of risk mitigation measures is required (see below).

### 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 SAE053H/01 in maize (1.5 L product/ha) considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)**

| | | | | | |
|---|---|---|---|---|---|
| **Intended use**<br>**Product**<br>**Application rate (g/ha)**<br>**MAF** | | **Maize**<br>SAE053H/01<br>1 x 1.5 L product/ha, i.e. 1470 g product/ha<br>1.00 | | | |
| **Buffer strip (m)** | **Drift rate (%)** | **PER$_{off-field}$ (L/ha)** | **PER$_{off-field}$ 50 % drift red. (L/ha)** | **PER$_{off-field}$ 75 % drift red. (L/ha)** | **PER$_{off-field}$ 90 % drift red. (L/ha)** |
| 1 | 2.77 | 0.04155 | 0.02078 | 0.01039 | 0.004155 |
| 5 | 0.57 | 0.00855 | 0.00428 | 0.00214 | 0.000855 |
| 10 | 0.29 | 0.00435 | 0.00218 | 0.00109 | 0.000435 |
| **Seedling emergence and growth** | | | | | |
| **Toxicity value**<br>$ER_{50}$ = 0.0577 L/ha | | **TER**<br>**criterion: TER ≥ 5** | | | |
| 1 | | **1.389** | **2.777** | 5.553 | 13.89 |
| 5 | | 6.749 | 13.48 | 26.96 | 67.49 |
| 10 | | 13.26 | 26.47 | 52.94 | 132.6 |
| **Vegetative vigour – worst-case toxicity estimate (deterministic approach)** | | | | | |
| **Toxicity value**<br>$ER_{50}$ = 0.00847 L/ha | | **TER**<br>**criterion: TER ≥ 5** | | | |
| 1 | | **0.204** | **0.408** | **0.815** | **2.038** |
| 5 | | **0.991** | **1.981** | **3.963** | 9.906 |
| 10 | | **1.947** | **3.894** | 7.789 | 19.47 |
| **Vegetative vigour – $HC_5$ (probabilistic approach)** | | | | | |
| **Toxicity value**<br>$HC_5$ = 0.00589 L/ha | | **TER**<br>**criterion: TER ≥ 1** | | | |
| 1 | | **0.142** | **0.284** | **0.567** | 1.418 |
| 5 | | **0.689** | 1.378 | 2.756 | 6.889 |
| 10 | | 1.354 | 2.708 | 5.416 | 13.54 |

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

Accordingly, the risk to non-target terrestrial plants from the use of SAE053H/01 in maize is indicated to be acceptable if risk mitigation measures are accounted for. Based on the probabilistic approach, minimum risk mitigation measures are

- 10 m drift buffer OR
- 5 m drift buffer plus 50% drift-reducing nozzles OR
- 90% drift-reducing nozzles.

The same risk mitigation measures would be required for the actual application rate of 1.2 L product/ha.

### 9.10.3 Overall conclusions

The risk from exposure to mesotrione and nicosulfuron applied as SAE053H/01 in maize (risk envelope: 1 x 1.5 L product/ha; i.e. 120 g a.s./ha mesotrione and 45 g a.s./ha nicosulfuron) is indicated to be acceptable for non-target plants based on Tier 2 data using the probabilistic approach with either a drift buffer zone of 10 m or a combination of 5 m drift buffer and 50% drift-reducing nozzles, or 90% drift-reducing nozzles.

**Review Comments:**

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002).

Based on the probabilistic risk assessment it can be concluded that the proposed use of SAE053H/01 poses acceptable risk to non-target plants, if applied according to the recommended use pattern. Particular precautions to reduce the environmental concentrations resulting from SAE053H/01 applications are required (10 m buffer zone or 5 m with 50% or 1 m with 90% drift reduction techniques).

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

No other relevant data were identified in the EU review of the active substances mesotrione and nicosulfuron.

## 9.12 Monitoring data (KCP 10.8)

No data.

## 9.13 Classification and Labelling

Classification and labeling of SAE053H/01 is proposed in accordance with Regulation 1272/2008/EC. Based on the lowest acute aquatic endpoint for *Lemna gibba* with $E_rC_{50}$ = 0.058 mg product/L, classification into the category Acute 1 is required. Also chronic classification into Chronic 1 is required, as both active substances, mesotrione and nicosulfuron, are considered to be not readily biodegradable due to lacking data.

| | | |
|---|---|---|
| **Hazard Class-and-Category:** | Aquatic Acute 1 | Acute (short-term) aquatic hazard |
| | Aquatic Chronic 1 | Chronic (long-term) aquatic hazard |

**GHS Pictogram, signal word, hazard statements and precautionary statements under Regulation 1272/2008:**

**GHS Pictograms:**

**Signal Word:** **Warning**

| | | |
|---|---|---|
| **Hazard statements** | H410 | Very toxic to aquatic life with long lasting effects |
| ~~**Precautionary Statement Prevention**~~ | ~~P273~~ | ~~Avoid release to the environment~~ |
| **Precautionary Statement Response** | P391 | Collect spillage |
| **Precautionary Statement Disposal** | P501 | Dispose of contents/ container in accordance with national law. |

# Appendix 1 Lists of data considered in support of the evaluation

**List of data submitted by the applicant and relied on**

| Data point | Author(s) | Year | **Title**<br>**Company Report No.**<br>**Source (where different from company)**<br>**GLP or GEP status**<br>**Published or not** | **Vertebrate study Y/N** | Owner |
|---|---|---|---|---|---|
| KCP 10.1.2.1 = KCP 7.1.1 | xxx | 2016 | Acute oral toxicity study of SAE053H/01 in rats<br>Report No.: 401-1-01-15025<br>xxxx<br>GLP<br>Unpublished<br>- filed in Part B Section 6 - | Y | Sumi Agro Europe Limited |
| KCP 10.1.2.2 = KCP 8.10/01 | Bakker, F. | 2016 | Magnitude of mesotrione residues in maize plants following one application in Southern and Northern Europe in 2016<br>Report No.: JS001LRM<br>Eurofins-MITOX, Amsterdam, The Netherlands<br>GLP<br>Unpublished | N | Sumi Agro Europe & Albaugh Europe Sàrl |
| KCP 10.1.2.2 = KCP 8.10/02 | van de Sandt, H.J. | 2019 | Decline of mesotrione residues in maize plants following one application in The Netherlands – 2017<br>Report No.: S17-05218<br>Eurofins de Bredelaar, Elst, The Netherlands<br>GLP<br>Unpublished | N | Albaugh Europe Sàrl |
| KCP 10.1.2.2/01 | Guckland, A., Wang, M. and Norman, S. | 2019 | Mesotrione: Toxicological endpoint for use in reproduction risk assessment for wild lagomorphs<br>Report No.: 19003-REC<br>WSC Scientific GmbH, Heidelberg, Germany<br>non-GLP<br>Unpublished | N | Sumi Agro Europe & Albaugh Europe Sàrl |
| KCP 10.1.2.2/02 | Cooke, J. | 2019 | Mesotrione: Kinetic assessment of residue decline in maize<br>Report No.: 0400477-KIN1 | N | Albaugh Europe Sàrl |

| Data point | Author(s) | Year | **Title**<br>**Company Report No.**<br>**Source (where different from company)**<br>**GLP or GEP status**<br>**Published or not** | **Vertebrate study Y/N** | **Owner** |
|---|---|---|---|---|---|
| | | | ERM, North Yorkshire, UK<br>non-GLP<br>Unpublished | | |
| KCP 10.2.1/01 | xxx | 2016 | SAE053H/01: Toxicity to the rainbow trout *Oncorhynchus mykiss* under laboratory conditions (acute toxicity test – static)<br>Report No.: S16-03041<br>xxxx<br>GLP<br>Unpublished | Y | Sumi Agro Europe Limited |
| KCP 10.2.1/02 | Zawadsky, C. | 2016 | SAE053H/01: Toxicity to the water flea *Daphnia magna* Straus under laboratory conditions (acute immobilisation test – static)<br>Report No.: S16-03042<br>Eurofins Agroscience Services GmbH, Niefern-Öschelbronn, Germany<br>GLP<br>Unpublished | N | Sumi Agro Europe Limited |
| KCP 10.2.1/03 | Falk, S. | 2016a | SAE053H/01: Toxicity to the single cell green alga *Pseudokirchneriella subcapitata* Hindák under laboratory conditions<br>Report No.: S16-03039<br>Eurofins Agroscience Services GmbH, Niefern-Öschelbronn, Germany<br>GLP<br>Unpublished | N | Sumi Agro Europe Limited |
| KCP 10.2.1/04 | Falk, S. | 2016b | SAE053H/01: Toxicity to the diatom *Navicula pelliculosa* under laboratory conditions<br>Report No.: S16-03040<br>Eurofins Agroscience Services GmbH, Niefern-Öschelbronn, Germany<br>GLP<br>Unpublished | N | Sumi Agro Europe Limited |
| KCP | Lang née Zawadsky, | 2016b | SAE053H/01: Toxicity to the duckweed *Lemna gibba* under laboratory conditions (acute test – semi- | N | Sumi Agro |

| Data point | Author(s) | Year | **Title**<br>**Company Report No.**<br>**Source (where different from company)**<br>**GLP or GEP status**<br>**Published or not** | **Vertebrate study Y/N** | **Owner** |
|---|---|---|---|---|---|
| 10.2.1/05 | C. | | static)<br>Report No.: S16-03044<br>Eurofins Agroscience Services GmbH, Niefern-Öschelbronn, Germany<br>GLP<br>Unpublished | | Europe Limited |
| KCP 10.2.1/06 | Gonsior, G. | 2016 | SAE053H/01: Growth inhibition of *Myriophyllum spicatum* in a water/sediment system<br>Report No.: S16-03045<br>Eurofins Agroscience Services GmbH, Niefern-Öschelbronn, Germany<br>GLP<br>Unpublished | N | Sumi Agro Europe Limited |
| KCP 10.2.1/07 | Bertrand, C. | 2019 | Mesotrione technical: Toxicity to the duckweed *Lemna gibba* under laboratory conditions (acute test – semi-static)<br>Report No.: S19-03470<br>Eurofins Agroscience Services GmbH, Niefern-Öschelbronn, Germany<br>GLP<br>Unpublished | N | Sumi Agro Europe & Albaugh Europe Sàrl |
| KCP 10.2.1/08 | Christmann, R. | 2021a | Mesotrione: Toxicity to the aquatic plant *Spirodela polyrhiza* in a growth inhibition test<br>Report No.: 218-31<br>Institut für Gewässerschutz MESOCOSM GmbH, Homberg, Germany<br>GLP<br>Unpublished | N | Sumi Agro Europe & Albaugh Europe Sàrl |
| KCP 10.2.1/09 | Christmann, R. | 2021b | Mesotrione: Toxicity to the aquatic plant *Wolffia arrhiza* in a growth inhibition test<br>Report No.: 218-32<br>Institut für Gewässerschutz MESOCOSM GmbH, Homberg, Germany<br>GLP<br>Unpublished | N | Sumi Agro Europe & Albaugh Europe Sàrl |
| KCP | Lang née Zawadsky, | 2016a | SAE053H/01: Toxicity to the water flea *Daphnia magna* under laboratory conditions (reproduction test) | N | Sumi Agro |

| Data point | Author(s) | Year | **Title**<br>**Company Report No.**<br>**Source (where different from company)**<br>**GLP or GEP status**<br>**Published or not** | **Vertebrate study Y/N** | **Owner** |
|---|---|---|---|---|---|
| 10.2.2/01 | C. | | Report No.: S16-03043<br>Eurofins Agroscience Services GmbH, Niefern-Öschelbronn, Germany<br>GLP<br>Unpublished | | Europe Limited |
| KCP 10.3.1.1/01 | Molitor, A. M. | 2016a | SAE053H/01 – Acute oral and contact toxicity to the honeybee, *Apis mellifera* L. under laboratory conditions<br>Report No.: S16-02516<br>Eurofins Agroscience Services GmbH, Niefern-Öschelbronn, Germany<br>GLP<br>Unpublished | N | Sumi Agro Europe Limited |
| KCP 10.3.1.2/01 | Molitor, A. M. | 2016b | SAE053H/01 – Assessment of effects on the adult honey bee, *Apis mellifera* L., in a 10 day chronic feeding test under laboratory conditions<br>Report No.: S16-02518<br>Eurofins Agroscience Services GmbH, Niefern-Öschelbronn, Germany<br>GLP<br>Unpublished | N | Sumi Agro Europe Limited |
| KCP 10.3.1.3/01 | Vergé, E. and Wagner, J. | 2016 | SAE053H/01 – Honey bee (*Apis mellifera* L.) larval toxicity test (repeated exposure)<br>Report No.: S16-02503<br>Eurofins Agroscience Services GmbH, Niefern-Öschelbronn, Germany<br>GLP<br>Unpublished | N | Sumi Agro Europe Limited |
| KCP 10.3.2.1/01 | Walter, C. | 2016a | SAE053H/01 – Toxicity to the aphid parasitoid *Aphidius rhopalosiphi* De Stefani Perez (Hymenoptera, Braconidae) under laboratory conditions<br>Report No.: S16-01607<br>Eurofins Agroscience Services GmbH, Niefern-Öschelbronn, Germany<br>GLP<br>Unpublished | N | Sumi Agro Europe Limited |

| Data point | Author(s) | Year | **Title**<br>**Company Report No.**<br>**Source (where different from company)**<br>**GLP or GEP status**<br>**Published or not** | **Vertebrate study Y/N** | **Owner** |
|---|---|---|---|---|---|
| KCP 10.3.2.1/02 | Walter, C. | 2016b | SAE053H/01 – Toxicity to the predatory mite *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) under laboratory conditions<br>Report No.: S16-01608<br>Eurofins Agroscience Services GmbH, Niefern-Öschelbronn, Germany<br>GLP<br>Unpublished | N | Sumi Agro Europe Limited |
| KCP 10.3.2.2/01 | Röhlig, U. | 2017a | Effects of SAE053H/01 on the parasitic wasp *Aphidius rhopalosiphi* DeStefani-Perez in an extended laboratory test (under semi-field conditions ages residues on potted maize plants)<br>Report No.: 17 48 NAR 0001<br>BioChem agrar, Labor für biologische und chemische Analytik GmbH, Gerichshain, Germany<br>GLP<br>Unpublished | N | Sumi Agro Europe Limited |
| KCP 10.3.2.2/02 | Röhlig, U. | 2017b | Effects of SAE053H/01 on the rove beetle *Aleochara bilineata* Gyll. in an extended laboratory test<br>Report No.: 17 48 NKE 0002<br>BioChem agrar, Labor für biologische und chemische Analytik GmbH, Gerichshain, Germany<br>GLP<br>Unpublished | N | Sumi Agro Europe Limited |
| KCP 10.4.1.1/01 | Wagenhoff, E. | 2016a | SAE053H/01: Sublethal toxicity to the earthworm *Eisenia fetida* (Annelida, Lumbricidae) in artificial soil with 10% peat<br>Report No.: S16-01484<br>Eurofins Agroscience Services GmbH, Niefern-Öschelbronn, Germany<br>GLP<br>Unpublished | N | Sumi Agro Europe Limited |
| KCP 10.4.2.1/01 | Häuser, R. | 2016 | SAE053H/01: Effects on the reproductive output of the springtail *Folsomia candida* Willem (Collembola, Isotomidae) in artificial soil<br>Report No.: S16-01485<br>Eurofins Agroscience Services GmbH, Niefern-Öschelbronn, Germany | N | Sumi Agro Europe Limited |

| Data point | Author(s) | Year | **Title**<br>**Company Report No.**<br>**Source (where different from company)**<br>**GLP or GEP status**<br>**Published or not** | **Vertebrate study Y/N** | **Owner** |
|---|---|---|---|---|---|
| | | | GLP<br>Unpublished | | |
| KCP 10.4.2.1/02 | Wagenhoff, E. | 2016b | SAE053H/01: Effects on the reproductive output of the predatory mite *Hypoaspis aculeifer* Canestrini (Acari: Laelapidae) in artificial soil<br>Report No.: S16-01486<br>Eurofins Agroscience Services GmbH, Niefern-Öschelbronn, Germany<br>GLP<br>Unpublished | N | Sumi Agro Europe Limited |
| KCP 10.5/01 | Duffner, A. | 2016 | SAE053H/01: Effects on the activity of soil microflora under laboratory conditions (Nitrogen transformation)<br>Report No.: S16-01487<br>Eurofins Agroscience Services GmbH, Niefern-Öschelbronn, Germany<br>GLP<br>Unpublished | N | Sumi Agro Europe Limited |
| KCP 10.6.2/01 | Gröning, C. | 2017a | SAE053H/01: Effects on the seedling emergence of ten non-target terrestrial plant species under greenhouse conditions<br>Report No.: S16-02421<br>Eurofins Agroscience Services GmbH, Niefern-Öschelbronn, Germany<br>GLP<br>Unpublished | N | Sumi Agro Europe Limited |
| KCP 10.6.2/02 | Gröning, C. | 2017b | SAE053H/01: Effects on the vegetative vigour of ten non-target terrestrial plant species under greenhouse conditions<br>Report No.: S16-02422<br>Eurofins Agroscience Services GmbH, Niefern-Öschelbronn, Germany<br>GLP<br>Unpublished | N | Sumi Agro Europe Limited |

# Appendix 2 Detailed evaluation of the new studies

**Review Comment:**

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

## A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

### A 2.1.1 KCP 10.1.1 Effects on birds

#### A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

No additional data submitted.

#### A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

No additional data submitted.

### 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

Please refer to Part B, Section 6 of this submission.

#### A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

Next to the studies summaries below, residue data (Bakker, 2016 and van de Sandt, 2019) is available which is summarised in Part B, Section 7 of this submission.

##### A 2.1.2.2.1 Study 1: Expert statement on mammalian reproduction endpoint for mesotrione

| | |
|---|---|
| Comments of zRMS: | The applicant's proposal to change the mammalian endpoint was not accepted. This issue was discussed at Pesticides Peer Review experts Meeting 136 in December 2015, where it was decided that the observed effects (e.g., litter size and pup survival) on the F2 generation should not disregard. Therefore the meeting agreed that the NOAEL of 0.3 mg/kg bw/day should be used in the risk assessment.<br><br>In zRMS opinion, the endpoint can be re-evaluated by using the benchmark dose approach. Further details can be found in the EFSA Journal 2017;15(1):4658. |

| | |
|---|---|
| Reference: | KCP 10.1.2.2/01 |
| Report | Mesotrione: Toxicological endpoint for use in reproduction risk assessment for wild lagomorphs, Guckland, A., Wang, M. and Norman, S., 2019, 19003-REC |
| Guideline(s): | not applicable |
| Deviations: | not applicable |
| GLP: | not applicable |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | not applicable |

**Material and Methods**

For the expert statement no laboratory material or methods have been used. Instead the most appropriate ecotoxicological endpoint for the reproduction risk assessment for wild mammals was determined based on public literature and the available toxicological studies from the EU review of mesotrione.

In a first step, the mode and mechanism of action of mesotrione was evaluated and described from the available literature. This includes the tyrosine catabolism and the consequences for the mode of action in mammals and its reversibility.

For the second step, the available toxicological studies, namely the rat multi-generation study and the mouse multi-generation study were evaluated in detail. No multi-generation study was available for lagomorphs to evaluate their sensitivity compared to rats and mice. However, several other toxicological studies were available for all three species, which allowed some comparison particularly about the enzymatic capacity of the three species.

Consequently, in the next step the sensitivity of all three species to nitisinone and mesotrione was compared based on the available data set. Some further comparisons were made for the influence of the sex (male vs. female rats and male vs. female mice). Finally, the overall difference in sensitivity of rats and mice as well as mice and rabbits was determined.

In a last and supporting step, also the gene-expression was studied since a higher gene expression results in a higher enzyme activity. In order to evaluate whether one of the metabolic pathways may be more pronounced in one of the species, the gene expression of TAT (tyrosine aminotransferase) and AAD (amino acid decarboxylase) were studied, which catalyse the first step of the transamination and decarboxylation pathway, respectively. The involved genes were obtained from the KEGG database and the gene expression was retrieved from the Bgee database presented as a rank score. The lower the score is, the more the gene is expressed. Since tyrosine is mainly metabolised in liver and kidney, those two tissues were considered most relevant.

Based on the overall evaluation of the points mentioned above and considering the application scheme of mesotrione as well as the degradation on foliage, a refined endpoint for the use in the reproductive risk assessment for wild mammals is proposed.

**Results and Discussion**

*Mode of action of mesotrione*

Mesotrione is a triketone (similar to nitisinone), which inhibits the enzyme HPPD (hydroxyphenylpyruvate dioxygenase). This enzyme is involved in the catabolism pathway of tyrosine, which catalyses the transformation of HPP (4-hydroxyphenylpyruvate) to homogentisic acid in humans and other mammals such as rats, mice and rabbits. Consequently, the tyrosine catabolism is the most relevant for comparison of those species with regard to sensitivity to mesotrione.

The major metabolic pathway of tyrosine was found to be transamination, with decarboxylation being the alternative pathway as soon as tyrosine levels are increasing (see figure below). It is noted that in the transamination pathway, the first step (transformation of tyrosine to HPP via TAT) is reversible, leading to elevated plasma concentrations of tyrosine after ingestion of mesotrione (tyrosinemia). Despite the alternative pathway, tyrosine levels in the blood plasma will further increase after high mesotrione doses leading to hypertyosinemia and associated symptoms. However, a study in male rats on the reversibility of elevated plasma tyrosine following cessation of 90 days of dietary exposure to mesotrione has shown that tyrosine levels returned to control levels after one week of cessation (earlier post-exposure time-points were not available) at 100 ppm mesotrione (7.5 mg/kg bw/d).

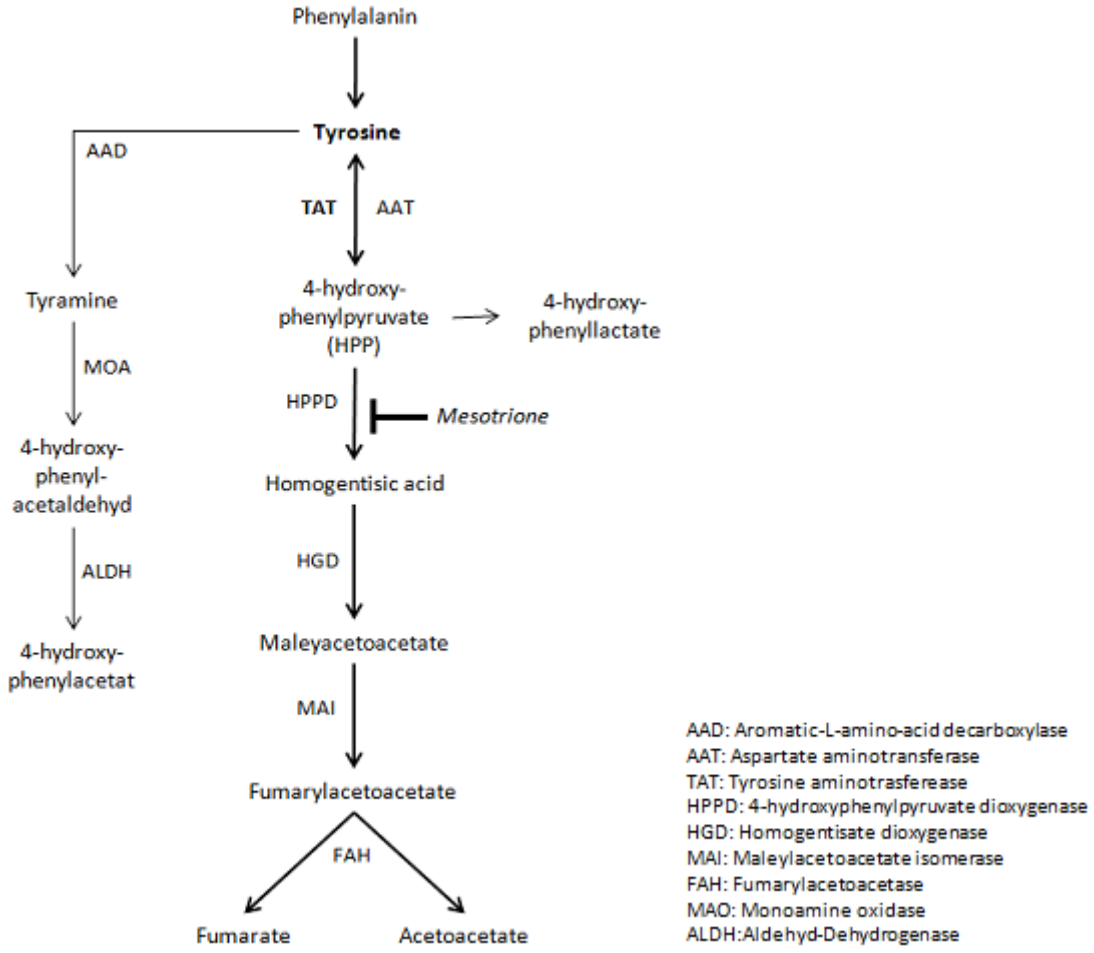



**Figure A 2.1.2.2-1: Tyrosine metabolism via transamination and decarboxylation (summarised from Chakrapani and Holme, 2006; Lock, 2017)**

*Multi-generation studies*

The rat multi-generation study on mesotrione was summarised and the results have been discussed. In contrast to the EU agreed NOEL of 0.3 mg/kg bw/d based on effects in the F2 pups, the outcome was that the NOEL from the study for a refined ecotoxicological risk assessment could be set at 100 ppm (9 mg/kg bw/d) when considering at the one hand the relevant exposure duration, i.e. effects on F2 pups are considered less relevant when considering that mesotrione is degraded quickly in the environment, and on the other hand the relevant parameters for reproductive success (i.e. population level). This value is supported by the value from the study on elevated tyrosine plasma levels mentioned above where reversal after 7 days after cessation was observed after daily intake of 7.5 mg/kg bw/d mesotrione.

The mouse multi-generation study on mesotrione was also summarised and discussed. The outcome, however, was the same as the one from the EU review, i.e. the NOAEL was set at 10 ppm (2 mg/kg bw/d) based on organ weight effects in adults and pups. It was however noted that there were no effects on reproductive performance or fertility in any of the tested doses.

The comparison of the two studies showed large differences in sensitivity between rats and mice, prompting the need to understand where lagomorphs may 'sit' in terms of sensitivity as lagomorphs were the critical focal species in the risk assessment for mesotrione. There was no multi-generation study available on lagomorphs (rabbits), however, other toxicological studies are available for all three species allowing some comparison in particular on their enzymatic capacity.

*Comparison of sensitivity (rat, mouse, lagomorph) for other toxicological studies*

First the sensitivity to nitisinone was compared, as the mode of action is the same as for mesotrione and additionally nitisinone is a much more potent inhibitor of HPPD. Studies conducted with the three species receiving the same dose of 10 mg/kg bw/d of nitisinone showed the highest plasma tyrosine concentrations in rats with up to 2700 nmol/mL. In mice and rabbits the maximum levels were lower (1200 and 1500 nmol/mL, respectively). Control levels were all in similar ranges. At higher doses, the tyrosine concentrations reached a plateau which was reached at lower doses in rats (≥ 0.5 mg/kg bw) compared to mice and rabbits (≥ 10 mg/kg bw). The differences in sensitivity between rats on the one side and mice and rabbits on the other side were also reflected by the measured activity of enzymes involved in the catabolism of tyrosine.

The comparison of sensitivity to mesotrione had a similar outcome. In several studies on rats, mice and rabbits it was found that much higher doses were required for mouse and rabbit to obtain similar blood plasma concentrations of tyrosine compared to rats.

*Further comparisons*

From the comparison between male and female rats it was concluded that male rats were considerably more sensitive compared to female rats based on the fact that the plasma tyrosine levels were much more elevated after a shorter duration of exposure.

For mice, males were only slightly more sensitive than females.

Comparing rats with mice, it was found that at 8.5 – 9.5 mg/kg bw/d intake level both male and female rats had been substantially more sensitive compared to both male and female mice.

Comparing the results for mice and rabbits, it was found that both had a similar sensitivity at 500 – 600 mg/kg bw/day intake level.

*Gene expression*

The results for the rank scores of both genes and both tissues for all three species are given in the table below.

**Table A 2.1.2.2-1: Expression of TAT- and AAD-gene from the Bgee gene expression database**

| Species | Ensembl ID of enzyme | Rank score in liver | Rank score in kidney |
|---|---|---|---|
| **TAT** | | | |
| Rat | ENSRNOG00000016348 | 315 | 16’900 |
| Mouse | ENSMUSG00000001670 | 202 | 21’300 |
| Rabbit | ENSOCUG00000009957 | 340 | 14’200 |
| **AAD** | | | |
| Rat | ENSRNOG00000004327 | 3140 | 3510 |
| Mouse | ENSMUSG00000020182 | 969 | 625 |
| Rabbit | ENSOCUG00000014968 | 2960 | 557 |

The lower the rank score, the more the gene is expressed.

As can be seen from the table, the rank score in kidney for the TAT-gene, being responsible for catalysing the main metabolic pathway of transamination, is very high compared to kidney for all three species, meaning that the gene expression in kidney is low and therefore negligible. When looking more detailed at the rank scores in liver, the rank score and thereby gene expression for rat and rabbit is similar, whereas for mice the rank score is lower, i.e. gene expression is higher. This indicates that for mice this pathway is more effective than for rat and rabbit, which however would not sufficiently explain the differences in maximum tyrosine levels in blood plasma particularly between rat and rabbit.
When, however, looking at the AAD-gene, being responsible for the catalysation of the alternative metabolic pathway of decarboxylation, it can be seen that for rats rank scores are high in liver and kidney, compared to mice (low rank scores in both tissues) and rabbits (low rank score in kidney, high rank score in liver). This means that for mice and rabbits, gene expression is high and medium, respectively, resulting into an effective pathway for these species, whereas for rats gene expression is low and therefore this pathway is not as effective as for the other species. Since mesotrione only inhibits the transamination pathway, mice and rabbits may switch to the alternative metabolism of tyrosine via decarboxylation to a larger extent compared to rats, leaving rats with higher tyrosine levels in the blood plasma.

*Proposal of refined endpoint*

Mesotrione is applied only once per season to maize fields in spring (BBCH 10-29) and has a short $DT_{50}$ on foliage (concluded to be less than one day based on residue decline studies). The lengths of gestation for wood mouse and brown hare were found to be 19-32 days and 37-44 days, respectively. Hence, the potential dietary exposure of a local population feeding on sprayed foliage would be on a single occasion for a short period.

Based on the comparison of multi-generation studies for rats and mice above and the conclusion from the further considerations on species sensitivity above that rats are much more sensitive compared to mice and rabbits, it is stated that for the lagomorph risk assessment the endpoint from the multi-generation study with mice is much more suitable compared to the endpoint from the multi-generation study in rats. Consequently, the proposed NOEL for the lagomorph refined risk assessment is 2 mg/kg bw/d (10 ppm in diet).

**Conclusion**

The expert statement provided a detailed evaluation of the available toxicological studies on mesotrione considering also the mode of action of mesotrione in mammals and the resulting differences in species sensitivity. In conclusion, the most suitable endpoint for the refined risk assessment for lagormorphs was

proposed to be the NOEL = 2 mg/kg bw/d from the multi-generation study in mice.

### A 2.1.2.2.2 Study 2: Kinetic evaluation of residue studies on mesotrione

| | |
|---|---|
| Comments of zRMS: | The two residue decline studies in maize (Bakker, 2016 and van de Sandt, 2019) have been evaluated and accepted in dRR Part B7. Thus, results of the 8 trials were used in the kinetic modelling (SFO, FOMC).<br>Due to expected rapid decline of mesotrione, the samplings were carried out at 1, 4, 8, 12, 24 and 36 hours, and 2, 3 and 4 days after application. The sampling schedule gave 9 data points for each trial, which is sufficient to perform the reliable kinetic analysis.<br>The evaluation was carried out with the tool CAKE v 3.2. With the residue decline data, specific $DT_{50}$ value for mesotrione was calculated according to best practice in environmental modelling. The residue data were analysed using the optimisation of the two parameters M0 (start concentration) and k (rate constant), which is a standard tool used for kinetic evaluation. The acceptability of kinetic fits was judged both visually and according to the $\chi 2$ error and the t-test functions according to FOCUS (2014). It is recommended (but not as an absolute cut-off criterion) that a $\chi 2$ error of less than 15% and a t-test probability of greater than 95% ($p < 0.05$) for estimated degradation rate constants indicate an acceptable fit.<br><br>The kinetic modelling of the laboratory data conducted using the CAKE (version 3.2) software package is accepted. The mesotrione modelling $DT_{50}$ values ranged from 0.13 to 0.92 days. The geometric mean was 0.46 days (zRMS calculations: taking to consideration all data the geomean is 0.45828; using data from central zone the geomean is 0.45887). |

| | |
|---|---|
| Reference: | KCP 10.1.2.2/02 |
| Report | Mesotrione: Kinetic assessment of residue decline in maize, Cooke, J., 2019, 0400477-KIN1 |
| Guideline(s): | not applicable |
| Deviations: | not applicable |
| GLP: | not applicable |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | not applicable |

**Material and Methods**

The residue data of two residue decline studies in maize (Bakker, 2016 and van de Sandt, 2019) have been evaluated in this kinetic assessment in accordance with FOCUS guidance (2014). The modelled $DT_{50}$ values were used to derive a crop dissipation half-life endpoint.

In a first step, the input data were generated in accordance with the recommendations in FOCUS guidance (2014). True replicates were available for each sampling time so all values were used individually in the optimisation. Values between LOD and LOQ were set to the actual measured value. In case this was not reported, 0.5*(LOQ+LOD) was used as value. The first value < LOD just after detectable amounts was set

to 0.5*LOD. All further values < LOD were omitted unless detections > LOQ were made later in the experiment. In that case samples were included up to the first non-detect (< LOD) which is not followed by later positive samples > LOQ.

All trials from both studies were included in the derivation of an overall $DT_{50}$ value, except for trials JS001LRM-04 and -05 where rapid dissipation in the first 6-12 hours after application was observed which may have been caused by rainfall.

The kinetic modelling was conducted using CAKE (version 3.2) software package. Data was evaluated with different models, namely single first order (SFO), first-order multi-compartment (FOMC) and, if necessary, with double first order in parallel (DFOP) and hockey-stick (HS) model.

In the first instance, the data were directly fitted, un-weighted, with the complete usable data set (as detailed above) and unconstrained initial concentration ($M_0$). To give the best chance of finding the global minimum (i.e., the true best-fit values) the model default initial parameters were examined and amended if necessary to provide appropriate starting values, as recommended by FOCUS (2014). The acceptability of kinetic fits was judged both visually (main tool as recommended by the guidance) and according to the $\chi^2$ error (< 15%) and the t-test functions ($p < 0.05$).

The FOCUS (2014) flowchart for calculating modelling endpoints has been followed. Each trial has been considered following the steps in the flowchart and the considerations were discussed in detail.

**Results and Discussion**

All trials (except the two mentioned above) have been evaluated with the kinetic models and the fit of the model was determined. The results are presented in the table below.

**Table A 2.1.2.2-2: Summary of modelling endpoints for mesotrione from residue decline studies in maize**

| Study | Trial | Step in flowchart | Kinetic model | Visual fit | $\chi^2$ error [%] | t-test | $DT_{50, mod}$ [days] |
|---|---|---|---|---|---|---|---|
| Bakker (2016) | JS001LRM-01 (France) | 1 | SFO | good | 13.6 | $p < 0.05$ | 0.69 |
| | JS001LRM-02 (France) | 1 | SFO | intermediate | 6.8 | $p < 0.05$ | 0.42 |
| | JS001LRM-03 (France) | 1 | SFO | excellent | 6.7 | $p < 0.05$ | 0.33 |
| | JS001LRM-06 (The Netherlands) | 1 | SFO | excellent | 4.6 | $p < 0.05$ | 0.45 |
| Van de Sandt (2019) | S17-05218-01 (The Netherlands) | 1 | SFO | good | 12.5 | $p < 0.05$ | 0.92 |
| | S17-05218-02 (The Netherlands) | 1 | SFO | good | 10.3 | $p < 0.05$ | 0.70 |
| | S17-05218-03 (The Netherlands) | 1 | SFO | intermediate | 19.5 | $p < 0.05$ | [a] |
| | | 2 | FOMC | good | 13.7 | n.a. | 0.54 [b] |
| | S17-05218-04 (The Netherlands) | 1 | SFO | poor | 29.0 | $p < 0.05$ | [a] |
| | | 2 | FOMC | excellent | 10.4 | n.a. | 0.13 [b] |

n.a. not applicable

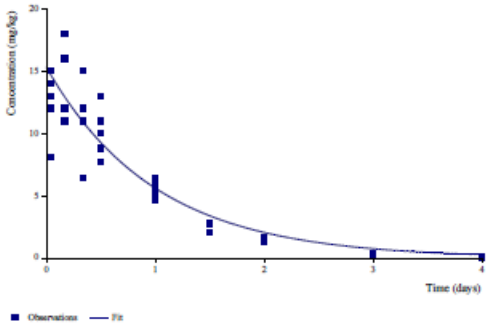
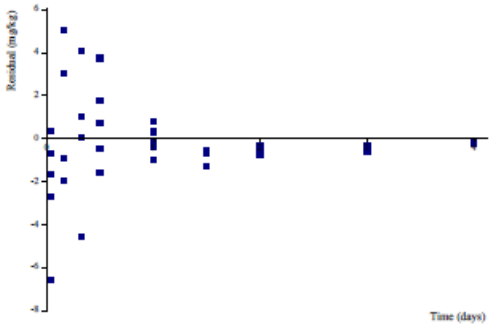
[a] The SFO model is not considered acceptable. Deviation from SFO is not due to outliers or experimental artefacts. Therefore, biphasic models were investigated.

[b] For FOMC modelling the $DT_{50, mod} = DT_{90}/3.32$, i.e. 1.8/3.32 = 0.54 and 0.42/3.32 = 0.13

The geometric mean $DT_{50}$ was calculated to be 0.46 days.

### 4.1 Decline of mesotrione in Trial JS001LRM-01 in France

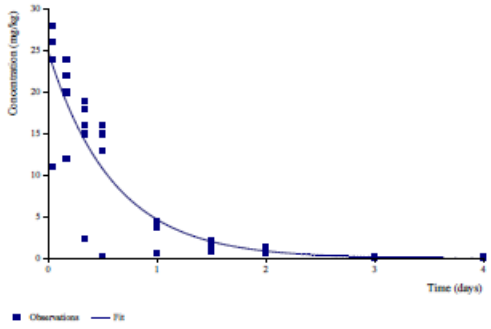
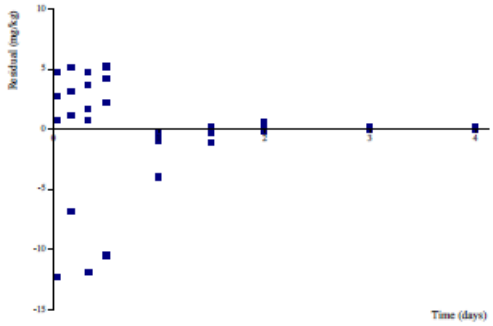
Step 1: Run SFO. SFO statistically and visually acceptable?

| | SFO |
|---|---|
| Plot |   |
| Residuals |   |
| Visual fit | Good |
| $\chi^2$ error (%) | 13.6 |
| t-test | *k*: $p < 0.05$ |
| $DT_{50}$ (days) | 0.69 |
| $DT_{90}$ (days) | 2.3 |
| Modelling $DT_{50}$ (days) | 0.69 |
| Assessment | Visual fit is good, $\chi^2$ error is acceptable and rate parameter differs significantly from zero. |
| Discussion | The SFO model is considered acceptable and should be used for modelling endpoints. |

Summary: Use SFO. $DT_{50\ mod}$ = 0.69 days.

## 4.2 Decline of mesotrione in Trial JS001LRM-02 in France

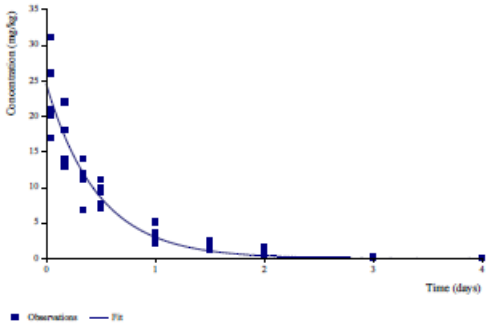
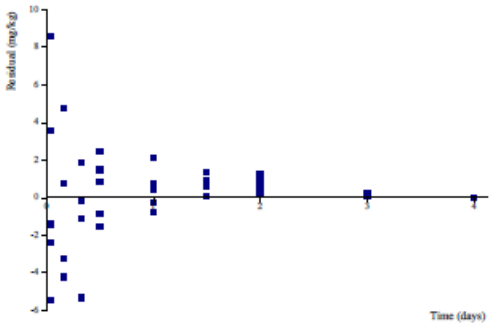
Step 1: Run SFO. SFO statistically and visually acceptable?

| | SFO |
|---|---|
| Plot |  |
| Residuals |  |
| Visual fit | Intermediate |
| $\chi^2$ error (%) | 6.8 |
| t-test | $k$: $p < 0.05$ |
| $DT_{50}$ (days) | 0.42 |
| $DT_{90}$ (days) | 1.4 |
| Modelling $DT_{50}$ (days) | 0.42 |
| Assessment | Visual fit is reasonable, $\chi^2$ error is low and rate parameter differs significantly from zero. |
| Discussion | The SFO model is considered acceptable and should be used for modelling endpoints. |

Summary: Use SFO. $DT_{50\ mod}$ = 0.42 days.

### 4.3 Decline of mesotrione in Trial JS001LRM-03 in France

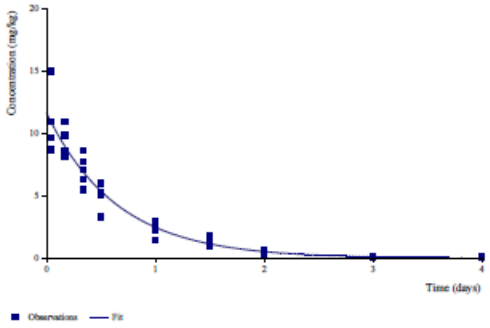
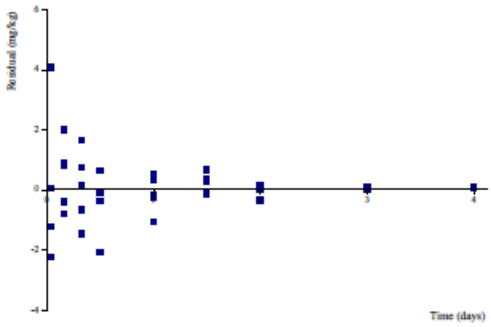
Step 1: Run SFO. SFO statistically and visually acceptable?

| | SFO |
|---|---|
| Plot |  |
| Residuals |  |
| Visual fit | Excellent |
| $\chi^2$ error (%) | 6.7 |
| t-test | *k*: $p < 0.05$ |
| $DT_{50}$ (days) | 0.33 |
| $DT_{90}$ (days) | 1.1 |
| Modelling $DT_{50}$ (days) | 0.33 |
| Assessment | Visual fit is excellent, $\chi^2$ error is low and rate parameter differs significantly from zero. |
| Discussion | The SFO model is considered acceptable and should be used for modelling endpoints. |

Summary: Use SFO. $DT_{50\ mod}$ = 0.33 days.

## 4.4 Decline of mesotrione in Trial JS001LRM-06 in The Netherlands

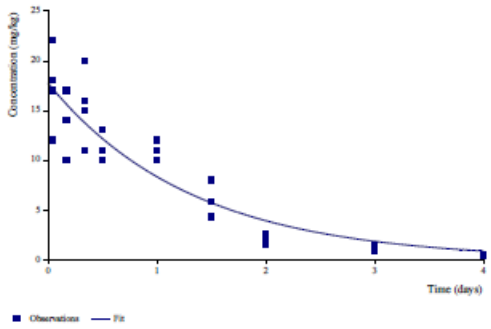
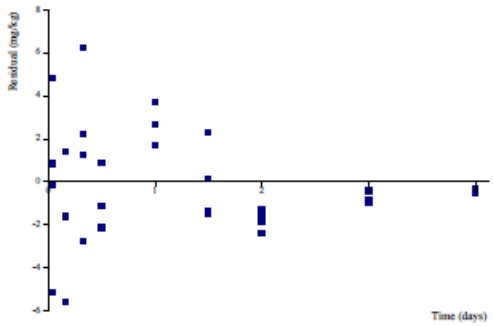
Step 1: Run SFO. SFO statistically and visually acceptable?

| | SFO |
|---|---|
| Plot |  |
| Residuals |  |
| Visual fit | Excellent |
| $\chi^2$ error (%) | 4.6 |
| t-test | *k*: $p < 0.05$ |
| $DT_{50}$ (days) | 0.45 |
| $DT_{90}$ (days) | 1.5 |
| Modelling $DT_{50}$ (days) | 0.45 |
| Assessment | Visual fit is excellent, $\chi^2$ error is low and rate parameter differs significantly from zero. |
| Discussion | The SFO model is considered acceptable and should be used for modelling endpoints. |

Summary: Use SFO. $DT_{50\ mod}$ = 0.45 days.

## 4.5 Decline of mesotrione in Trial S17-05218-01 in The Netherlands

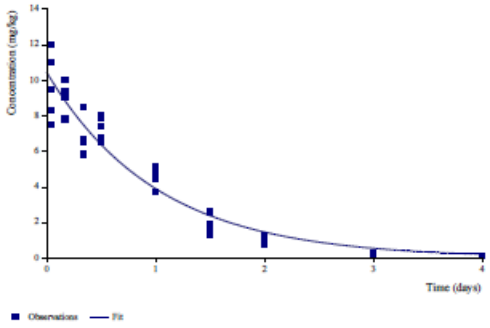
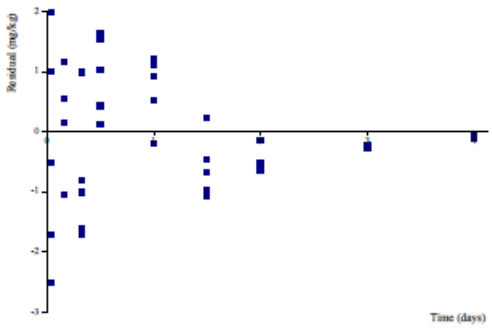
Step 1: Run SFO. SFO statistically and visually acceptable?

| | SFO |
|---|---|
| Plot |   |
| Residuals |   |
| Visual fit | Good |
| $\chi^2$ error (%) | 12.5 |
| t-test | *k*: $p < 0.05$ |
| $DT_{50}$ (days) | 0.92 |
| $DT_{90}$ (days) | 3.1 |
| Modelling $DT_{50}$ (days) | 0.92 |
| Assessment | Visual fit is good, $\chi^2$ error is acceptable and rate parameter differs significantly from zero. |
| Discussion | The SFO model is considered acceptable and should be used for modelling endpoints. |

Summary: Use SFO. $DT_{50\ mod}$ = 0.92 days.

## 4.6 Decline of mesotrione in Trial S17-05218-02 in The Netherlands

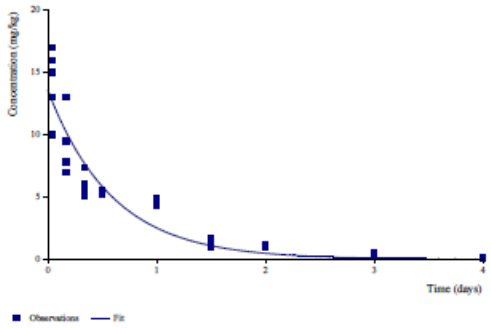
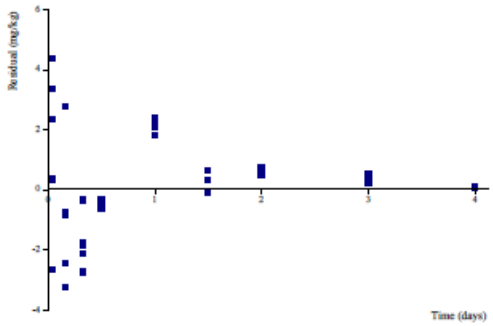
Step 1: Run SFO. SFO statistically and visually acceptable?

| | SFO |
|---|---|
| Plot |   |
| Residuals |   |
| Visual fit | Good |
| $\chi^2$ error (%) | 10.3 |
| t-test | *k*: $p < 0.05$ |
| $DT_{50}$ (days) | 0.70 |
| $DT_{90}$ (days) | 2.3 |
| Modelling $DT_{50}$ (days) | 0.70 |
| Assessment | Visual fit is good, $\chi^2$ error is acceptable and rate parameter differs significantly from zero. |
| Discussion | The SFO model is considered acceptable and should be used for modelling endpoints. |

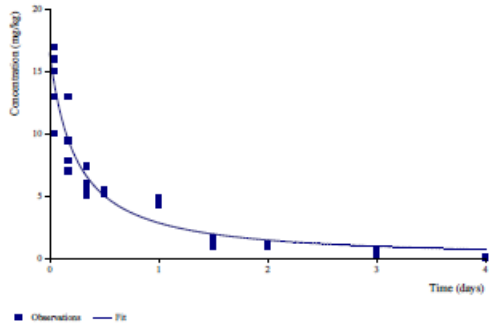
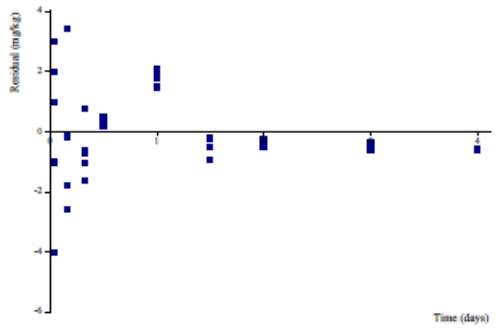
Summary: Use SFO. $DT_{50\ mod}$ = 0.70 days.

### 4.7 Decline of mesotrione in Trial S17-05218-03 in The Netherlands

Step 1: Run SFO. SFO statistically and visually acceptable?

| | SFO |
|---|---|
| Plot |  |
| Residuals |  |
| Visual fit | Intermediate |
| $\chi^2$ error (%) | 19.5 |
| t-test | *k*: $p < 0.05$ |
| $DT_{50}$ (days) | 0.41 |
| $DT_{90}$ (days) | 1.4 |
| Modelling $DT_{50}$ (days) | 0.70 |
| Assessment | Visual fit is reasonable and rate parameter differs significantly from zero, however, $\chi^2$ error is high. |
| Discussion | The SFO model is not considered acceptable. Deviation from SFO is not due to outliers or experimental artefacts. Therefore, biphasic models should be investigated. |

Step 2: 10% initially measured concentration reached within experimental period. Run FOMC. FOMC statistically and visually acceptable?

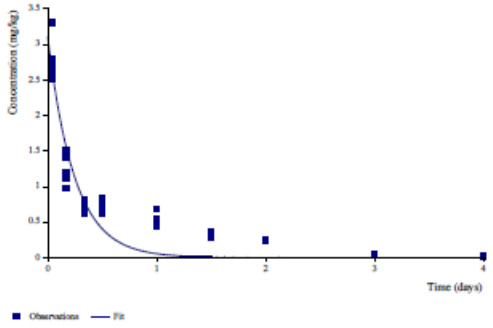
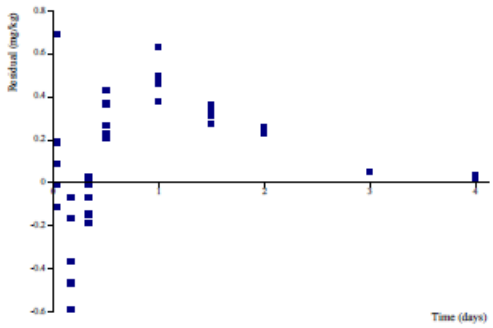
| | FOMC |
|---|---|
| Plot |  |
| Residuals |  |
| Visual fit | Good |
| $\chi^2$ error (%) | 13.7 |
| t-test | NA |
| $DT_{50}$ (days) | 0.23 |
| $DT_{90}$ (days) | 1.8 |
| Modelling $DT_{50}$ (days)[1] | 0.54 |
| Assessment | Visual fit is good and $\chi^2$ error is acceptable. |
| Discussion | The FOMC model is considered acceptable and should be used for modelling endpoints. |

[1] For FOMC modelling $DT_{50} = DT_{90} / 3.32$

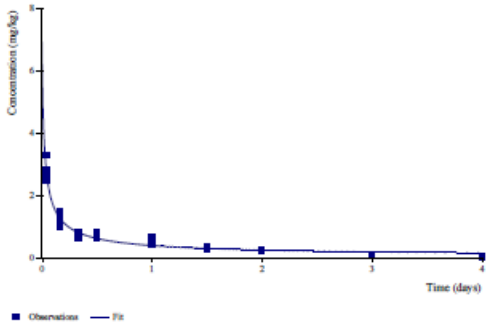
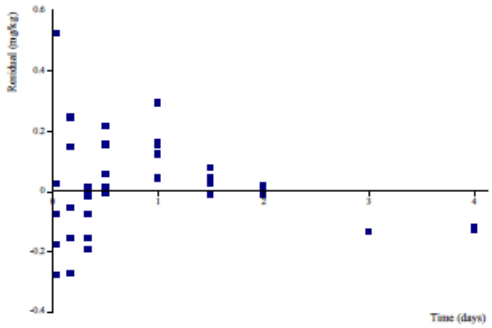
Summary: Use FOMC. $DT_{50\,mod} = 0.54$ days.

## 4.8 Decline of mesotrione in Trial S17-05218-04 in The Netherlands

Step 1: Run SFO. SFO statistically and visually acceptable?

| | SFO |
|---|---|
| Plot |  |
| Residuals |  |
| Visual fit | Poor |
| $\chi^2$ error (%) | 29.0 |
| t-test | *k*: $p < 0.05$ |
| $DT_{50}$ (days) | 0.17 |
| $DT_{90}$ (days) | 0.57 |
| Modelling $DT_{50}$ (days) | 0.17 |
| Assessment | Rate parameter differs significantly from zero, however, visual fit is poor and $\chi^2$ error is high. |
| Discussion | The SFO model is not considered acceptable. Deviation from SFO is not due to outliers or experimental artefacts. Therefore, biphasic models should be investigated. |

Step 2: 10% initially measured concentration reached within experimental period. Run FOMC. FOMC statistically and visually acceptable?

| | FOMC |
|---|---|
| Plot |   |
| Residuals |   |
| Visual fit | Excellent |
| $\chi^2$ error (%) | 10.4 |
| t-test | NA |
| $DT_{50}$ (days) | 0.03 |
| $DT_{90}$ (days) | 0.42 |
| Modelling $DT_{50}$ (days)[1] | 0.13 |
| Assessment | Visual fit is excellent and $\chi^2$ error is acceptable. |
| Discussion | The DFOP model provides the best fit and should be used. |

[1] For FOMC modelling $DT_{50} = DT_{90} / 3.32$

Summary: Use FOMC. $DT_{50\ mod} = 0.13$ days.

**Conclusion**

The overall geometric mean $DT_{50}$ from the usable residue data of the two residue decline trials in maize

(Bakker, 2016 and van de Sandt, 2019) was determined as 0.46 days.

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

No additional data submitted.

## A 2.2 KCP 10.2 Effects on aquatic organisms

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

#### A 2.2.1.1 Study 1: Acute toxicity to Rainbow trout

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 203 and according to the principles of GLP. In the definitive test all the validity criteria were met.<br>The study is considered to be reliable and suitable for the risk assessment. All results refer to nominal concentrations. |

| | |
|---|---|
| Reference: | KCP 10.2.1/01 |
| Report | SAE053H/01: Toxicity to the rainbow trout *Oncorhynchus mykiss* under laboratory conditions (acute toxicity test – static), xxx, 2016, S16--03041 |
| Guideline(s): | Yes, OECD 203 (1992) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication<br>(if vertebrate study) | No, representative product study. |

**Materials and Methods**

**A. MATERIALS**

| | |
|---|---|
| **1. Test material** | SAE053H/01<br>(Other names: Mesotrione/Nicosulfuron 80/30 OD) |
| **Description** | White to beige liquid/cream, OD (oil dispersion) |
| **Lot/Batch #** | 54606-101 |
| **Purity** | Nicosulfuron: 30 g/L nominal; 30.7 g/L analysed<br>Mesotrione: 80 g/L nominal; 81.7 g/L analysed<br>Density: 0.98 g/cm³ |
| **Stability of test material** | Stable under storage conditions (ambient temperature, 5°C – 30°C, in a dark and dry place)<br>Expiry date: 20 Mar 2018 |

**2. Vehicle and/or positive control** Vehicle control: test water; no positive control required

**3. Test organism**

| | |
|---|---|
| **Species** | Rainbow trout (*Oncorhynchus mykiss* Walbaum); Salmoniformes: Salmonidae |
| **Strain** | Not applicable |
| **Source** | Forellenzucht Peter Störk, Bad Saulgau, Germany |
| **Age** | Not reported; Size: 4 - 6 cm |
| **Acclimation period** | The fish were acclimatised to the test water and temperature for at least 12 days under continuous renewal of water. No mortality above 5% was observed throughout the acclimatisation period. |
| **Feeding** | During holding and acclimatisation until two days before the start of the test, the fish were fed daily with granular rearing food from the fish supplier once daily. The fish were not fed during the test. |
| **Test units** | 25 L glass aquaria containing 15 L test medium. The loading rate was 0.914 g fish/L test medium. |

**4. Environmental conditions**

| | |
|---|---|
| **Test water** | Mixture of dechlorinated drinking water and deionised water, conductivity 663 µS/cm |
| **Hardness** | 13° dH (german hardness), corresponding to 232 mg/L (as $CaCO_3$) |
| **Water temperature** | nominal: 13 – 17 °C; actual: 15.9 – 16.5 °C |
| **Lighting** | 16 hours daily |
| **Shaking** | Continuous aeration with a membrane pump using a Pasteur pipette |

## B. STUDY DESIGN AND METHODS

**1. In-life dates** 13 Jun 2016 to 19 Jul 2016

**2. Experimental conditions**

*Test design*

Rainbow trout (*Oncorhynchus mykiss*) were exposed in a static 96 h test to the test substance at five concentrations and a test water control. The recorded effects after 0, 4, 24, 48, 72 and 96 hours were mortality and visible abnormalities of the test fish.

*Number of animals per treatment*

Seven fish were used per test substance treatment and control.

*Test conditions*

The test was conducted in a mixture of dechlorinated drinking water and deionised water. The water temperature was maintained at 15.9 – 16.5 °C and the test systems were illuminated for 16 hours daily. The dissolved oxygen concentration in the test media and control was above 68% of air saturation. The pH values in the test solutions and control were between 7.94 and 8.52. The measured water hardness was 13° dH corresponding to 232 mg/L $CaCO_3$.

*Test concentrations*

Nominal test substance concentrations were 3.42, 7.51, 16.5, 36.4 and 80.0 mg product/L, corresponding to 0.285, 0.626, 1.37, 3.03 and 6.66 mg a.s./L mesotrione and 0.107, 0.235, 0.520, 1.14 and 2.50 mg a.s./L nicosulfuron based on the analysed content of active substances in the formulation and the product density. In addition, a control group with untreated test water was used. The concentrations were chosen based on a range-finding test with concentrations of 1.00, 10.0 and 100 mg product/L.

*Treatment/Application*

The necessary amount of test item was weighed and transferred into the test medium directly. Afterwards the aquaria were stirred. At the concentrations of 7.51, 16.5, 36.4 and 80.0 mg product/L, the test medium became turbid. In the beginning, a light precipitation was observed which could be dissolved again after stirring.

*Analytics*

The concentrations of mesotrione and nicosulfuron were analysed in the control and all test item solutions in the fresh (t = 0 h) and aged (t = 24, 72 or 96 h depending on effects) solutions. Measurements were performed via HPLC-MS/MS. Details to the analytical method are summarized in Part B, Section 5.

**3. Sampling and measurements**

The test fish were observed for mortality and visual abnormalities 0, 4, 24, 48, 72 and 96 hours after test start. Water temperature, pH and oxygen saturation were measured in all vessels at the beginning of the test and every 24 hours in the test solutions. Water hardness was determined at test start in the untreated control.

**4. Calculation of toxicity**

Mortality was determined for each treatment and observation time. Dead fish were removed from the test vessels and length and weight was recorded. At test end, all fish were euthanized, weighed and measured. Abnormalities in appearance and behaviour were evaluated such as loss of equilibrium, swimming behaviour, respiratory function, pigmentation and other events.

**5. Statistics**

The $LC_{50}$ values after 24, 48, 72 and 96 h were calculated with Probit analysis using linear maximum likelihood regression. The NOEC was established based on the highest test concentration at which no mortality above the allowed control mortality was observed. For evaluation, the statistical programme ToxRat Professional 3.2.1 was used. The level of significance was set to $\alpha = 0.05$.

**Results and Discussion**

The measured concentrations of mesotrione in the fresh test item solutions ranged from 77 to 88% of nominal and in the aged solution from 80 to 88% of nominal. For nicosulfuron, the measured concentrations in the fresh solutions ranged from 91 to 102% and in the aged solution from 90 to 100% of the nominal concentrations (see following table). Toxicological endpoints were evaluated using nominal concentrations of the test item since the mean of measured concentrations of mesotrione and nicosulfuron in all test solutions were 84 and 96% of the nominal concentrations.

**Table A 2.2.1-1: Concentrations of mesotrione and nicosulfuron in the test media during the test**

| Nominal test concentration [mg product/L] | Nominal test concentration [mg a.s./L] | Sampling [h] | Measured concentration of active substance [mg a.s./L] | Measured concentration of active substance [% nominal] | Mean [%] |
|---|---|---|---|---|---|
| Control | | 0 | n.d. | - | - |
| | | 96 | n.d. | - | |
| **Active substance: mesotrione** | | | | | |
| 3.42 | 0.285 | 0 | 0.244 | 86 | 87 |
| | | 96 | 0.250 | 88 | |
| 7.51 | 0.626 | 0 | 0.480 | 77 | 79 |
| | | 72 | 0.501 | 80 | |
| 16.5 | 1.37 | 0 | 1.16 | 85 | 87 |
| | | 96 | 1.20 | 88 | |
| 36.4 | 3.03 | 0 | 2.56 | 84 | 84 |
| | | 24 | 2.52 | 83 | |
| 80.0 | 6.66 | 0 | 5.85 | 88 | 87 |
| | | 24 | 5.64 | 85 | |
| **Active substance: nicosulfuron** | | | | | |
| 3.42 | 0.107 | 0 | 0.0971 | 91 | 91 |
| | | 96 | 0.0965 | 90 | |
| 7.51 | 0.235 | 0 | 0.216 | 92 | 94 |
| | | 72 | 0.224 | 95 | |
| 16.5 | 0.520 | 0 | 0.507 | 98 | 99 |
| | | 96 | 0.519 | 100 | |
| 36.4 | 1.14 | 0 | 1.09 | 96 | 95 |
| | | 24 | 1.07 | 94 | |
| 80.0 | 2.50 | 0 | 2.54 | 102 | 100 |
| | | 24 | 2.44 | 98 | |

- = not applicable; n.d. = not determined; LOQ = 0.0250 mg a.s./L mesotrione, 0.00939 mg a.s./L nicosulfuron

In the control no mortality was observed until the end of the test. In the test item concentrations, mortality ranged from 14 to 100% at the end of the test (see table below).

In the lowest test item concentration of 3.42 mg product/L, six fish showed pigmentation after 24 h and 48 h. This number declined to five fish after 72 h and to two fish after 96 h. After 72 h, four fish and after 96 h two fish showed reduced activity. Additionally, after 72 h one fish showed loss of equilibrium.
At 7.51 mg product/L, one fish showed reduced activity after 4 h.
At 16.5 mg product/L all of the seven fish showed a loss of equilibrium after 4 h and were upside down with loss of equilibrium, showing only movement of gills as a sign of life after 24 h. After 48 h all seven fish improved their activity, but still suffered loss of equilibrium. After 72 h, only three fish showed sublethal effects by reduced activity (one fish) or dark pigmentation (two fish).
At 36.4 mg product/L, all of the seven fish were upside down with movement of gills as only sign of life after 4 h.
At the highest test item concentration of 80 mg product/L, all of the seven fish were upside down with loss of equilibrium, showing only movement of gills as a sign of life 4 h.
In the control, one fish lost equilibrium after 48 h and one fish showed reduced activity after 72 h.

The average weight of the test organisms was 1.48 ± 0.47 g and the average length was 52 ± 4 mm.

The 96-hour $LC_{50}$ of SAE053H/01 in rainbow trout was extrapolated and determined to be 2.15 mg product/L (nominal concentration). The 96-hour NOEC could not be determined; the 72 h NOEC was 3.42 mg product/L (nominal concentration).

**Table A 2.2.1-2: Acute mortality of SAE053H/01 in rainbow trout**

| Nominal test concentration [mg product/L] | Cumulative mortality at time point [%] | | | | |
|---|---|---|---|---|---|
| | 4 h | 24 h | 48 h | 72 h | 96 h |
| Control | 0 | 0 | 0 | 0 | 0 |
| 3.42 | 0 | 0 | 0 | 14 | 57 |
| 7.51 | 0 | 0 | 0 | 100 | 100 |
| 16.5 | 0 | 0 | 0 | 14 | 86 |
| 36.4 | 0 | 100 | 100 | 100 | 100 |
| 80.0 | 0 | 100 | 100 | 100 | 100 |
| **Endpoints [mg product/L] based on nominal concentrations** | | | | | |
| $LC_{50}$ (95% Confidence limit) | > 80.0 (-) | 24.0 (19.2 – 30.5) | 24.0 (19.2 – 30.5) | 7.91 (-) | 2.15 (-) |
| NOEC | 80.0 | 16.5 | 16.5 | 3.42 | n.a. |

n.a. no NOEC observed; - not calculable

The mortality in the control was 0% at test end (required according to test guideline OECD 203 ≤ 10% in the controls). Dissolved oxygen concentrations in the test solutions were ≥ 68% of air saturation (required according to the test guideline ≥ 60%). Thus, the study did fulfil all validity criteria of OECD test guideline 203.

**Conclusion**

The 96-hour $LC_{50}$ of SAE053H/01 in rainbow trout was extrapolated and determined to be 2.15 mg product/L and the NOEC after 72 h was 3.42 mg product/L based on nominal concentrations. No NOEC could be determined after 96 h. All validity criteria were met in the study.

### A 2.2.1.2 Study 2: Acute toxicity to *Daphnia magna*

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 202 and according to the principles of GLP. In the definitive test all the validity criteria were met.<br>The study is considered to be reliable and suitable for the risk assessment. All results refer to nominal concentrations. |

| | |
|---|---|
| Reference: | KCP 10.2.1/02 |
| Report | SAE053H/01: Toxicity to the water flea *Daphnia magna* Straus under laboratory conditions (acute immobilisation test – static), Zawadsky, C., 2016, S16-03042 |
| Guideline(s): | Yes, OECD 202 (2004) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | - |

**Materials and Methods**

**A. MATERIALS**

| | |
|---|---|
| **1. Test material** | SAE053H/01<br>(Other names: Mesotrione/Nicosulfuron 80/30 OD) |
| **Description** | White to beige liquid/cream, OD (oil dispersion) |
| **Lot/Batch #** | 54606-101 |
| **Purity** | Nicosulfuron: 30 g/L nominal; 30.7 g/L analysed<br>Mesotrione: 80 g/L nominal; 81.7 g/L analysed<br>Density: 0.98 g/cm³ |
| **Stability of test material** | Stable under storage conditions (ambient temperature, 5°C – 30°C, in a dark and dry place)<br>Expiry date: 20 Mar 2018 |
| **2. Vehicle and/or positive control** | Vehicle control: test water<br>Positive control: The reference item potassium dichromate was tested around the same time period as the study (Jun 2016) and resulted in an $EC_{50}$ (24 h) between 1.0 and 2.0 mg/L. |
| **3. Test organism** | |
| **Species** | *Daphnia magna* (Straus) |
| **Strain** | Clone V |
| **Source** | Bred at the test site and originally purchased from the Umweltbundesamt (Federal Environment Agency) in Berlin, Germany. |
| **Age** | < 24 hours |
| **Acclimation period** | Rearing of daphnids was performed under environmental conditions similar to those in the test. |
| **Feeding** | During holding, daphnids were fed with *Desmodesmus subspicatus* algae at least three times a week. Daphnids were not fed during the test. |
| **Test units** | 100-mL glass beakers filled with 50 mL test medium covered with a glass plate to reduce evaporation |
| **4. Environmental conditions** | |
| **Test water** | Elendt M4 medium |
| **Hardness** | 13° dH corresponding to 232 mg/L as $CaCO_3$ |
| **Water temperature** | nominal: 18 – 22°C, actual: 18.9 – 20.4 °C |
| **Lighting** | 16 hours photoperiod daily |
| **Aeration** | None |

**B. STUDY DESIGN AND METHODS**

| | |
|---|---|
| **1. In-life dates** | 07 Jun 2016 to 19 Jul 2016 |

## 2. Experimental conditions

*Test design*

*Daphnia magna* of less than 24 hours old were exposed in a static 48-hour test to the test substance at five test concentrations and a test water control. A reference item was tested at two concentrations around the same time period as the study. The recorded effect was mortality and immobility of the daphnids after 24 and 48 hours.

*Number of animals per treatment*

Twenty daphnids per treatment, five daphnids/replicate, four replicates/test substance treatment and test water control. One additional replicate for measurements was established without organisms for each test concentration.

*Test conditions*

The test was conducted in Elendt M4 medium with a hardness of 13° dH (232 mg/L as $CaCO_3$). The test systems were illuminated for 16 hours photoperiod daily. The water temperature was maintained at 18.9 – 20.4°C. The dissolved oxygen concentration was at least 8.6 mg/L and the pH ranged between 7.67 and 8.38.

*Test concentrations*

Concentrations of nominal 3.01, 4.06, 5.49, 7.41 and 10.0 mg product/L were tested. These concentrations correspond to nominal 0.251, 0.339, 0.458, 0.618 and 0.834 mg a.s./L mesotrione and 0.0942, 0.127, 0.172, 0.232 and 0.313 mg a.s./L nicosulfuron based on the analysed content of active substances and the product density. A test water control was tested additionally. The concentrations were chosen based on a GLP range-finding test with test item concentrations of 6.25, 12.5, 25.0, 50.0 and 100 mg product/L.

*Treatment/Application*

A stock solution (equally to the highest tested concentration) was prepared with test medium. This solution was slightly turbid. For the remaining test solutions, the stock solution was serially diluted with test medium and thoroughly mixed.

*Analytics*

Analytical samples were taken from all test concentrations and control at test start (fresh) and after 48 hours (aged). Samples were analysed using HPLC-MS/MS. The analytical method is summarized in Part B, Section 5.

## 3. Sampling and measurements

Observations for *Daphnia* immobilisation and mortality were made after 24 and 48 hours. All daphnids not able to swim within 15 seconds after gentle agitation of the test vessel were considered to be immobilised. Behavioural changes and abnormalities in appearance were recorded as well. The test temperature and the pH value as well as the oxygen saturation rate were measured in the fresh and in the aged medium after 24 and 48 hours in the additional replicate without organisms.

### 4. Calculation of toxicity

Immobility or mortality was determined for each treatment and observation time.

### 5. Statistics

The 24 and 48 h $EC_{50}$ were determined by logit analysis using linear maximum likelihood regression. The NOEC was determined as the highest concentration at which the immobilisation was not higher than the allowed control immobilisation. The statistical evaluation was performed using ToxRat Professional 3.2.1.

## Results and Discussion

The measured concentrations of mesotrione in the fresh test item solutions ranged from 89 to 101% of nominal with a mean initial concentration of 94% of nominal. The measured concentration in the aged solution was between 89 and 107% of nominal with a mean concentration of 98% of the nominal test concentration. The measured concentrations of nicosulfuron in the fresh test item solutions ranged from 87 to 96% of nominal with a mean concentration of 91% of nominal. The measured concentration in the aged solution was between 93 and 99% of nominal with a mean concentration of 96% of the nominal concentration (Table A 2.2.1-3). Toxicological endpoints were evaluated using nominal concentrations of the product.

**Table 9.10.3-3: Concentrations of mesotrione and nicosulfuron in the test media during the exposure period**

| Nominal test concentration | | Sampling | Measured concentration of active substance | |
|---|---|---|---|---|
| [mg product/L] | [mg a.s./L] | [h] | [mg a.s./L] | [% nominal] |
| Control | | 0 | n.d. | - |
| | | 48 | n.d. | - |
| **Active substance: mesotrione** | | | | |
| 3.01 | 0.251 | 0 | 0.235 | 94 |
| | | 48 | 0.247 | 98 |
| 4.06 | 0.339 | 0 | 0.302 | 89 |
| | | 48 | 0.301 | 89 |
| 5.49 | 0.458 | 0 | 0.421 | 92 |
| | | 48 | 0.441 | 96 |
| 7.41 | 0.618 | 0 | 0.587 | 95 |
| | | 48 | 0.661 | 107 |
| 10.0 | 0.834 | 0 | 0.842 | 101 |
| | | 48 | 0.842 | 101 |
| **Active substance: nicosulfuron** | | | | |
| 3.01 | 0.0942 | 0 | 0.0815 | 87 |
| | | 48 | 0.0873 | 93 |
| 4.06 | 0.127 | 0 | 0.117 | 92 |
| | | 48 | 0.124 | 98 |
| 5.49 | 0.172 | 0 | 0.159 | 92 |
| | | 48 | 0.162 | 94 |
| 7.41 | 0.232 | 0 | 0.207 | 89 |
| | | 48 | 0.224 | 97 |
| 10.0 | 0.313 | 0 | 0.301 | 96 |
| | | 48 | 0.311 | 99 |

- = not applicable; n.d. = not determined; LOQ = 0.0250 mg a.s./L mesotrione, 0.00939 mg a.s./L nicosulfuron

In the control no immobilisation occurred after 48 hours. In the test item concentrations the immobilisation rate was 10, 20, 80, 100 and 95% at 3.01, 4.06, 5.49, 7.41 and 10.0 mg product/L, respectively, at the end of the test (Table A 2.2.1-4). The 48-hour $EC_{50}$ of SAE053H/01 in *Daphnia magna* was determined to be 4.64 mg product/L based on nominal concentrations. The 48-hour NOEC was determined to be 3.01 mg product/L based on nominal concentrations.

The test with the reference item potassium dichromate resulted in an $EC_{50}$ (24 h) between 1.0 and 2.0 mg/L.

**Table A 2.2.1-4: Acute toxicity of SAE053H/01 to *Daphnia magna***

| Nominal test concentration [mg product/L] | Dead and immobile test animals 24 hours No. | 24 hours [%] | 48 hours No. | 48 hours [%] |
|---|---|---|---|---|
| Control | 0 | 0 | 0 | 0 |
| 3.01 | 0 | 0 | 2 | 10 |
| 4.06 | 0 | 0 | 4 | 20 |
| 5.49 | 8 | 40 | 16 | 80 |
| 7.41 | 14 | 70 | 20 | 100 |
| 10.0 | 16 | 80 | 19 | 95 |
| **Endpoint [mg product/L] based on nominal concentrations** | | | | |
| $EC_{50}$ (95% confidence limit) | 6.63 (5.91 – 7.51) | | 4.64 (2.10 – 8.38) | |
| NOEC | 4.06 | | 3.01 | |

In the control the immobility was 0% at test end (required according to test guideline OECD 202 ≤ 10%) and dissolved oxygen concentration was ≥ 8.6 mg/L (required ≥ 3 mg/L). Therefore, the validity criteria were fulfilled.

**Conclusion**

The 48 h $EC_{50}$ of SAE053H/01 in *Daphnia magna* determined to be 4.64 (95% CL: 2.10 – 8.38) mg product/L based on nominal concentrations. The 48 h NOEC was determined to be 3.01 mg product/L based on nominal concentrations. All validity criteria were fulfilled.

### A 2.2.1.3 Study 3: Toxicity to the alga *Pseudokirchneriella subcapitata*

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 201 and according to the principles of GLP. In the definitive test all the validity criteria were met.<br>The study is considered to be reliable and suitable for the risk assessment.<br>Toxicological endpoints were evaluated using nominal concentrations of the test item since the mean of measured concentrations of mesotrione and nicosulfuron in all test solutions were 80.5 and 91.4% of the nominal concentrations. |

| | |
|---|---|
| Reference: | KCP 10.2.1/03 |
| Report | SAE053H/01: Toxicity to the single cell green alga *Pseudokirchneriella subcapitata* Hindák under laboratory conditions, Falk, S., 2016a, S16-03039 |

| | |
|---|---|
| Guideline(s): | Yes, OECD 201 (2011) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | - |

**Materials and Methods**

**A. MATERIALS**

**1. Test material** — SAE053H/01
(Other names: Mesotrione/Nicosulfuron 80/30 OD)

| | |
|---|---|
| **Description** | White to beige liquid/cream, OD (oil dispersion) |
| **Lot/Batch #** | 54606-101 |
| **Purity** | Nicosulfuron: 30 g/L nominal; 30.7 g/L analysed<br>Mesotrione: 80 g/L nominal; 81.7 g/L analysed<br>Density: 0.98 g/cm³ |
| **Stability of test material** | Stable under storage conditions (ambient temperature, 5°C – 30°C, in a dark and dry place)<br>Expiry date: 20 Mar 2018 |

**2. Vehicle and/or positive control** — Vehicle control: test water
Positive control: Potassium dichromate is tested as reference item twice a year to confirm the sensitivity. In the most recent test with *Pseudokirchneriella subcapitata* in June 2016, the 72-hour $E_rC_{50}$ was determined at 1.16 mg/L.

**3. Test organism**

| | |
|---|---|
| **Species** | *Pseudokirchneriella subcapitata* |
| **Strain** | SAG 61.81 |
| **Source** | MBM Sciencebridge GmbH, Hans-Adolf-Krebs-Weg 1, 37077 Göttingen, Germany |
| **Age** | Algae cells were taken from a semi-continuous liquid stock culture with exponential growth. |
| **Acclimation period** | Three days before test start, algae were held under test conditions in exponential growth stage to produce a pre-culture. |
| **Test units** | 100 mL Erlenmeyer flasks with aluminium caps containing 50 mL of test solution. |

**4. Environmental conditions**

**Test water** — The algae were tested in AAP-medium (according to OECD 201) with the following concentrations:

| | |
|---|---|
| $NaHCO_3$ | 15 mg/L |
| $KH_2PO_4$ | 1.044 mg/L |

| | |
|---|---|
| $MgSO_4 \cdot 7H_2O$ | 14.6 mg/L |
| $NaNO_3$ | 25.5 mg/L |
| $CaCl_2 \cdot 2H_2O$ | 4.41 mg/L |
| $MgCl_2 \cdot 6H_2O$ | 12.16 mg/L |
| $H_3BO_3$ | 0.186 mg/L |
| $MnCl_2 \cdot 4H_2O$ | 0.415 mg/L |
| $ZnCl_2$ | 0.00327 mg/L |
| $CoCl_2 \cdot 6H_2O$ | 0.00143 mg/L |
| $CuCl_2 \cdot 2H_2O$ | $1.2 \cdot 10^{-5}$ mg/L |
| $Na_2MoO_4 \cdot 2H_2O$ | 0.00726 mg/L |
| $FeCl_3 \cdot 6H_2O$ | 0.160 mg/L |
| $Na_2EDTA \cdot 2H_2O$ | 0.300 mg/L |
| The pH was adjusted to 7.5 ± 0.1. | |

| | |
|---|---|
| **Water temperature** | nominal: 21 – 24 °C; actual: 22.3 – 23.2°C |
| **Lighting** | Continuous illumination from the side; light intensity nominal: 67.7 – 91.7 $\mu Em^{-2}s^{-1}$; actual: 73.2 – 83.8 $\mu Em^{-2}s^{-1}$ |
| **Shaking** | Yes |

## B. STUDY DESIGN AND METHODS

**1. In-life dates** 04 Jul 2016 to 28 Jul 2016

**2. Experimental conditions**

*Test design*

The single cell green alga *Pseudokirchneriella subcapitata* was exposed in a static 72-hour test to the test substance at five concentrations and to a control, each test concentration with three replicates and the control with six replicates. The inhibition of algal growth was quantified based on yield and growth rates of the algae.

*Inoculum at test start*

The cell density was adjusted to $0.5 \times 10^4$ cells/mL in all treatments and in the control at start of the exposure period.

*Test conditions*

The test temperature was maintained at 22.3 – 23.2 °C during the exposure phase. The test systems were continuously illuminated at 73.2 – 83.8 $\mu Em^{-2}s^{-1}$. The pH in the test medium of the control was between 7.21 and 7.86.

*Test concentrations*

Nominal test substance concentrations were 0.477, 1.53, 4.88, 15.6 and 50.0 mg product/L, corresponding to nominal 0.0397, 0.127, 0.407, 1.30 and 4.17 mg a.s./L mesotrione and 0.0149, 0.0479, 0.153, 0.488 and 1.57 mg a.s./L nicosulfuron based on the analysed content of active substances in the formulation and the product density. In addition, a control group with untreated test medium was tested. The concentrations were chosen based on a non-GLP range finder with test item concentrations of 0.0100, 0.100, 1.0, 10.0 and 100 mg product/L.

*Treatment/Application*

A stock solution was prepared by dissolving 125 mg in test medium. The higher test item concentrations (4.88, 15.6 and 50 mg product/L) were prepared by diluting the stock solution with test medium to obtain the required test concentrations. The two lower concentrations were prepared by diluting the test solution of 50.0 mg product/L with test medium.

*Analytics*

The concentrations of mesotrione and nicosulfuron were analysed in the test solutions of all concentration levels and the control at the start (t = 0 h) and end (t = 72 h) of the test by HPLC-MS/MS. Details to the analytical method are summarized in Part B, Section 5.

**3. Sampling and measurements**

The daily fluorescence measurements were performed with a fluorescence microplate reader (infinite 200Pro) with an emission wavelength of 670 nm and evaluated with Tecan i-control (Software for Tecan Readers Tecan i-control, 1.11.1.0). At defined dates (24, 48 and 72 hours), the number of cells in each replicate was determined in duplicate. The determination was performed by fluorescence measurement. By the means of a calibration curve, where fluorescence signals were plotted versus cell numbers, the cell numbers were derived from the fluorescence signals. To establish a calibration curve, the cell numbers were counted with a Neubauer chamber after preparation of a dilution series of a logarithmic growing *Pseudokirchneriella subcapitata* culture. Additionally, the morphological appearance of the algae cells was observed microscopically at the end of the test.

The pH was measured at the beginning and at the end of the test. The temperature in the test was determined daily. The light intensity was measured at test start.

**4. Calculation of toxicity**

The average specific growth rate for a specific period was calculated as the logarithmic increase of the cell numbers for each single vessel of controls and treatments. The percentage inhibition of growth rates (% $I_\mu$) was calculated as the difference between the growth rates of the control ($\mu_c$) and the growth rates in the treatment ($\mu_t$).

Yield was calculated as the cell numbers at the end of the test minus the starting cell numbers for each single vessel of controls and treatments. For each test concentration and control, a mean value for yield along with variance estimates was calculated. The percent inhibition in yield (% $I_y$) was calculated for each treatment replicate.

**5. Statistics**

A test for normality of the data was performed by calculating the Shapiro-Wilk's statistic and the homogeneity of variance of the data was evaluated by using the Levene's Test. The NOEC and LOEC were determined by Jonckheeere-Terpstra-test for growth rate and Bonferroni-Holms corrected Welch test for yield. The $EC_{50}$, $EC_{20}$ and $EC_{10}$-values for growth rate and yield were determined by Probit analysis following the normal and Gompertz distribution, respectively. Only concentrations within a clear dose response were used for calculations. Due to statistical reasons the inhibition-values above 100% were set to 100 and values below zero were set to zero. The statistical evaluation for the 72 hours period was performed for growth rate and yield using SAS ® (2002–2010).

**Results and Discussion**

Analytically measured concentrations of mesotrione and nicosulfuron were determined in the test solution samples from all test concentrations and the control. The measured concentrations of mesotrione in the fresh solutions were between 73 and 89% of nominal. At test end the measured concentrations in the aged solutions were between 80 and 85% of nominal. The measured concentrations of nicosulfuron in the fresh solutions were between 80 and 95% of nominal and between 90 and 101% in the aged solutions (Table A 2.2.1-5). The biological results were based on the nominal test concentrations of the product.

After the exposure period of 72 hours, the test substance had a statistically significant inhibitory effect on the yield and growth rate of the algae at the nominal test concentrations of ≥ 4.88 and ≥ 15.6 mg product/L, respectively (Table A 2.2.1-6 and Table A 2.2.1-7). The 72-hour NOEC for yield was determined to be 1.53 mg product/L. The 72-hour NOEC value for growth rate was determined to be nominal 4.88 mg product/L. The 72-hour $E_yC_{50}$, $E_yC_{20}$ and $E_yC_{10}$ were determined to be 4.81, 4.58 and 4.44 mg product/L, respectively. The 72-hour $E_rC_{50}$, $E_rC_{20}$ and $E_rC_{10}$ were 5.46, 4.81 and 4.50 mg product/L, respectively. The cells were considered normal for the control and up to and including the test item concentration of 1.53 mg product/L. At a test item concentration of 4.88 mg product/L isolated cells were observed. No cells were observed at the test item concentration of 15.6 and 50 mg product/L.

For the reference item potassium dichromate, which was tested in a separate study, an $E_rC_{50}$ of 1.16 mg/L was determined.

**Table A 2.2.1-5: Concentrations of mesotrione and nicosulfuron in the test media during the test**

| Nominal test concentration [mg product/L] | Nominal test concentration [mg a.s./L] | Sampling [h] | Measured concentration of active substance [mg a.s./L] | Measured concentration of active substance [% nominal] |
|---|---|---|---|---|
| Control | | 0 | n.d. | - |
| | | 72 | n.d. | - |
| **Active substance: mesotrione** | | | | |
| 0.477 | 0.0397 | 0 | 0.0353 | 89 |
| | | 72 | 0.0339 | 85 |
| 1.53 | 0.127 | 0 | 0.094 | 74 |
| | | 72 | 0.101 | 80 |
| 4.88 | 0.407 | 0 | 0.336 | 83 |
| | | 72 | 0.327 | 80 |
| 15.6 | 1.30 | 0 | 1.01 | 78 |
| | | 72 | 1.05 | 81 |
| 50.0 | 4.17 | 0 | 3.05 | 73 |
| | | 72 | 3.46 | 83 |
| **Active substance: nicosulfuron** | | | | |
| 0.477 | 0.0149 | 0 | 0.0142 | 95 |
| | | 72 | 0.0144 | 97 |
| 1.53 | 0.0479 | 0 | 0.0410 | 86 |
| | | 72 | 0.0429 | 90 |
| 4.88 | 0.153 | 0 | 0.137 | 90 |
| | | 72 | 0.147 | 96 |
| 15.6 | 0.488 | 0 | 0.147 | 85 |
| | | 72 | 0.468 | 96 |
| 50.0 | 1.57 | 0 | 1.25 | 80 |
| | | 72 | 1.59 | 101 |

- = not applicable; n.d. = not determined; $LOQ_{mesotrione}$: 0.00417 mg/L; $LOQ_{nicosulfuron}$: 0.00157 mg/L

**Table A 2.2.1-6: Biomass of algae exposed to SAE053H/01 during the exposure period**

| Nominal concentration [mg product/L] | Mean cell number [a] [x $10^4$/mL] | | | |
|---|---|---|---|---|
| | 0 hours | 24 hours | 48 hours | 72 hours |
| Control | 0.49 | 2.20 | 9.78 | 36.95 |
| 0.477 | 0.49 | 2.50 | 13.36 | 50.61 |
| 1.53 | 0.49 | 2.69 | 12.32 | 53.66 |
| 4.88 | 0.49 | 1.38 | 4.60 | 13.89 |
| 15.6 | 0.49 | 0.98 | 0.37 | 0.34 |
| 50.0 | 0.49 | 0.31 | 0.18 | -0.34 |

| 72-hour endpoints | | |
|---|---|---|
| | Growth rate (µ) | Yield (y) |
| | Nominal concentration [mg product/L] | Nominal concentration [mg product/L] |
| $EC_{50}$ (95% CL) | 5.46 ( - ) | 4.81 (4.75 – 4.86) |
| $EC_{20}$ (95% CL) | 4.81 ( - ) | 4.58 (4.54 – 4.63) |
| $EC_{10}$ (95% CL) | 4.50 ( - ) | 4.44 (4.40 – 4.49) |
| NOEC | 4.88 | 1.53 |
| LOEC | 15.6 | 4.88 |

[a] Mean of three replicates for the treatments and of six replicates in the control
- = not determined due to mathematical reasons

**Table A 2.2.1-7: Inhibition of average growth rate and yield of algae exposed to SAE053H/01**

| Nominal test concentration [mg product/L] | Percent inhibition of growth rate [a] | | | Percent inhibition of yield [a] | | |
|---|---|---|---|---|---|---|
| | 0 – 24 h | 0 – 48 h | 0 – 72 h | 0 – 24 h | 0 – 48 h | 0 – 72 h |
| Control | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.477 | -7.9 | -10.2 | -7.2 [b] | -17.5 | -38.5 | -37.5 [b] |
| 1.53 | -12.8 | -7.6 | -8.6 [c] | -28.7 | -27.3 | -45.8 [c] |
| 4.88 | 31.7 | 25.3 | 22.8 | 48.0 | 55.8 | 63.2* |
| 15.6 | 56.4 | 111.6 | 110.6* [d] | 71.3 | 101.3 | 100.4* [d] |
| 50.0 | 132.7 | > 100 [e] | > 100* [b,e] | 110.5 | 103.3 | 102.3* [b] |

[a] Mean of three replicates for the treatments and of six replicates in the control; negative values indicate increase compared to the control
[b] Values was omitted for $EC_x$ calculation
[c] Values were set to zero for $EC_x$ calculation
[d] Values were set to 100 for $EC_x$ calculation
[e] Due to negative cell numbers, the value was not calculable but was above 100
* Statistically significantly different compared to the control

In the control the biomass had increased by a factor of 75.4 after 72 hours (required factor ≥ 16 according to test guideline OECD 201). The mean coefficient of variation of the daily growth rates in the control (section-by-section growth rates) during 72 hours was 9% (required according to test guideline ≤ 35%). The coefficient of variation of the average specific growth rates in the replicates of the control was 4.0% after 72 hours (required according to test guideline ≤ 7%). Thus, the study did fulfil all validity criteria of OECD test guideline 201.

## Conclusion

The effects of SAE053H/01 on the growth and biomass of the single cell green alga *Pseudokirchneriella subcapitata* were assessed. The 72-hour $E_rC_{50}$ was 5.46 mg product/L (95% CL not determined). The 72-hour $E_yC_{50}$was determined to be 4.81 (95% CL: 4.75 – 4.86) mg product/L. The NOEC values for growth

rate and yield were determined to be 4.88 and 1.53 mg product/L, respectively. All validity criteria were met in the study.

### A 2.2.1.4 Study 4: Toxicity to diatom *Navicula pelliculosa*

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 201 and according to the principles of GLP. In the definitive test all the validity criteria were met.<br>The study is considered to be reliable and suitable for the risk assessment. All results refer to nominal concentrations. |

| | |
|---|---|
| Reference: | KCP 10.2.1/04 |
| Report | SAE053H/01: Toxicity to the diatom *Navicula pelliculosa* under laboratory conditions, Falk, S., 2016b, S16-03040 |
| Guideline(s): | Yes, OECD 201 (2011) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication<br>(if vertebrate study) | - |

**Materials and Methods**

**A. MATERIALS**

**1. Test material** SAE053H/01
(Other names: Mesotrione/Nicosulfuron 80/30 OD)

**Description** White to beige liquid/cream, OD (oil dispersion)
**Lot/Batch #** 54606-101
**Purity** Nicosulfuron: 30 g/L nominal; 30.7 g/L analysed
Mesotrione: 80 g/L nominal; 81.7 g/L analysed
Density: 0.98 g/cm³
**Stability of test material** Stable under storage conditions (ambient temperature, 5°C – 30°C, in a dark and dry place)
Expiry date: 20 Mar 2018

**2. Vehicle and/or positive control** Vehicle control: test water
Positive control: 3,5-dichlorophenol is tested as reference item twice a year to confirm the sensitivity of the test organisms. In the most recent test with *Navicula pelliculosa* in July 2016, the 72-hour $E_rC_{50}$ was determined at 2.22 mg/L.

**3. Test organism**

**Species** *Navicula pelliculosa*
**Strain** SAG 1050-3

| | |
|---|---|
| **Source** | MBM Sciencebridge GmbH, Hans-Adolf-Krebs-Weg 1, 37077 Göttingen, Germany |
| **Age** | Diatoms were taken from a semi-continuous liquid stock culture with exponential growth. |
| **Acclimation period** | Three days before test start, algae were held under test conditions in exponential growth stage to produce a pre-culture. |
| **Test units** | 100 mL Erlenmeyer flasks with aluminium caps containing 50 mL of test solution. |

**4. Environmental conditions**

**Test water**

The algae were tested in OECD-medium (according to OECD 201) with the following concentrations:

| | |
|---|---|
| $NaHCO_3$ | 50 mg/L |
| $K_2HPO_4$ | 1.60 mg/L |
| $MgSO_4 \cdot 7H_2O$ | 15.0 mg/L |
| $NH_4Cl$ | 15.0 mg/L |
| $CaCl_2 \cdot 2H_2O$ | 18.0 mg/L |
| $MgCl_2 \cdot 6H_2O$ | 12.0 mg/L |
| $H_3BO_3$ | 0.185 mg/L |
| $MnCl_2 \cdot 4H_2O$ | 0.415 mg/L |
| $ZnCl_2$ | 0.00300 mg/L |
| $CoCl_2 \cdot 6H_2O$ | 0.00150 mg/L |
| $CuCl_2 \cdot 2H_2O$ | 0.00001 mg/L |
| $Na_2MoO_4 \cdot 2H_2O$ | 0.00700 mg/L |
| $FeCl_3 \cdot 6H_2O$ | 0.0640 mg/L |
| $Na_2EDTA \cdot 2H_2O$ | 0.100 mg/L |
| $Na_2SiO_3 \cdot 5H_2O$ | 10.5 mg/L |

The pH was adjusted to 8.1 ± 0.1.

| | |
|---|---|
| **Water temperature** | nominal: 21 – 24 °C; actual: 22.3 – 23.2°C |
| **Lighting** | Continuous illumination from the side; light intensity nominal: 67.7 – 91.7 $\mu Em^{-2}s^{-1}$; actual: 73.2 – 83.8 $\mu Em^{-2}s^{-1}$ |
| **Shaking** | Yes |

**B. STUDY DESIGN AND METHODS**

**1. In-life dates** 04 Jul 2016 to 29 Jul 2016

**2. Experimental conditions**

*Test design*

The diatom *Navicula pelliculosa* was exposed in a static 72-hour test to the test substance at five concentrations and to a control, each test concentration with three replicates and the control with six replicates. The inhibition of algal growth was quantified based on yield and growth rates of the algae.

*Inoculum at test start*

The cell density was adjusted to 1 x $10^4$ cells/mL in all treatments and in the control at the start of the exposure period.

*Test conditions*

The test temperature was maintained at 22.3 – 23.2 °C during the exposure phase. The test systems were continuously illuminated at 73.2 – 83.8 $\mu Em^{-2}s^{-1}$. The pH in the test medium of the control was between 7.95 and 8.07.

*Test concentrations*

Nominal test substance concentrations were 6.25, 12.5, 25.0, 50.0 and 100 mg product/L, corresponding to nominal 0.521, 1.04, 2.08, 4.17 and 8.33 mg a.s./L mesotrione and 0.196, 0.391, 0.783, 1.57 and 3.13 mg a.s./L nicosulfuron based on the analysed content of active substances in the formulation and the product density. In addition, a control group with untreated test medium was tested. The concentrations were chosen based on a non-GLP range finder with test item concentrations of 0.0100, 0.100, 1.0, 10.0 and 100 mg product/L.

*Treatment/Application*

A stock solution was prepared by dissolving 250 mg in test medium. All test item concentrations except the lowest one were prepared by diluting the stock solution with test medium to obtain the required test concentrations. The lowest concentration was prepared by diluting the test solution of 100 mg product/L with test medium.

*Analytics*

The concentrations of mesotrione and nicosulfuron were analysed in the test solutions of all concentration levels and the control at the start (t = 0 h) and end (t = 72 h) of the test by HPLC-MS/MS. Details to the analytical method are summarized in Part B, Section 5.

**3. Sampling and measurements**

The daily fluorescence measurements were performed with a fluorescence microplate reader (infinite 200Pro) with an emission wavelength of 670 nm and evaluated with Tecan i-control (Software for Tecan Readers Tecan i-control, 1.11.1.0). At defined dates (24, 48 and 72 hours), the number of cells in each replicate was determined in duplicate. The determination was performed by fluorescence measurement. By the means of a calibration curve, where fluorescence signals were plotted versus cell numbers, the cell numbers were derived from the fluorescence signals. To establish a calibration curve, the cell numbers were counted with a Neubauer chamber after preparation of a dilution series of a logarithmic growing *Navicula pelliculosa* culture. Additionally, the morphological appearance of the algae cells was observed microscopically at the end of the test.

The pH was measured at the beginning and at the end of the test. The temperature in the test was determined daily. The light intensity was measured at test start.

**4. Calculation of toxicity**

The average specific growth rate for a specific period was calculated as the logarithmic increase of the cell numbers for each single vessel of controls and treatments. The percentage inhibition of growth rates (% $I_\mu$)

was calculated as the difference between the growth rates of the control ($\mu_c$) and the growth rates in the treatment ($\mu_t$).

Yield was calculated as the cell numbers at the end of the test minus the starting cell numbers for each single vessel of controls and treatments. For each test concentration and control, a mean value for yield along with variance estimates was calculated. The percent inhibition in yield (% $I_y$) was calculated for each treatment replicate.

**5. Statistics**

A test for normality of the data was performed by calculating the Shapiro-Wilk's statistic and the homogeneity of variance of the data was evaluated by using the Levene's Test. The NOEC and LOEC were determined by Dunnett's t-test (left-sided). The $EC_{50}$, $EC_{20}$ and $EC_{10}$-values for growth rate and yield were determined by Probit analysis following normal distribution. Only concentrations within a clear dose response were used for calculations. Due to statistical reasons the inhibition-values below zero were set to zero. The statistical evaluation for the 72 hours period was performed for growth rate and yield using SAS ® (2002–2010).

**Results and Discussion**

Analytically measured concentrations of mesotrione and nicosulfuron were determined in the test solution samples from all test concentrations and the control. The measured concentrations of mesotrione in the fresh solutions were between 85 and 114% of nominal. At test end the measured concentrations in the aged solutions were between 80 and 107% of nominal. The measured concentrations of nicosulfuron in the fresh solutions were between 80 and 105% of nominal and between 90 and 105% in the aged solutions (Table A 2.2.1-8). The biological results were based on the nominal test concentrations of the product.

After the exposure period of 72 hours, the test substance had a statistically significant inhibitory effect on the yield and growth rate of the algae at the nominal test concentrations of ≥ 25.0 mg product/L (Table A 2.2.1-9 and Table A 2.2.1-10). The 72-hour NOEC for yield and growth rate was determined to be nominal 12.5 mg product/L. The 72-hour $E_yC_{50}$, $E_yC_{20}$ and $E_yC_{10}$ were determined to be 34.3, 19.7 and 14.7 mg product/L, respectively. The 72-hour $E_rC_{50}$, $E_rC_{20}$ and $E_rC_{10}$ were 64.9, 32.0 and 22.2 mg product/L, respectively. The cells were considered normal for the control and up to and including the test item concentration of 50.0 mg product/L. At a test item concentration of 50.0 mg product/L a reduction of cells was observed. No cells were observed at the test item concentration of 100 mg product/L.

For the reference item 3,5-dichlorophenol, which was tested in a separate study, an $E_rC_{50}$ of 2.22 mg/L was determined.

**Table A 2.2.1-8: Concentrations of mesotrione and nicosulfuron in the test media during the test**

| Nominal test concentration [mg product/L] | Nominal test concentration [mg a.s./L] | Sampling [h] | Measured concentration of active substance [mg a.s./L] | Measured concentration of active substance [% nominal] |
|---|---|---|---|---|
| Control | | 0 | n.d. | - |
| | | 72 | n.d. | - |
| **Active substance: mesotrione** | | | | |
| 6.25 | 0.521 | 0 | 0.592 | 114 |
| | | 72 | 0.555 | 107 |
| 12.5 | 1.04 | 0 | 0.885 | 85 |
| | | 72 | 0.932 | 90 |
| 25.0 | 2.08 | 0 | 1.77 | 85 |
| | | 72 | 1.66 | 80 |
| 50.0 | 4.17 | 0 | 3.53 | 85 |
| | | 72 | 3.55 | 85 |
| 100 | 8.33 | 0 | 7.35 | 88 |
| | | 72 | 7.90 | 95 |
| **Active substance: nicosulfuron** | | | | |
| 6.25 | 0.196 | 0 | 0.205 | 105 |
| | | 72 | 0.205 | 105 |
| 12.5 | 0.391 | 0 | 0.314 | 80 |
| | | 72 | 0.352 | 90 |
| 25.0 | 0.783 | 0 | 0.684 | 87 |
| | | 72 | 0.727 | 93 |
| 50.0 | 1.57 | 0 | 1.54 | 98 |
| | | 72 | 1.46 | 93 |
| 100 | 3.13 | 0 | 2.73 | 87 |
| | | 72 | 2.87 | 92 |

- = not applicable; n.d. = not determined; $LOQ_{mesotrione}$: 0.0250 mg/L; $LOQ_{nicosulfuron}$: 0.00939 mg/L

**Table A 2.2.1-9: Biomass of algae exposed to SAE053H/01 during the exposure period**

| Nominal concentration [mg product/L] | Mean cell number [a] [x $10^4$/mL] 0 hours | 24 hours | 48 hours | 72 hours |
|---|---|---|---|---|
| Control | 1.14 | 3.04 | 7.96 | 25.28 |
| 6.25 | 1.14 | 3.55 | 9.11 | 26.85 |
| 12.5 | 1.14 | 3.42 | 7.06 | 25.17 |
| 25.0 | 1.14 | 2.30 | 5.75 | 14.20 |
| 50.0 | 1.14 | 1.15 | 4.08 | 8.73 |
| 100 | 1.14 | 0.29 | 1.12 | 3.00 |

| 72-hour endpoints | Growth rate (µ) | Yield (y) |
|---|---|---|
| | Nominal concentration [mg product/L] | Nominal concentration [mg product/L] |
| $EC_{50}$ (95% CL) | 64.9 (48.3 – 101) | 34.3 (21.4 – 55.3) |
| $EC_{20}$ (95% CL) | 32.0 (19.9 – 43.1) | 19.7 (7.57 – 29.3) |
| $EC_{10}$ (95% CL) | 22.2 (11.1 – 31.3) | 14.7 (4.01 – 23.1) |
| NOEC | 12.5 | |
| LOEC | 25.0 | |

- = not determined due to mathematical reasons

**Table A 2.2.1-10: Inhibition of average growth rate and yield of algae exposed to SAE053H/01**

| Nominal test concentration [mg product/L] | Percent inhibition of growth rate [a] | | | Percent inhibition of yield [a] | | |
|---|---|---|---|---|---|---|
| | 0 – 24 h | 0 – 48 h | 0 – 72 h | 0 – 24 h | 0 – 48 h | 0 – 72 h |
| Control | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 6.25 | -19.1 | -7.0 | -1.8 [b] | -26.8 | -16.9 | -6.5 [b] |
| 12.5 | -16.1 | 6.1 | 0.3 | -20.0 | 13.2 | 0.5 |
| 25.0 | 27.5 | 16.6 | 19.2* | 38.9 | 32.4 | 45.9* |
| 50.0 | 99.7 | 34.3 | 34.5* | 99.5 | 56.9 | 68.6* |
| 100 | 212.7 | 101.6 | 69.3* | 144.7 | 100.3 | 92.3* |

[a] Mean of three replicates for the treatments and of six replicates in the control; negative values indicate increase compared to the control
[b] Values were set to zero for $EC_x$ calculation
* Statistically significantly different compared to the control

In the control the biomass had increased by a factor of 22.2 after 72 hours (required factor ≥ 16 according to test guideline OECD 201). The mean coefficient of variation of the daily growth rates in the control (section-by-section growth rates) during 72 hours was 31% (required according to test guideline ≤ 35%). The coefficient of variation of the average specific growth rates in the replicates of the control was 5.0% after 72 hours (required according to test guideline ≤ 7%). Thus, the study did fulfil all validity criteria of OECD test guideline 201.

**Conclusion**

The effects of SAE053H/01 on the growth and biomass of the diatom *Navicula pelliculosa* were assessed. The 72-hour $E_rC_{50}$ was 64.9 (95% CL: 48.3 – 101) mg product/L. The 72-hour $E_yC_{50}$was determined to be 34.3 (95% CL: 21.4 – 55.3) mg product/L. The NOEC values for growth rate and yield was determined to be 12.5 mg product/L, respectively. All validity criteria were met in the study.

### A 2.2.1.5 Study 5: Toxicity to the macrophyte *Lemna gibba*

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 221 and according to the principles of GLP. In the definitive test all the validity criteria were met.<br>The study is considered to be reliable and suitable for the risk assessment. The justification of deviation with measured concentrations of active substances was accepted. All results refer to nominal concentrations. |

| | |
|---|---|
| Reference: | KCP 10.2.1/05 |
| Report | SAE053H/01: Toxicity to the duckweed *Lemna gibba* under laboratory conditions (acute test – semi-static), Lang née Zawadsky, C., 2016b, S16-03044 |
| Guideline(s): | Yes, OECD 221 (2006) |
| Deviations: | Yes, the measured concentrations for nicosulfuron were outside the range of 80 – 120% of nominal. However, for mesotrione the values were within the range and the product has a defined ratio of both active substances. Furthermore, effects were present at concentrations above 25.0 µg product/L, only. For these nominal concentrations, the measured concentrations deviated only slightly from the required range. It is therefore not expected that the slight deviation will impact the validity and integrity of the study. |

| | |
|---|---|
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | - |

**Materials and Methods**

**A. MATERIALS**

**1. Test material**

SAE053H/01
(Other names: Mesotrione/Nicosulfuron 80/30 OD)

| | |
|---|---|
| **Description** | White to beige liquid/cream, OD (oil dispersion) |
| **Lot/Batch #** | 54606-101 |
| **Purity** | Nicosulfuron: 30 g/L nominal; 30.7 g/L analysed<br>Mesotrione: 80 g/L nominal; 81.7 g/L analysed<br>Density: 0.98 g/cm³ |
| **Stability of test material** | Stable under storage conditions (ambient temperature, 5°C – 30°C, in a dark and dry place)<br>Expiry date: 20 Mar 2018 |

**2. Vehicle and/or positive control**

Vehicle control: test water
Positive control: 3,5-dichlorophenol is tested as reference item twice a year to confirm the sensitivity of the test organisms. In the most recent test with *Lemna gibba* in March 2016, the 72-hour $E_rC_{50}$ were determined at 7.17 mg/L (based on frond numbers) and 7.24 mg/L (based on dry weight).

**3. Test organism**

| | |
|---|---|
| **Species** | Duckweed *Lemna gibba* (Alismatales: Araceae) |
| **Strain** | G3 |
| **Source** | Cultured at the test site (original source: Dr. Janet Slovin, Horticulture Crops Quality Laboratory, U.S. Department of Agriculture. BARC-West, Bldg. 050 HH-4, Beltsville, MD 20705, U.S.A.) |
| **Age** | Young, light-green plants of similar size and comprising 2-4 fronds were transferred onto fresh medium and cultured for 7-10 days prior to testing, with two further transfers onto fresh medium before initiating the test. |
| **Acclimation period** | As described above. |
| **Test units** | 250-mL glass beakers containing 150 mL of test medium |

**4. Environmental conditions**

**Test water**

The plants were cultivated and tested in reconstituted test water (20x AAP medium) with the following nominal concentrations:

Macro-nutrients

| | |
|---|---|
| $NaHCO_3$ | 300.0 mg/L |
| $K_2HPO_4$ | 22.9 mg/L |

| | |
|---|---|
| $MgSO_4 \times 7\ H_2O$ | 290 mg/L |
| $NaNO_3$ | 510 mg/L |
| $MgCl_2 \times 6\ H_2O$ | 240 mg/L |
| $CaCl_2 \times 2\ H_2O$ | 90 mg/L |

Trace elements

| | |
|---|---|
| $H_3BO_3$ | 3.7 mg/L |
| $MnCl_2 \times 4\ H_2O$ | 8.3 mg/L |
| $ZnCl_2$ | 0.066 mg/L |
| $CoCl_2 \times 6\ H_2O$ | 0.029 mg/L |
| $CuCl_2 \times 2\ H_2O$ | $2.4 \times 10^{-4}$ mg/L |
| $Na_2MoO_4 \times 2\ H_2O$ | 0.145 mg/L |
| $FeCl_3 \times 6\ H_2O$ | 3.2 mg/L |
| $Na_2EDTA \times 2\ H_2O$ | 6.0 mg/L |

The pH was adjusted to 7.5 ± 0.1.

**Water temperature** Nominal: 24 ± 2 °C; actual: 24.2 – 24.5°C

**Lighting** Continuous illumination at an average light intensity of 7900 Lux using Light tubes: Econlux SolarStringer SunStrip LEDs (daylight, 10 W – 500 mm)

**Shaking** None

## B. STUDY DESIGN AND METHODS

**1. In life dates** 08 Jul 2016 to 29 Aug 2016

**2. Experimental conditions**

*Test design*

The freshwater aquatic macrophyte *Lemna gibba* was exposed in a semi-static 7-day test to SAE053H/01 at five concentrations each with three replicates and a test water control with six replicates. The recorded effect was inhibition of plant growth (yield and growth rate) based on frond numbers and dry weight.

*Inoculum at test start*

Young, light-green plants of similar size and comprising 2 - 4 fronds were transferred onto fresh medium and cultured for 7 – 10 days prior to testing, with two further transfers onto fresh medium before initiating the test.

Colonies consisting of 2-4 fronds were transferred from the inoculum culture into the test vessels containing a total of 12 fronds, each. The size of plants and fronds were nearly identical in each test vessel.

*Test conditions*

The water temperature was maintained at 24.2 – 24.5°C and the test systems were continuously illuminated at approximately 7900 Lux. The pH of the fresh solutions was 7.40 – 7.80 and of the aged test solutions 8.59 – 9.31.

*Concentrations tested*

SAE053H/01 was tested at nominally 6.25, 12.5, 25.0, 50.0 and 100 µg product/L, corresponding to nominal 0.521, 1.04, 2.08, 4.17 and 8.33 µg a.s./L mesotrione and 0.196, 0.301, 0.783, 1.57 and 3.13 µg a.s./L nicosulfuron based on the analysed content of active substances in the formulation and the product density. In addition, a control group with untreated test medium was tested. The concentrations were chosen based on a non-GLP range-finding test.

*Treatment/Application*

The necessary amount of test item for preparing the stock solution (10.0 mg/L) was weighed and transferred to a volumetric flask. Deionised water was added up to the bench mark and the solution was homogenised by shaking. The stock solution was slightly turbid. Test solutions were prepared by serial dilution of the stock solution. The preparation procedure of the test solutions was the same at day 0, 3 and 5.

*Analytics*

Samples were taken at t = 0 d fresh, t = 3 d aged, t = 3 d fresh, t = 5 d aged and t = 7 d aged from all tested concentrations and control. The samples were analysed using HPLC-MS/MS. Details to the analytical method are summarized in Part B, Section 5.

**3. Sampling and measurements**

Frond numbers in each test vessel were determined at the start of the test. Frond numbers and the appearance of the colonies were checked on t = 0, 3, 5 and 7 days as well as any change in plant development, frond size, necrosis and additional observations of test media or other abnormalities.

The dry weight of the fronds was determined at the end of the test after drying at 60°C for about 72 hours. A representative batch of six times 12 fronds from the culture used for the test was dried to determine the dry weight for the test start.

The test temperature was measured continuously in a surrogate vessel held under the same conditions as the test vessels and recorded after 0, 3, 5 and 7 days. The pH-value of the test solutions were measured in the control and each test concentration in one replicate of test solution on t = 0, 3, 5 and 7 days (fresh and aged solutions). Light intensity was measured at test start.

**4. Calculation of toxicity**

Means of frond number for each test concentration at each observation time were calculated. Means of dry weight for each test concentration at day 7 were calculated. The specific growth rate ($\mu$) for frond number and dry weight was calculated as the logarithmic increase for each replicate for control and treatments. The percentage inhibition of growth rate (% $I_R$) was calculated as the difference between the mean growth rate of the control ($\mu_C$) and the mean growth rate in the treatments ($\mu_T$).
The mean doubling times ($T_d$) of the culture for the control and treatments were calculated.

Effects on yield were determined on the basis of total frond numbers and dry weight in each vessel at the start and end of the test. For the test concentrations, a mean value for yield was calculated. The mean percent inhibition in yield was calculated for each treatment group.

**5. Statistics**

The statistical evaluation for day 7 was performed for yield of frond numbers, growth rate of frond numbers, growth rate of dry weight and yield of dry weight. Normality of the data was tested using Shapiro-Wilk's test, homogeneity of the data was checked using Levene's test. The NOEC and LOEC were determined by using a multiple comparison method (Dunnett's t-test, left-sided for yield and growth rate of frond numbers and for growth rate of dry weight, Bonferroni-Holms corrected Welch test, left-sided for yield of dry weight).

The $EC_{10}$, $EC_{20}$, $EC_{50}$ values were determined by Probit analysis following normal distribution. Negative values of inhibition were set to zero due to statistical reasons. The evaluation of data was performed by SAS® (2002-2010).

**Results and Discussion**

The concentrations of mesotrione and nicosulfuron were measured at all concentration levels and the control at test start (t = 0), after 3 days (aged and fresh solution), after 5 days (fresh solution) and after 7 days (aged solution). Measured concentrations of mesotrione in the fresh test medium were between 89 and 155% of nominal, the mean initial concentration was 102% of nominal. In the aged solutions, the measured concentrations were between 86 and 105% of nominal with a mean measured concentration of 96% of nominal. The fresh concentration of mesotrione in the t = 0d samples of the nominal concentration of 6.25 µg product/L was the only initial value out of the range of 80-120% of nominal. Therefore the correct application of the test item is proven. Measured concentrations of nicosulfuron in the fresh test medium were between 92 and 165% of nominal, the mean initial concentration was 122% of nominal. In the aged solutions, the measured concentrations were between 105 and 133% of nominal with a mean measured concentration of 121% of nominal (Table A 2.2.1-11). All toxicological endpoints were evaluated using nominal concentrations of the test item.

The mean frond numbers and dry weights are presented in table A 2.2.1-12, the effects of SAE053H/01 on the growth rates and yield of *Lemna gibba* during the 7-day test are presented in Table 2.2.1-13. After 7 days of exposure, significant inhibitory effects were determined for yield and growth rate of frond numbers and dry weight at 25.0, 50.0 and 100 µg product/L. The overall LOEC was therefore determined to be 25.0 µg product/L, the corresponding NOEC at 12.5 µg product/L. The $E_rC_{50}$ were determined as 58.0 µg product/L for frond numbers and > 100 µg product/L for dry weight. The $E_yC_{50}$ values were 30.0 µg product/L for frond numbers and 31.3 µg product/L for dry weight. Further toxicity values are presented in Table 2.2.1-14.

On day 3, partly single fronds and shortened roots were observed at 25.0 µg product/L and above.
On day 5 shortened and less roots were observed at concentrations of 12.5 to 100 µg product/L. Additionally, in the concentrations of 25.0 to 100 µg product/L deformed fronds, gibbosity and small offshoots were observed. At 25.0 and 50.0 µg product/L partially single fronds and patchy fronds were noted.
On day 7 shortened and less roots and partly small offshoots were observed at concentrations of 12.5 to 100 µg product/L. Additionally, at concentrations of 25.0 to 100 µg product/L single and deformed fronds were found and gibbosity was observed. In the concentrations of 50.0 and 100 µg product/L tightly assembled fronds were noted. At 50.0 µg product/L fronds were yellow. At 100 µg product/L fronds were partly patchy.

**Table A 2.2.1-11: Measured concentrations of mesotrione and nicosulfuron in the test media**

| Nominal concentration | | Measured concentration of active substance | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 days fresh | | 3 days aged | | 3 days fresh | | 5 days fresh | | 7 days aged | |
| [µg prod./L] | [µg a.s./L] | [µg/L] | [%] nom | [µg/L] | [%] nom | [µg/L] | [%] nom | [µg/L] | [%] nom | [µg/L] | [%] nom |
| Control | | n.d. | - | n.d. | - | n.d. | - | n.d. | - | n.d. | - |
| **Active substance: mesotrione** | | | | | | | | | | | |
| 6.25 | 0.521 | 0.807 | 155 | 0.468 | 90 | 0.466 | 89 | 0.556 | 107 | 0.546 | 105 |
| 12.5 | 1.04 | 1.21 | 116 | 0.893 | 86 | 0.932 | 90 | 1.06 | 102 | 1.08 | 104 |
| 25.0 | 2.08 | 2.22 | 107 | 1.97 | 95 | 1.99 | 96 | 2.13 | 102 | 2.09 | 100 |
| 50.0 | 4.17 | 3.92 | 94 | 3.67 | 88 | 4.02 | 96 | 4.06 | 97 | 4.13 | 99 |
| 100 | 8.33 | 7.64 | 92 | 7.49 | 90 | 7.61 | 91 | 8.31 | 100 | 8.15 | 98 |
| **Active substance: nicosulfuron** | | | | | | | | | | | |
| 6.25 | 0.196 | 0.324 | 165 | 0.222 | 113 | 0.180 | 92 | 0.257 | 131 | 0.261 | 133 |
| 12.5 | 0.391 | 0.511 | 131 | 0.411 | 105 | 0.363 | 93 | 0.480 | 123 | 0.480 | 123 |
| 25.0 | 0.783 | 1.08 | 138 | 0.948 | 121 | 0.825 | 105 | 1.00 | 128 | 0.993 | 127 |
| 50.0 | 1.57 | 1.93 | 123 | 1.76 | 112 | 1.99 | 127 | 2.05 | 131 | 2.05 | 131 |
| 100 | 3.13 | 3.70 | 118 | 3.76 | 120 | 3.44 | 110 | 3.79 | 121 | 3.90 | 125 |

LOQ (mesotrione) = 0.250 µg/L, LOQ (nicosulfuron) = 0.0939 µg/L
- = not calculated; n.d. = not detectable

**Table A 2.2.1-12: Mean frond number and dry weights of *Lemna gibba* during the 7-day test**

| Nominal concentration | Mean of frond numbers | | | | | Mean dry weight [g] | | |
|---|---|---|---|---|---|---|---|---|
| [µg product/L] | 0 d | 3 d | 5 d | 7 d | 7 d – 0 d | 0 d | 7 d | 7 d – 0 d |
| Control | 12 | 35 | 100 | 297 | 285 | 0.0025 | 0.0480 | 0.0455 |
| 6.25 | 12 | 35 | 201 | 299 | 287 | | 0.0498 | 0.0473 |
| 12.5 | 12 | 36 | 97 | 293 | 281 | | 0.0462 | 0.0737 |
| 25.0 | 12 | 27 | 67 | 152 | 140* | | 0.0188 | 0.0163* |
| 50.0 | 12 | 24 | 42 | 68 | 56* | | 0.0138 | 0.0113* |
| 100 | 12 | 17 | 25 | 31 | 19* | | 0.0113 | 0.0088* |

* Statistically significantly lower than in the control (according to Dunnett's t-test or Bonferroni corrected Welch test)

**Table A 2.2.1-13: Effects of SAE053H/01 on growth of *Lemna gibba* during the 7-day test**

| Nominal concentrations | Based on frond number | | | | Based on dry weight | |
|---|---|---|---|---|---|---|
| | Yield inhibition [%] | Mean growth rates µ [$d^{-1}$] / Inhibition of growth rates [%] | | | Yield inhibition [%] | Mean growth rates µ [$d^{-1}$] / Inhibition of growth rates [%] |
| [µg prod./L] | 7d – 0d | 3d | 5d | 7d | 7d – 0 d | 7d – 0 d |
| Control | - | 0.3550 / - | 0.4236 / - | 0.4579 / - | - | 0.4215 / - |
| 6.25 | -0.7 [a)] | 0.3535 / 0.4 | 0.4279 / -1.0 | 0.4591 / -0.3 [a)] | -4.0 [a)] | 0.4271 / -1.3 [a)] |
| 12.5 | 1.4 | 0.3631 / -2.3 | 0.4183 / 1.3 | 0.4562 / 0.4 | 4.0 | 0.4166 / 1.2 |
| 25.0 | 50.9 | 0.2651 / 25.3 | 0.3441 / 18.8 | 0.3626* / 20.8 | 64.2 | 0.2878* / 31.7 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 50.0 | 80.4 | 0.2284 / 35.7 | 0.2496 / 41.1 | 0.2456* / 46.4 | 75.2 | 0.2442* / 42.1 |
| 100 | 93.3 | 0.1225 / 65.5 | 0.1435 / 66.1 | 0.1369* / 70.1 | 80.7 | 0.2144* / 49.1 |

A negative value indicates increase in growth relative to the control
* Statistically significantly lower than in the control (according to Dunnett's t-test or Bonferroni corrected Welch test)

**Table A 2.2.1-14: Toxicity of SAE053H/01 for *Lemna gibba* after 7 days of exposure**

| 7-day endpoints [µg product/L] | | | | |
|---|---|---|---|---|
| | Based on frond numbers | | Based on dry weight | |
| | Growth rate (r) | Yield (y) | Growth rate (r) | Yield (y) |
| $EC_{50}$ (95% CL) | 58.0 (43.5 – 86.1) | 30.3 (18.1 – 49.0) | > 100 (-) | 31.3 (0.681 – 1706) |
| $EC_{20}$ (95% CL) | 28.8 (17.8 – 38.6) | 18.0 (6.43 – 27.0) | 27.1 (0.00277 – 68.4) | 15.1 ($6.46*10^{-15}$ – 33.2) |
| $EC_{10}$ (95% CL) | 19.9 (10.1 – 28.2) | 13.7 (3.43 – 21.5) | 15.8 ($1.84*10^{-8}$ – 34.5) | 10.3 ($5.40*10^{-23}$ – 23.7) |
| NOEC | 25.0 | | | |
| LOEC | 12.5 | | | |

The doubling time ($T_d$) of frond number in the control was calculated to be 1.514 days ($T_d$ = ln2 / r), hence, clearly fulfilling the validity criterion given in the guideline ($T_d$ < 2.5 d) and indicating satisfactory growth of *Lemna* under test conditions.

**Conclusion**

The $E_rC_{50}$ values of SAE053H/01 for the freshwater aquatic macrophyte *Lemna gibba* were determined to be 58.0 (95% CL: 43.5 – 86.1) µg product/L for frond numbers and > 100 µg product/L (no CL calculable) for dry weight. The $E_yC_{50}$ were determined to be 30.3 (95% CL: 18.1 – 49.0) µg product/L for frond numbers and 31.3 (95% CL: 0.681 – 1706) µg product/L for dry weight. The validity criterion was fulfilled.

### A 2.2.1.6 Study 6: Toxicity to the macrophyte *Myriophyllum spicatum*

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 239 and according to the principles of GLP. In the definitive test all the validity criteria were met.<br>The study is considered to be reliable and suitable for the risk assessment. All results refer to nominal concentrations. |

| | |
|---|---|
| Reference: | KCP 10.2.1/06 |
| Report | SAE053H/01: Growth inhibition of *Myriophyllum spicatum* in a water/sediment system, Gonsior, G., 2016, S16-03045 |
| Guideline(s): | OECD 239 (2014) |
| Deviations: | In deviation to the guideline recommendation which only evaluates the shoot biomass, the total plant biomass comprising roots and shoots was assessed to avoid underestimation of effects on rooted aquatic macrophytes, especially for test items which may affect root development. |
| GLP: | Yes |
| Acceptability: | Yes |

| | |
|---|---|
| Duplication<br>(if vertebrate study) | - |

## Materials and Methods

### A. MATERIALS

**1. Test material**

SAE053H/01
(Other names: Mesotrione/Nicosulfuron 80/30 OD)

| | |
|---|---|
| **Description** | White to beige liquid/cream, OD (oil dispersion) |
| **Lot/Batch #** | 54606-101 |
| **Purity** | Nicosulfuron: 30 g/L nominal; 30.7 g/L analysed<br>Mesotrione: 80 g/L nominal; 81.7 g/L analysed<br>Density: 0.98 g/cm³ |
| **Stability of test material** | Stable under storage conditions (ambient temperature, 5°C – 30°C, in a dark and dry place)<br>Expiry date: 20 Mar 2018 |

**2. Vehicle and/or positive control**

Vehicle control: test water
Reference item: not reported

**3. Test organism**

| | |
|---|---|
| **Species** | *Myriophyllum spicatum* L. (Haloragaceae) |
| **Strain** | Not stated |
| **Source** | Cultured at the test facility; originally obtained from Federal Environment Agency Berlin, Germany based on a culture of Landesanstalt für Gewässerkunde Koblenz, Germany |
| **Age** | Nine days prior to test initiation, submerged apical shoots of the same size (5 cm in length and without side shoots) were planted in a tub of stainless steel. |
| **Acclimation period** | Culturing takes place under the same environmental conditions as used in the test. |
| **Test units** | 2 L glass-beakers measuring approx. 12 cm in diameter and 24 cm height. Only one shoot per test vessel was planted. The volume of added water was recorded, and the level marked on the outside of the test vessels. |

**4. Environmental conditions**

**Test water**

The plants were cultivated in modified Andrews solution and tested in Smart and Barko medium. The test medium contained the following components:

| | |
|---|---|
| $CaCl_2 \times 2\ H_2O$ | 91.7 mg/L |
| $MgSO_4 \times 7\ H_2O$ | 69.0 mg/L |
| $NaHCO_3$ | 58.4 mg/L |
| $KHCO_3$ | 15.4 mg/L |

**Sediment**

The test was performed in the presence of artificial sediment with the following composition:

| | |
|---|---|
| Sphagnum peat | 4-5% |
| Kaolin clay | 20 ± 1% |

| | |
|---|---|
| | Quartz sand 75-76%<br>Calcium carbonate was added to adjust the pH to 7.0 ± 0.5. The organic carbon content of the final mixture was 2 ± 0.5%. |
| **Water temperature** | Nominal: 20 ± 2°C, actual: 18.1 – 21.3°C |
| **Lighting** | 16-hour light (light intensity: nominal 140 ± 20 $\mu Em^{-2}s^{-1}$, actual 120 - 160 $\mu Em^{-2}s^{-1}$ at the water surface) to 8-hour dark photoperiod |
| **Shaking** | None |

## B. STUDY DESIGN AND METHODS

**1. In-life dates** 20 Jul 2016 to 31 Aug 2016

**2. Experimental conditions**

*Test design*

The freshwater aquatic macrophyte *Myriophyllum spicatum* was exposed in a static water/sediment system for 14 days to the test substance at five concentrations each with five replicates and ten replicates of a test water control. The recorded effect was inhibition of plant growth based on shoot length, plant fresh and dry weight. In addition, the number and length of side shoots was assessed.

*Test conditions*

During the whole test, the light intensity was in the range of 120 – 160 $\mu Em^{-2}s^{-1}$ and the test temperature was maintained at 18.1 – 21.3°C. The pH was 7.42 – 9.49 during the exposure phase. Oxygen saturation was 87 – 156% during the test.

*Test concentrations*

Nominal test substance concentrations were 0.00954, 0.0305, 0.0977, 0.313 and 1.00 mg product/L, corresponding to nominal 0.000795, 0.00254, 0.00814, 0.0261 and 0.0833 mg a.s./L mesotrione and 0.000299, 0.000955, 0.00306, 0.00980 and 0.0313 mg a.s./L nicosulfuron based on the analysed content of active substances in the formulation and the product density. In addition, a control group with untreated test medium was tested. The concentrations were chosen based on a non-GLP range-finding test.

*Treatment/Application*

Approximately 350 g of moist sediment was transferred to the test vessels. The surface was overlaid with moist sediment without ammonium chloride and sodium phosphate and a thin layer of washed quartz sand to minimise displacement of the sediment when the medium was added. Afterwards the test vessels were filled carefully with growth medium (1.5 L) to a depth of 14 cm. One day after preparation of the test vessels and before application, one rooted apical shoot per vessel was planted carefully, ensuring the plant was rooted into the sediment. Shortly afterwards, application of the test item was performed and mixed in with gentle stirring.
For preparation of the test item solutions, a stock solution was prepared by dispersing 150 mg test item in test medium. The solution was shaken until the oily film disappeared; however, the substance was still not homogeneously distributed. Furthermore, the stock solution was turbid and foam formation was observed. The test solutions were prepared by serial dilution of the stock solution with test medium. The test item solutions of all concentrations except for the lowest were still turbid.

*Analytics*

The contents of mesotrione and nicosulfuron were measured in the overlaying water at test start and test end in all test item concentrations and the control. The sediment samples were analysed at all concentrations and the control at test end. The porewater samples were measured at the highest test concentration at test end. Details to the analytical method are summarized in Part B, Section 5.

**3. Sampling and measurements**

Assessments of plant growth were made on days 0, 7 and 14 during the test. On day 0 fifteen additional plants, representative of those used in the test, were selected from the available plant material. The plants were blotted dry prior to assessment of plant fresh weight and shoot length. The plants were placed separately in labelled glass beakers and dried at 60°C for > 48 hours. The weight of the dry plant samples was recorded.

On day 14 plants were harvested from each treatment group for assessment of biomass (plant fresh weight and plant dry weight), shoot length and number and length of side shoots. In addition observations on shoot and root development (e.g. necrosis, deformation) were documented.

Water temperature, pH and dissolved oxygen content were recorded on days 0, 7 and 14. Light intensity on the water surface was measured at test start.

**4. Calculation of toxicity**

Specific growth rate and % inhibition for specific growth rate was calculated. Additionally, mean values for yield and the mean percent inhibition in yield were calculated for each treatment group. The mean doubling times of the culture for the control and treatment was calculated.

**5. Statistics**

For NOEC/LOEC determination data were subjected to ANOVA. A test for normality of the data was carried out by calculating the Shapiro-Wilk's statistic. For homogeneity of variances across treatment groups a Bartlett's or Levene's test was performed. As data were normally distributed and variance was homogeneous a Dunnett's t-test was performed to determine significant differences from controls (SAS® Proprietary Software 9.3).
The $EC_x$ values were calculated using Probit analysis. Values below zero were set to zero and only concentration within a clear dose response were used for calculations.

**Results and Discussion**

The concentrations of mesotrione and nicosulfuron were measured in the overlaying water at all concentration levels and the control at test start and end. Measured concentrations of mesotrione in the test medium were between 93 and 113% of nominal at test start, the mean measured content for all concentrations was 102% of nominal. At test end, the concentrations were between 89 and 108% of nominal and the mean measured concentration was 98%. In the sediment, concentrations of mesotrione were 8% of the applied amount and could only be quantified in the three highest concentration levels. In the porewater less than 1% of the applied amount was found in the highest concentration level. For nicosulfuron the concentration in the overlaying water at test start was between 89 and 105% of nominal and the mean measured concentration was 98% of nominal. At test end the concentrations were between 86 and 98% of nominal while the mean measured concentration was 92% of nominal. In the sediment, 10 to 11% of the applied amount was found but could only be quantified in the two highest concentration levels. In the

porewater, less than 1% of the applied amount was found in the highest concentration level (Table A 2.2.1-15).
As the concentrations of mesotrione and nicosulfuron were between 80 and 120% of nominal at test start, the toxicological endpoints were related to nominal concentrations of the test item.

The effects of SAE053H/01 on the growth of *Myriophyllum spicatum* during the exposure phase are presented in Table A 2.2.1-16. After 14 days of exposure, the test substance had a statistically significantly inhibitory effect on plant growth based on the parameters shoot length, plant fresh and dry weight (calculated as growth rate and yield for all three parameters) at concentrations of 0.0977 mg product/L and above. The overall 14-day NOEC and LOEC were therefore determined to be 0.0305 and 0.0977 mg product/L, respectively. The lowest 14-day $EC_{50}$ values were calculated to be 0.179 mg product/L for yield (based on dry weight) and 0.334 mg product/L for growth rate (based on dry weight). Further toxicity values are presented in Table A 2.2.1-16.

For the test concentrations ≥ 0.0977 mg product/L a reduced length of shoot was observed on day 14; at the highest concentration additionally light reddish apicals and side shoots were observed on day 7. Furthermore only moderate root development was observed at the two highest concentrations.

**Table A 2.2.1-15: Concentrations of mesotrione and nicosulfuron in the test media (overlying water, sediment and porewater) during the exposure phase**

| Nominal test concentration | | Sampling | Measured concentration of active substance in | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | overlaying water | | sediment | | porewater | |
| [mg prod./L] | [mg a.s./L] | [d] | [mg a.s./L] | [% nominal] | [mg a.s./kg] | [% applied] | [mg a.s./L] | [% applied] |
| Control | | 0 | n.d. | - | n.d. | - | - | - |
| | | 14 | n.d. | - | n.d. | - | - | - |
| **Active substance: mesotrione** | | | | | | | | |
| 0.00954 | 0.000795 | 0 | 0.000737 | 93 | - | - | - | - |
| | | 14 | 0.000858 | 108 | n.d. | - | - | - |
| 0.0305 | 0.00254 | 0 | 0.00263 | 104 | - | - | - | - |
| | | 14 | 0.00257 | 101 | < LOQ | - | - | - |
| 0.0977 | 0.00814 | 0 | 0.00844 | 104 | - | - | - | - |
| | | 14 | 0.00749 | 92 | 0.00165 | 8 | - | - |
| 0.313 | 0.0261 | 0 | 0.0257 | 98 | - | - | - | - |
| | | 14 | 0.0232 | 89 | 0.00504 | 8 | - | - |
| 1.00 | 0.0833 | 0 | 0.0944 | 113 | - | - | - | - |
| | | 14 | 0.0813 | 98 | 0.0179 | 8 | 0.0373 | < 1 |
| Mean concentration in overlaying water at 0 d [%] | | | 102 | | | | | |
| **Active substance: nicosulfuron** | | | | | | | | |
| 0.00954 | 0.000299 | 0 | 0.000267 | 89 | - | - | - | - |
| | | 14 | 0.000293 | 98 | n.d. | - | - | - |
| 0.0305 | 0.000955 | 0 | 0.000956 | 100 | - | - | - | - |
| | | 14 | 0.000918 | 96 | < LOQ | - | - | - |
| 0.0977 | 0.00306 | 0 | 0.00298 | 97 | - | - | - | - |
| | | 14 | 0.00275 | 90 | < LOQ | - | - | - |
| 0.313 | 0.00980 | 0 | 0.00969 | 99 | - | - | - | - |
| | | 14 | 0.00842 | 86 | 0.00236 | 10 | - | - |
| 1.00 | 0.0313 | 0 | 0.0328 | 105 | - | - | - | - |
| | | 14 | 0.0282 | 90 | 0.00871 | 11 | 0.0118 | < 1 |
| Mean concentration in overlaying water at 0 d [%] | | | 98 | | | | | |

n.d. not detectable; - not determined
LOQ in water: LOQ (mesotrione) = 0.000250 mg/L; LOQ (nicosulfuron) = 0.0000939 mg/L
LOQ in sediment: 0.00100 mg/kg for mesotrione and nicosulfuron

**Table A 2.2.1-16: Effects of SAE053H/01 on growth of *Myriophyllum spicatum***

| Nominal concentration [mg product/L] | Inhibition of total shoot length [%] | | fresh weight [%] | | dry weight [%] | |
|---|---|---|---|---|---|---|
| | Growth rate | Yield | Growth rate | Yield | Growth rate | Yield |
| 0.00954 | 9.6 | 19.4 | 2.6 | 5.3 | -2.7 | -5.1 |
| 0.0305 | 1.0 | 1.8 | 4.4 | 9.0 | 8.7 | 14.1 |
| 0.0977 | 23.3* | 41.5* | 22.5* | 39.4* | 32.7* | 46.4* |
| 0.313 | 41.5* | 63.8* | 42.8* | 64.0* | 57.3* | 70.7* |
| 1.00 | 53.1* | 74.1* | 46.2* | 66.8* | 62.7* | 74.3* |

* Inhibition statistically significant (according to Dunnett's t-test)

**Table A 2.2.1-17: Toxicity endpoints of SAE053H/01 for effects on *Myriophyllum spicatum***

| 14-day endpoints [mg product/L] | | | | | | |
|---|---|---|---|---|---|---|
| | Based on shoot length | | Based on fresh weight | | Based on dry weight | |
| | Growth rate (r) | Yield (y) | Growth rate (r) | Yield (y) | Growth rate (r) | Yield (y) |
| $EC_{50}$ (95% CL) | 0.634 (0.466 – 0.954) | 0.232 (0.186 – 0.293) | > 1.00 | 0.248 (0.191 – 0.333) | 0.334 (0.259 – 0.448) | 0.179 (0.144 – 0.227) |
| $EC_{20}$ (95% CL) | 0.122 (0.0831 – 0.164) | 0.0598 (0.0410 – 0.0797) | 0.110 (0.0758 – 0.152) | 0.0440 (0.0301 – 0.0596) | 0.0681 (0.0485 – 0.0896) | 0.0419 (0.0300 – 0.0549) |
| $EC_{10}$ (95% CL) | 0.0518 (0.0287 – 0.0771) | 0.0294 (0.0175 – 0.0427) | 0.0390 (0.0220 – 0.0587) | 0.0178 (0.0105 – 0.0265) | 0.0296 (0.0185 – 0.0423) | 0.0196 (0.0125 – 0.0276) |
| NOEC | 0.0305 | | | | | |
| LOEC | 0.0977 | | | | | |

The study fulfils the validity criteria of OECD 238, since the CV for yield of fresh weight and shoot length was below 35% and a doubling of shoot biomass and length was reached within the test duration. The mean control growth rates and variability were considered acceptable.

**Conclusion**

The lowest 14-day $E_yC_{50}$ and $E_rC_{50}$ values of SAE053H/01 for the freshwater aquatic macrophyte *Myriophyllum spicatum* were determined to be 0.179 (95% CL: 0.144 – 0.227) and 0.334 (95% CL: 0.259 – 0.448) mg product/L (both based on dry weight), respectively. The NOEC of the study was 0.0305 mg product/L. The study did fulfil the validity criteria.

### A 2.2.1.7 Study 7: Toxicity to the macrophyte *Lemna gibba* – mesotrione

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 221 and according to the principles of GLP. In the definitive test all the validity criteria were met.<br>The study is considered to be reliable and suitable for the risk assessment. All results refer to nominal concentrations. |

| | |
|---|---|
| Reference: | KCP 10.2.1/07 |
| Report | Mesotrione technical: Toxicity to the duckweed *Lemna gibba* under laboratory conditions (acute test – semi-static), Bertrand, C., 2019, S19-03470 |
| Guideline(s): | OECD 221 (2006) |
| Deviations: | The pH was not adjusted at day 2 by mistake and therefore pH variation in the controls was above 1.5 units. However, as the validity criteria were met, the study is still considered valid and acceptable in accordance with OECD 221 (2006). |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication<br>(if vertebrate study) | - |

**Materials and Methods**

**A. MATERIALS**

| | |
|---|---|
| **1. Test material** | Mesotrione technical |
| **Description** | Pale yellow solid |
| **Lot/Batch #** | 20130715 |
| **Purity** | 99.07 % w/w analysed<br>Density: not applicable |
| **Stability of test material** | Stable under storage conditions (ambient ≤ 30°C, dark, dry)<br>Expiry date: 31 Jan 2020 |
| **2. Vehicle and/or positive control** | Vehicle control: Test medium<br>Vehicle solvent control: Test medium with 0.01 % (v/v) DMF<br>Positive control: 3,5-dichlorophenol is tested as reference item twice a year to confirm the sensitivity of the test organisms. In the most recent test with *Lemna gibba* in August 2019, the 72-hour $E_rC_{50}$ values were determined at 9.79 mg/L (based on frond numbers) and 8.14 mg/L (based on dry weight). |
| **3. Test organism** | |
| **Species** | Duckweed *Lemna gibba* (Alismatales: Araceae) |
| **Strain** | G3 |
| **Source** | Cultured at the test site (original source: Dr. Janet Slovin, Horticulture Crops Quality Laboratory, U.S. Department of Agriculture. BARC-West, Bldg. 050 HH-4, Beltsville, MD 20705, U.S.A.) |

**Age** Young, light-green plants of similar size and comprising 2-4 fronds were transferred onto fresh medium and cultured for 7-10 days prior to testing, with two further transfers onto fresh medium before initiating the test.

**Acclimation period** Culturing was done under conditions similar to the test, i.e. 24 ± 2°C and 6500 – 10000 Lux.

**Test units** 250-mL glass beakers, covered with a glass plate, containing 150 mL of test medium

**4. Environmental conditions**

**Test water** The plants were cultivated and tested in reconstituted test water (20x AAP medium) with the following nominal concentrations:

| Macro-nutrients | | Trace elements | |
|---|---|---|---|
| $NaHCO_3$ | 300 mg/L | $H_3BO_3$ | 3.7 mg/L |
| $K_2HPO_4$ | 22.9 mg/L | $MnCl_2 \times 4\ H_2O$ | 8.3 mg/L |
| $MgSO_4 \times 7\ H_2O$ | 290 mg/L | $ZnCl_2$ | 0.066 mg/L |
| $NaNO_3$ | 510 mg/L | $CoCl_2 \times 6\ H_2O$ | 0.029 mg/L |
| $MgCl_2 \times 6\ H_2O$ | 240 mg/L | $CuCl_2 \times 2\ H_2O$ | $2.4 \times 10^{-4}$ mg/L |
| $CaCl_2 \times 2\ H_2O$ | 90 mg/L | $Na_2MoO_4 \times 2\ H_2O$ | 0.145 mg/L |
| | | $FeCl_3 \times 6\ H_2O$ | 3.2 mg/L |
| | | $Na_2EDTA \times 2\ H_2O$ | 6.0 mg/L |

The pH was adjusted to 7.5 ± 0.1.

**Water temperature** Nominal: 24 ± 2 °C; actual: 23.67 – 24.13°C

**pH** Nominal: increase by less than 1.5 units in the control; actual: 7.59 – 9.47 (control) and 7.59 – 9.46 (solvent control)

**Lighting** Continuous illumination (light intensity nominal: 6500 – 10000 Lux, variation < ± 15%; actual: average of 8473 Lux using light tubes: T8-fluorescent tubes, LT30W/865 daylight)

**Shaking** None

## B. STUDY DESIGN AND METHODS

**1. In life dates** 29 Jul 2019 – 02 Sep 2019

**2. Experimental conditions**

*Test design*

The freshwater aquatic macrophyte *Lemna gibba* was exposed in a semi-static 7-day test to mesotrione technical at seven concentrations each with three replicates, a test water control and a solvent control with each six replicates. The recorded effect was inhibition of plant growth (yield and growth rate) based on frond numbers and dry weight.

*Inoculum at test start*

Young, light-green plants of similar size and comprising 2 - 4 fronds were transferred onto fresh medium and cultured for 7 – 10 days prior to testing, with two further transfers onto fresh medium before initiating the test.

Colonies consisting of 2-4 fronds were transferred from the inoculum culture into the test vessels containing a total of 12 fronds, each. The size of plants and fronds were similar in each test vessel.

*Concentrations tested*

Mesotrione technical was tested at nominally 0.698, 2.24, 7.15, 22.9, 73.2, 234 and 750 µg a.s./L. In addition, a control group with untreated test medium was tested and a solvent control containing test medium and DMF at 0.01 % (v/v). The test concentrations were chosen based on a non-GLP range-finding test.

*Treatment/Application*

To prepare the stock solution (= highest test item concentration), 75 mg of test item was weighed and transferred to a volumetric flask. DMF was added up to the bench mark and the solution was homogenised by shaking. The stock solution was clear and transparent. Remaining test item solutions were prepared by serial dilution. After preparation, an amount of 15 µL was applied to the test vessels containing 150 mL test medium. The control was prepared with test medium only. The solvent control was prepared by adding 15 µL DMF to 150 mL test medium.

*Analytics*

Samples were taken at t = 0 d fresh, t = 2 d aged, t = 2 d fresh, t = 5 d aged, t = 5 d fresh and t = 7 d aged from all tested concentrations and both controls. The samples were analysed for the actual content of mesotrione using HPLC-MS/MS. Details to the analytical method are summarized in Part B, Section 5.

**3. Sampling and measurements**

Frond numbers in each test vessel were determined at the start of the test. Frond numbers and the appearance of the colonies were checked on t = 0, 2, 5 and 7 days as well as any change in plant development, frond size, necrosis and additional observations of test media or other abnormalities.

The dry weight of the fronds was determined at the end of the test after drying at 60°C for about 48 hours. A representative batch of six times 12 fronds from the culture used for the test was dried to determine the dry weight for the test start.

The test temperature was measured continuously in a surrogate vessel held under the same conditions as the test vessels and recorded after 0, 2, 5 and 7 days. The pH-value of the test solutions was measured in the controls and each test concentration in one replicate of test solution on t = 0, 2, 5 and 7 days (on day 2 and 5 both in fresh and aged solutions). Light intensity was measured at test start.

**4. Calculation of toxicity**

For determination of the effects on growth rates, means of frond number for each test concentration and the controls at each observation time were calculated. Means of dry weight for each test concentration and controls at day 7 were calculated. The specific growth rate for frond number and dry weight was calculated as the logarithmic increase for each replicate for controls and treatments. The percentage inhibition of

growth rate was calculated as the difference between the mean growth rate of the solvent control and the mean growth rate in the treatments.

Effects on yield were determined on the basis of total frond numbers and dry weight in each vessel at the start and end of the test. For the test concentrations and controls, a mean value for yield was calculated. The mean percent inhibition in yield was calculated for each treatment group.

The mean doubling times of the culture for the controls and treatments were calculated.

**5. Statistics**

The statistical evaluation for day 7 was performed for yield of frond numbers, growth rate of frond numbers, growth rate of dry weight and yield of dry weight.

Control and solvent control were compared by calculation of the Shapiro-Wilks's statistic, a test for homogeneity of the data was performed according to F-Test. Significant differences were determined by using a pairwise method (t-test pooled, left-sided). In the following, treatment groups were compared to the solvent control.

A test for normality of the data was performed by calculating the Shapiro-Wilk's statistic, a test for homogeneity of the data was performed according to Levene. The NOEC and LOEC were determined by using a multiple comparison method (Jonckheere-Terpstra left sided for all parameters, except Welch Bonferroni-Holms corrected for growth rate of dry weight). The $EC_{10,20,50}$-values for yield of frond numbers and yield of dry weight, growth rate of frond numbers, and growth rate of dry weight were determined by probit analysis following Normal and logistic distribution. The evaluation of data was performed by SAS® (2016).

## Results and Discussion

Measured concentrations of mesotrione in the fresh test medium were between 100 and 116% of nominal. In the aged solutions, the measured concentrations were between 92 and 135% of nominal (see table below). All toxicological endpoints were evaluated using nominal and geometric mean measured concentrations of the test item.

**Table A 2.2.1-18: Measured concentrations of mesotrione in the test media**

| Nominal concentration [a)] [µg a.s./L] | Measured concentration of mesotrione | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 days fresh | | 2 days aged | | 2 days fresh | | 5 days fresh | | 5 days fresh | | 7 days aged | |
| | [µg/L] | [%] nom | [µg/L] | [%] nom | [µg/L] | [%] nom | [µg/L] | [%] nom | [µg/L] | [%] nom | [µg/L] | [%] nom |
| Control | < LOD | - | < LOQ [b)] | - | < LOD | - | < LOD | - | < LOD | - | < LOD | - |
| | Geometric mean concentration [%]: - ; actual concentration: - | | | | | | | | | | | |
| Solvent control | < LOD | - | < LOQ | - | < LOD | - | < LOD | - | < LOD | - | < LOD | - |
| | Geometric mean concentration [%]: - ; actual concentration: - | | | | | | | | | | | |
| 0.692 | 0.768 | 111 | 0.822 | 119 | 0.720 | 104 | 0.858 | 124 | 0.756 | 109 | 0.858 | 124 |
| | Geometric mean concentration [%]: 115 ; actual concentration: 0.803 µg test item/L | | | | | | | | | | | |
| 2.22 | 2.36 | 106 | 2.86 | 129 | 2.39 | 108 | 2.69 | 121 | 2.57 | 116 | 3.00 | 135 |
| | Geometric mean concentration [%]: 119 ; actual concentration: 2.67 µg test item/L | | | | | | | | | | | |
| 7.08 | 7.20 | 102 | 6.48 | 92 | 7.80 | 110 | 8.82 | 125 | 8.10 | 114 | 8.94 | 126 |
| | Geometric mean concentration [%]: 111 ; actual concentration: 7.94 µg test item/L | | | | | | | | | | | |
| 22.7 | 22.8 | 100 | 24.2 | 107 | 25.9 | 114 | 30.0 | 132 | 24.7 | 109 | 27.8 | 122 |
| | Geometric mean concentration [%]: 114 ; actual concentration: 26.1 µg test item/L | | | | | | | | | | | |
| 72.5 | 79.8 | 110 | 81.6 | 113 | 72.6 | 100 | 83.4 | 115 | 77.4 | 107 | 81.0 | 112 |
| | Geometric mean concentration [%]: 109 ; actual concentration: 79.8 µg test item/L | | | | | | | | | | | |
| 232 | 238 | 103 | 264 | 114 | 233 | 100 | 271 | 117 | 247 | 106 | 252 | 109 |
| | Geometric mean concentration [%]: 108 ; actual concentration: 253 µg test item/L | | | | | | | | | | | |
| 743 | 768 | 103 | 870 | 117 | 780 | 105 | 834 | 112 | 792 | 107 | 852 | 115 |
| | Geometric mean concentration [%]: 110 ; actual concentration: 825 µg test item/L | | | | | | | | | | | |

LOD = 0.0209 µg a.s./L; LOQ = 0.0698 µg a.s./L
- = not applicable
[a)] Corrected for purity of active substance
[b)] Measured amount was 0.0346 µg a.s./L which is slightly above the LOD of 0.0209 µg a.s./L. No mesotrione technical was detectable at 0 d in the fresh sample. Based on the analytical results of the test where mesotrione technical is stable between 0 d fresh and 2 d aged it can be assumed that the measured amount in the 2d aged control samples was not in the sample itself.

The mean frond numbers and dry weights are presented in table A 2.2.1-19, the effects of mesotrione on the growth rates and yield of *Lemna gibba* during the 7-day test are presented in Table 2.2.1-20. After 7 days of exposure, significant inhibitory effects were determined for yield and growth rate of frond numbers and dry weight at 2.67 µg test item/L (geometric mean concentration) and above. The endpoints are presented in Table 2.2.1-21.

On day 5 and 7 chlorosis leaves were observed at 2.67 µg test item/L (geometric mean concentration) and above. On day 5 and 7 deformed leaves, shortened roots and offshoot, tightly assembled fronds and isolated leaves were observed at 2.67 µg test item/L (geometric mean concentration) and above.

**Table A 2.2.1-19: Mean frond number and dry weights of *Lemna gibba* during the 7-day test**

| Nominal concentration | Mean of frond numbers | | | | | Mean dry weight [g] | | |
|---|---|---|---|---|---|---|---|---|
| [µg test item/L] | 0 d | 2 d | 5 d | 7 d | 7 d – 0 d | 0 d | 7 d | 7 d – 0 d |
| Control | 12 | 19 | 109 | 234 | 222 | 0.0018 | 0.0209 | 0.0191 |
| Solvent control | 12 | 24 | 107 | 221 | 209 | | 0.0295 | 0.0277 |
| 0.698 | 12 | 21 | 97 | 178 | 166 | | 0.0298 | 0.0280 |
| 2.24 | 12 | 20 | 57 | 94 | 82* | | 0.0110 | 0.0092* |
| 7.15 | 12 | 17 | 40 | 71 | 59* | | 0.0082 | 0.0064* |
| 22.9 | 12 | 18 | 26 | 47 | 35* | | 0.0060 | 0.0042* |
| 73.2 | 12 | 15 | 29 | 33 | 21* | | 0.0022 | 0.0004* |
| 234 | 12 | 15 | 32 | 30 | 18* | | 0.0023 | 0.0005* |
| 750 | 12 | 14 | 29 | 29 | 17* | | 0.0024 | 0.0006* |

* Statistically significantly lower than in the solvent control (according to Jonckheere-Terpstra test, left sided)

**Table A 2.2.1-20: Effects of mesotrione on growth of *Lemna gibba* during the 7-day test**

| Nominal concentrations | Based on frond number | | | | Based on dry weight | |
|---|---|---|---|---|---|---|
| | Yield inhibition [%] | Mean growth rates µ [$d^{-1}$] / Inhibition of growth rates [%] | | | Yield inhibition [%] | Mean growth rates µ [$d^{-1}$] / Inhibition of growth rates [%] |
| [µg test item/L] | 7d – 0d | 2d | 5d | 7d | 7d – 0 d | 7d – 0 d |
| Control | -6.2 | 0.2243 / 35.8 | 0.4407 / -1.1 | 0.4239 / -2.2 | 31.0 | 0.3478 / 12.5 |
| Solvent control | - | 0.3495 / - | 0.4357 / - | 0.4147 / - | - | 0.3977 / - |
| 0.698 | 20.6 | 0.2783 / 20.4 | 0.4168 / 4.3 | 0.3844 / 7.3 | -1.1 | 0.4007 / -0.8 |
| 2.24 | 60.8 | 0.2465 / 29.5 | 0.3111 / 28.6 | 0.2943* / 29.0 | 66.8 | 0.2590* / 34.9 |
| 7.15 | 71.8 | 0.1617 / 53.7 | 0.2395 / 45.0 | 0.2545* / 38.6 | 76.9 | 0.2154* / 45.8 |
| 22.9 | 83.3 | 0.1899 / 45.7 | 0.1518 / 65.2 | 0.1950* / 53.0 | 84.8 | 0.1662* / 58.2 |
| 73.2 | 90.0 | 0.1223 / 65.0 | 0.1763 / 59.5 | 0.1454* / 64.9 | 98.6 | 0.0306* / 92.3 |
| 234 | 91.4 | 0.1086 / 68.9 | 0.1908 / 56.2 | 0.1248* / 69.9 | 98.2 | 0.0309* / 92.2 |
| 750 | 91.9 | 0.0870 / 75.1 | 0.1764 / 59.5 | 0.1260* / 69.6 | 97.8 | 0.0274* / 93.1 |

A negative value indicates increase in growth relative to the solvent control

* Statistically significantly lower than in the control (according to Jonckheere-Terpstra test, left sided or Bonferroni-Holms corrected Welch test, left sided)

**Table A 2.2.1-21: Toxicity of mesotrione to *Lemna gibba* after 7 days of exposure**

| 7-day endpoints based on geometric mean concentrations [µg test item/L] | | | | |
|---|---|---|---|---|
| | Based on frond numbers | | Based on dry weight | |
| | Growth rate (r) | Yield (y) | Growth rate (r) | Yield (y) |
| **$EC_{50}$** (95% CL) | 35.4 (13.4 – 101) | 2.47 (0.469 – 6.20) | 11.3 (4.07 – 27.5) | 3.21 (0.371 – 10.8) |
| **$EC_{20}$** (95% CL) | 1.28 (0.102 – 4.23) | n.a. [a)] | 2.10 (0.257 – 5.39) | 0.916 (0.00342 – 2.70) |
| **$EC_{10}$** (95% CL) | 0.227 (0.00569 – 1.16) | n.a. [a)] | 0.784 (0.0421 – 2.52) | 0.440 (0.000166 – 1.59) |
| **NOEC** | 0.803 | | | |
| **LOEC** | 2.67 | | | |

n.a. not applicable, 20.6% inhibition at the lowest concentrations

The doubling time ($T_d$) of frond number in the control was calculated to be 1.635 days in the control and 1.671 days in the solvent control (required: < 2.5 d). Therefore, the study fulfilled the validity criterion of OECD 221 (2006).

**Conclusion**

The $E_rC_{50}$ values of meostrione for the freshwater aquatic macrophyte *Lemna gibba* were determined to be 11.3 (95% CL: 4.07 – 27.5) µg test item/L for dry weight and 35.4 (95% CL: 13.4 - 101) µg test item/L for frond numbers. The validity criterion of OECD 221 (2006) was fulfilled.

## A 2.2.1.8 Study 8: Toxicity to the macrophyte *Spirodela polyrhiza* – mesotrione

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 221 and according to the principles of GLP. In the definitive test all the validity criteria were met.<br>The study is considered to be reliable and suitable for the risk assessment. All results refer to geometric mean measured concentrations. |

| | |
|---|---|
| Reference: | KCP 10.2.1/08 |
| Report | Mesotrione: Toxicity to the aquatic plant *Spirodela polyrhiza* in a growth inhibition test, Christmann, R., 2021a, 218-31 |
| Guideline(s): | OECD 221 (2006) adapted for *S. polyrhiza* |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | - |

**Materials and Methods**

**A. MATERIALS**

**1. Test material** Mesotrione technical

| | |
|---|---|
| **Description** | Pale yellow solid |
| **Lot/Batch #** | MST1603001 |
| **Purity** | 99.11 % w/w analysed |
| **Stability of test material** | Stable under storage conditions (ambient, dark)<br>Expiry date: 20 Sep 2021 |

**2. Vehicle and/or positive control** Vehicle control: Test medium
Positive control: None

**3. Test organism**

| | |
|---|---|
| **Species** | *Spirodela polyrhiza* |
| **Source** | Cultured at the test site |
| **Age** | Colonies consisting of three fronds were used for the test. |
| **Acclimation period** | The pre-culture was held at 24 ± 2 °C and 6500 - 10000 Lux since Oct 2020 which is in agreement with the test conditions. |
| **Test units** | 2000-mL glass beakers, covered with perforated cling film, containing 500 mL of test medium |

**4. Environmental conditions**

**Test water** The plants were cultivated and tested in Steinberg medium with the following nominal concentrations:

| **Macro-nutrients** | | **Micro-nutrients** | |
|---|---|---|---|
| $KNO_3$ | 350.0 mg/L | $H_3BO_3$ | 0.120 mg/L |
| $Ca(NO_3)_2 \times 4\ H_2O$ | 295.0 mg/L | $ZnSO_4 \times 7\ H_2O$ | 0.180 mg/L |
| $KH_2PO_4$ | 90.0 mg/L | $Na_2MoO_4 \times 2\ H_2O$ | 0.044 mg/L |
| $K_2HPO_4$ | 12.6 mg/L | $MnCl_2 \times 4\ H_2O$ | 0.180 mg/L |
| $MgSO_4 \times 7\ H_2O$ | 100.0 mg/L | $FeCl_3 \times 6\ H_2O$ | 0.760 mg/L |
| | | $Na_2EDTA \times 2\ H_2O$ | 1.500 mg/L |

The pH of the medium was 5.5.

| | |
|---|---|
| **Water temperature** | Nominal: 24 ± 2 °C; actual: 23.7 - 25.0 °C |
| **pH** | Nominal: increase by less than 1.5 units in the control; actual: 5.52 - 5.64 in the fresh and 5.81 - 6.94 in the aged solutions of the control |
| **Lighting** | Continuous illumination (light intensity nominal: 6500 – 10000 Lux, variation < ± 15%; actual: 7831 - 8382 Lux) |
| **Oxygen content** | ≥ 7.38 mg/L in all solutions (fresh and aged) |

## B. STUDY DESIGN AND METHODS

**1. In life dates** 03 Mar 2020 - 17 Mar 2020 (main test 1, biological + analytical phase), 04 Nov 2020 - 12 Feb 2021 (main test 2, biological + analytical phase)

**2. Experimental conditions**

*Test design*

The aquatic macrophyte *Spirodela polyrhiza* was exposed in a semi-static 7-day test to mesotrione technical at six concentrations each with five replicates and a test water control with ten replicates. Medium was changed after 2 and 5 days of exposure. The recorded effect was inhibition of plant growth (yield and growth rate) based on frond numbers and dry weight. In addition, any phytotoxic symptoms were recorded.

*Inoculum at test start*

Two weeks before test start, the plants were kept under the same test conditions as in the test.

Colonies consisting of 3 fronds were at test start transferred from the inoculum culture into the test vessels containing a total of 12 fronds, each.

*Concentrations tested*

Mesotrione technical was tested at nominally 0.238, 0.763, 2.44, 7.81, 25.0 and 80.0 µg a.s./L. In addition, a control group with untreated test medium was tested. The test concentrations were chosen based on a non-GLP range-finding test.

*Treatment/Application*

To prepare the stock solution (= highest test concentration), 25 mg of test item was weighed and dissolved in 1000 mL test medium using a magnetic stirrer. Remaining test item application solutions were prepared by serial dilution. From each test item application solution 1.6 mL were given to 500 mL of test medium to obtain the final test media. The control was prepared with test medium only. The procedure was repeated for every test medium change (on day 0, 2 and 5). The test item was clear throughout the whole test (checked on day 0, 2, 5 and 7).

*Analytics*

Samples to verify the exposure to the test item were taken at t = 0 d fresh, t = 2 d aged, t = 2 d fresh, t = 5 d aged, t = 5 d fresh and t = 7 d aged from all tested concentrations and the control from pooled replicates. For each sampling point and concentration, two samples were analysed for the actual content of mesotrione using LC-MS/MS. Details to the analytical method are summarized in Part B, Section 5.

**3. Sampling and measurements**

Frond numbers in each test vessel were determined at the start of the test. Frond numbers and the appearance of the colonies were checked on t = 0, 2, 5 and 7 days as well as any change in plant development, frond size, necrosis and additional observations of test media or other abnormalities.

The dry weight of the fronds was determined at the end of the test after drying at 72.2 - 77.3 °C for 48 hours. A representative batch of six times 12 fronds from the culture used for the test was dried (48 hours at 65.0 - 78.0 °C) to determine the dry weight for the test start.

The test temperature was recorded daily. The pH-value of the test solutions was measured in the control and each test concentration on t = 0, 2, 5 and 7 days (on day 2 and 5 both in fresh and aged solutions). Light intensity was measured on days 0, 2, 5, 6 and 7

## 4. Calculation of toxicity

The average specific growth rate and yield were determined based on both parameters, frond numbers and dry weight. For each treatment group mean values and variance estimates were calculated together with the percent inhibition compared to the control. In addition, the doubling time of the average specific growth rate in the control treatment was determined.

## 5. Statistics

The statistical evaluation for day 7 was performed for all parameters.

$EC_x$ values were determined using non-linear regression analysis (3-parametric normal CDF).

The data for all parameters was tested for normality (Shapiro-Wilks test, $\alpha = 0.01$) and for homogeneity of data (Levenes test, $\alpha = 0.01$). The monotonicity of the data was checked using the Contrast test ($\alpha = 0.05$). All parameters were finally evaluated using ANOVA followed by Williams t-test ($\alpha = 0.05$, one-sided smaller).
Statistical evaluations were performed using ToxRat Professional 3.3.0.

## Results and Discussion

*A. ANALYTICAL RESULTS*

Measured concentrations of mesotrione in the fresh test medium were between 64 and 137% of nominal. In the aged solutions, the mean measured concentrations were between 78 and 115% of nominal (see table below). Since recoveries were outside the range of 80 to 120% of nominal, endpoints were based on geometric mean measured values.

**Table A 2.2.1-22: Measured concentrations of mesotrione in the test media**

| Nominal test concentration | Sampling | Measured concentration of mesotrione | | Mean fresh media | Mean aged media | Overall geometric mean | |
|---|---|---|---|---|---|---|---|
| [µg a.s./L] | [d] | [µg a.s./L] [a)] | [%] of nominal | [%] of nominal | [%] of nominal | [%] of nominal | [µg a.s./L] |
| Control | 0, 2, 5 (fresh), 2, 5, 7 (aged) | < LOD | - | - | - | - | - |
| 0.238 | 0 (fresh) | 0.307 / 0.326 | 129 / 137 | 124 | 107 | 115 | 0.275 |
| | 2 (aged) | 0.247 / 0.253 | 104 / 106 | | | | |
| | 2 (fresh) | 0.292 / 0.293 | 123 / 123 | | | | |
| | 5 (aged) | 0.273 / 0.266 | 115 / 112 | | | | |
| | 5 (fresh) | 0.285 / 0.262 | 120 / 110 | | | | |
| | 7 (aged) | 0.244 / 0.242 | 102 / 102 | | | | |
| 0.763 | 0 (fresh) | 0.862 / 0.849 | 113 / 111 | 110 | 93 | 100 | 0.767 |
| | 2 (aged) | 0.706 / 0.745 | 93 / 98 | | | | |
| | 2 (fresh) | 0.829 / 0.790 | 109 / 104 | | | | |
| | 5 (aged) | 0.673 / 0.640 | 88 / 84 | | | | |
| | 5 (fresh) | 0.862 / 0.842 | 113 / 110 | | | | |
| | 7 (aged) | 0.764 / 0.732 | 100 / 96 | | | | |
| 2.44 | 0 (fresh) | 2.787 / 2.722 | 114 / 112 | 109 | 85 | 96 | 2.33 |
| | 2 (aged) | 2.145 / 2.100 | 88 / 86 | | | | |

| Nominal test concentration | Sampling | Measured concentration of mesotrione | | Mean fresh media | Mean aged media | Overall geometric mean | |
|---|---|---|---|---|---|---|---|
| [µg a.s./L] | [d] | [µg a.s./L] [a)] | [%] of nominal | [%] of nominal | [%] of nominal | [%] of nominal | [µg a.s./L] |
| | 2 (fresh) | 2.379 / 2.547 | 97 / 104 | | | | |
| | 5 (aged) | 1.912 / 1.983 | 78 / 81 | | | | |
| | 5 (fresh) | 2.826 / 2.755 | 116 / 113 | | | | |
| | 7 (aged) | 2.249 / 2.035 | 92 / 83 | | | | |
| 7.81 | 0 (fresh) | 7.876 / 8.305 | 101 / 106 | 106 | 84 | 94 | 7.35 |
| | 2 (aged) | 6.590 / 6.411 | 84 / 82 | | | | |
| | 2 (fresh) | 8.091 / 8.234 | 104 / 105 | | | | |
| | 5 (aged) | 5.062 / 6.347 | 76 / 81 | | | | |
| | 5 (fresh) | 8.519 / 8.448 | 109 / 108 | | | | |
| | 7 (aged) | 6.983 / 7.305 | 89 / 94 | | | | |
| 25 | 0 (fresh) | 28.981 / 27.552 | 116 / 110 | 110 | 85 | 96 | 24.1 |
| | 2 (aged) | 21.656 / 23.085 | 87 / 92 | | | | |
| | 2 (fresh) | 27.373 / 25.944 | 109 / 104 | | | | |
| | 5 (aged) | 19.512 / 20.763 | 78 / 83 | | | | |
| | 5 (fresh) | 27.373 / 27.373 | 109 / 109 | | | | |
| | 7 (aged) | 20.227 / 22.728 | 81 / 91 | | | | |
| 80 | 0 (fresh) | 70.327 / 75.687 | 88 / 95 | 89 | 69 | 78 | 62.5 |
| | 2 (aged) | 60.144 / 57.464 | 75 / 72 | | | | |
| | 2 (fresh) | 68.719 / 68.719 | 86 / 86 | | | | |
| | 5 (aged) | 51.193 / 53.659 | 64 / 67 | | | | |
| | 5 (fresh) | 70.863 / 71.399 | 89 / 89 | | | | |
| | 7 (aged) | 55.320 / 55.320 | 69 / 69 | | | | |

LOD = 0.05 µg a.s./L; LOQ = 0.18 µg a.s./L
- = not applicable
[a)] two samples were measured for each time point/concentration; calculated concentration considering measured concentration and dilution factor

*B. BIOLOGICAL RESULTS*

The mean frond numbers and dry weights together with the effects of mesotrione on the inhibition of growth rates and yield of *Spirodela polyrhiza* during the 7-day test are presented in the tables below. After 7 days of exposure, significant inhibitory effects were determined for yield of frond numbers at all concentrations while for the remaining parameters (yield of dry weight and growth rate of frond numbers and dry weight) significant effects were observed at nominal 0.763 µg a.s./L and above. The endpoints are presented in the tables below.

On day 2 tested plants showed chlorosis at the concentration of 7.81 (slight chlorosis), 25.0 and 80.0 µg a.s./L. On day 5 and 7 chlorosis and slight necrosis could be observed at plants growing in 7.81 µg a.s./L and above. Furthermore, shorter roots were observed for test item concentrations of 2.44 µg a.s./L and above.

**Table A 2.2.1-23: Mean frond number and dry weights of *Spirodela polyrhiza* during the 7-day test**

| Nominal concentration | Mean of frond numbers | | | | | Mean dry weight [mg] | | |
|---|---|---|---|---|---|---|---|---|
| [µg a.s./L] | 0 d | 2 d | 5 d | 7 d | 7 d – 0 d | 0 d | 7 d | 7 d – 0 d |
| Control | 12 | 20.4 | 63.2 | 127.5 | 116 | 6.1 | 90.3 | 84.3 |
| 0.238 | 12 | 20.4 | 62.6 | 125.2 | 113 | | 89.5 | 83.4 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 0.763 | 12 | 20.6 | 64.0 | 123.2 | 111 | | 83.2 | 77.1 |
| 2.44 | 12 | 18.4 | 55.8 | 104.8 | 92.8 | | 64.7 | 58.6 |
| 7.81 | 12 | 16.0 | 31.6 | 33.4 | 21.4 | | 27.5 | 21.4 |
| 25.0 | 12 | 14.4 | 26.4 | 28.8 | 16.8 | | 17.9 | 11.8 |
| 80.0 | 12 | 13.8 | 25.2 | 25.6 | 13.6 | | 18.8 | 12.7 |

**Table A 2.2.1-24: Effects of mesotrione on growth of *Spirodela polyrhiza* during the 7-day test**

| **Nominal concentrations** [µg a.s./L] | **Based on frond number** | | **Based on dry weight** | |
|---|---|---|---|---|
| | **Inhibition of yield** [%] | **Inhibition of growth rates** [%] | **Inhibition of yield** [%] | **Inhibition of growth rates** [%] |
| Control | - | - | - | - |
| 0.238 | 2.0 * | 0.8 | 1.0 | 0.4 |
| 0.763 | 3.7 * | 1.4 | 8.5 * | 3.0 * |
| 2.44 | 19.7 * | 8.3 | 30.4 * | 12.4 * |
| 7.81 | 81.5 * | 56.7 | 74.6 * | 44.3 * |
| 25.0 | 85.5 * | 63.0 | 86.0 * | 60.1 * |
| 80.0 | 88.2 * | 67.9 | 84.9 * | 58.2 * |

* Statistically significantly different from the control (according to Williams t-test, one-sided, $\alpha = 0.05$)

**Table A 2.2.1-25: Toxicity of mesotrione to *Spirodela polyrhiza* after 7 days of exposure**

| **7-day endpoints based on geometric mean concentrations [µg a.s./L]** | | | | |
|---|---|---|---|---|
| | **Based on frond numbers** | | **Based on dry weight** | |
| | **Growth rate (r)** | **Yield (y)** | **Growth rate (r)** | **Yield (y)** |
| **$EC_{50}$** (95% CL) | 12.0 (5.26 - 26.9) | 4.16 (3.16 - 5.48) | 18.1 (8.20 - 39.0) | 0.677 (0.449 - 1.02) |
| **$EC_{20}$** (95% CL) | 2.08 (1.06 - 4.06) | 2.33 (1.86 - 2.93) | 2.24 (1.20 - 4.23) | 1.25 (0.845 - 1.86) |
| **$EC_{10}$** (95% CL) | 0.829 (0.418 - 1.65) | 1.72 (1.36 - 2.18) | 0.753 (0.400 - 1.42) | 4.03 (2.48 - 6.52) |
| **NOEC** | 0.275 | < 0.275 | 0.275 | |
| **LOEC** | 0.767 | ≤ 0.275 | 0.767 | |

*C. VALIDITY CRITERIA*

The doubling time ($T_d$) of frond numbers in the control was calculated to be 2.1 days (required: < 2.5 d). Therefore, the study fulfilled the validity criterion of OECD 221 (2006).

**Conclusion**

The $E_rC_{50}$ values of meostrione for the freshwater aquatic macrophyte *Spirodela polyrhiza* were determined to be 12.0 (95% CL: 5.26 - 26.9) µg a.s./L for frond numbers and 18.1 (95% CL: 8.10 - 39.0) µg a.s./L for dry weight. The validity criterion of OECD 221 (2006) was fulfilled.

## A 2.2.1.9 Study 9: Toxicity to the macrophyte *Wolffia arrhiza* – mesotrione

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 221 and according to the principles of GLP. In the definitive test all the validity criteria were met.<br>The study is considered to be reliable and suitable for the risk assessment. All results refer to geometric mean measured concentrations. |

| | |
|---|---|
| Reference: | KCP 10.2.1/09 |
| Report | Mesotrione: Toxicity to the aquatic plant *Wolffia arrhiza* in a growth inhibition test, Christmann, R., 2021b, 218-32 |
| Guideline(s): | OECD 221 (2006) |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | - |

**Materials and Methods**

**A. MATERIALS**

| | |
|---|---|
| **1. Test material** | Mesotrione technical |
| **Description** | Pale yellow solid |
| **Lot/Batch #** | MST1603001 |
| **Purity** | 99.11 % w/w analysed |
| **Stability of test material** | Stable under storage conditions (ambient, dark)<br>Expiry date: 20 Sep 2021 |
| **2. Vehicle and/or positive control** | Vehicle control: Test medium<br>Positive control: None |
| **3. Test organism** | |
| **Species** | *Wolffia arrhiza* |
| **Source** | Natural pond at the test facility |
| **Age** | Colonies consisting of one frond were used for the test. |
| **Acclimation period** | The pre-culture was held at 24 ± 2 °C and 6500 - 10000 Lux since Oct 2020 which is in agreement with the test conditions. |
| **Test units** | 2000-mL glass beakers, covered with perforated cling film, containing 500 mL of test medium |
| **4. Environmental conditions** | |
| **Test water** | The plants were cultivated and tested in Steinberg medium with the following nominal concentrations: |

| **Macro-nutrients** | | **Micro-nutrients** | |
|---|---|---|---|
| $KNO_3$ | 350.0 mg/L | $H_3BO_3$ | 0.120 mg/L |
| $Ca(NO_3)_2 \times 4\ H_2O$ | 295.0 mg/L | $ZnSO_4 \times 7\ H_2O$ | 0.180 mg/L |
| $KH_2PO_4$ | 90.0 mg/L | $Na_2MoO_4 \times 2\ H_2O$ | 0.044 mg/L |

| | | | |
|---|---|---|---|
| $K_2HPO_4$ | 12.6 mg/L | $MnCl_2 \times 4\ H_2O$ | 0.180 mg/L |
| $MgSO_4 \times 7\ H_2O$ | 100.0 mg/L | $FeCl_3 \times 6\ H_2O$ | 0.760 mg/L |
| | | $Na_2EDTA \times 2\ H_2O$ | 1.500 mg/L |

The pH of the medium was 5.5.

| | |
|---|---|
| **Water temperature** | Nominal: 24 ± 2 °C; actual: 23.7 - 25.0 °C |
| **pH** | Nominal: increase by less than 1.5 units in the control; actual: 5.52 - 5.62 in the fresh and 5.58 - 6.73 in the aged solutions of the control |
| **Lighting** | Continuous illumination (light intensity nominal: 6500 – 10000 Lux, variation < ± 15%; actual: 7876 - 8332 Lux) |
| **Oxygen content** | ≥ 8.02 mg/L in all solutions (fresh and aged) |

## B. STUDY DESIGN AND METHODS

**1. In life dates**

03 Mar 2020 - 19 Mar 2020 (main test 1, biological + analytical phase), 04 Nov 2020 - 07 Feb 2021 (main test 2, biological + analytical phase)

**2. Experimental conditions**

*Test design*

The aquatic macrophyte *Wolffia arrhiza* was exposed in a semi-static 7-day test to mesotrione technical at six concentrations each with five replicates and a test water control with ten replicates. Medium was changed after 2 and 5 days of exposure. The recorded effect was inhibition of plant growth (yield and growth rate) based on frond numbers and dry weight. In addition, any phytotoxic symptoms were recorded.

*Inoculum at test start*

Two weeks before test start, the plants were kept under the same test conditions as in the test.

Colonies consisting of 1 frond were transferred at test start from the inoculum culture into the test vessels containing a total of 50 fronds, each.

*Concentrations tested*

Mesotrione technical was tested at nominally 0.238, 0.763, 2.44, 7.81, 25.0 and 80.0 µg a.s./L. In addition, a control group with untreated test medium was tested. The test concentrations were chosen based on a non-GLP range-finding test.

*Treatment/Application*

To prepare the stock solution (= highest test concentration), 25 mg of test item was weighed and dissolved in 1000 mL test medium using a magnetic stirrer. Remaining test item application solutions were prepared by serial dilution. From each test item application solution 1.6 mL were given to 500 mL of test medium to obtain the final test media. The control was prepared with test medium only. The procedure was repeated for every test medium change (on day 0, 2 and 5). The test item was clear throughout the whole test (checked on day 0, 2, 5 and 7).

*Analytics*

Samples to verify the exposure to the test item were taken at t = 0 d fresh, t = 2 d aged, t = 2 d fresh, t = 5 d aged, t = 5 d fresh and t = 7 d aged from all tested concentrations and the control from pooled replicates. For each sampling point and concentration, two samples were analysed for the actual content of mesotrione using LC-MS/MS. Details to the analytical method are summarized in Part B, Section 5.

**3. Sampling and measurements**

Frond numbers in each test vessel were determined at the start of the test. Frond numbers and the appearance of the colonies were checked on t = 0, 2, 5 and 7 days as well as any change in plant development, frond size, necrosis and additional observations of test media or other abnormalities.

The dry weight of the fronds was determined at the end of the test after drying at 72.2 - 77.3 °C for 48 hours. A representative batch of six times 50 fronds from the culture used for the test was dried (48 hours at 65.0 - 78.0 °C) to determine the dry weight for the test start.

The test temperature was recorded daily. The pH-value of the test solutions was measured in the control and each test concentration on t = 0, 2, 5 and 7 days (on day 2 and 5 both in fresh and aged solutions). Light intensity was measured on days 0, 2, 5, 6 and 7

**4. Calculation of toxicity**

The average specific growth rate and yield were determined based on both parameters, frond numbers and dry weight. For each treatment group mean values and variance estimates were calculated together with the percent inhibition compared to the control. In addition, the doubling time of the average specific growth rate in the control treatment was determined.

**5. Statistics**

The statistical evaluation for day 7 was performed for all parameters.

$EC_x$ values were determined using non-linear regression analysis (3-parametric normal CDF).

The data for all parameters was tested for normality (Shapiro-Wilks test, $\alpha = 0.01$) and for homogeneity of data (Levenes test, $\alpha = 0.01$). The monotonicity of the data was checked using the Contrast test ($\alpha = 0.05$). Growth rate and yield for frond numbers as well as yield of dry weight were finally evaluated using ANOVA followed by Williams t-test ($\alpha = 0.05$, one-sided smaller). Growth rate of dry weight was evaluated using ANOVA followed by Dunnetts t-test ($\alpha = 0.05$, one-sided smaller).
Statistical evaluations were performed using ToxRat Professional 3.3.0.

## Results and Discussion

*A. ANALYTICAL RESULTS*

Measured concentrations of mesotrione in the fresh test medium were between 105 and 144% of nominal. In the aged solutions, the mean measured concentrations were between 75 and 110% of nominal (see table below). Since recoveries were outside the range of 80 to 120% of nominal, endpoints were based on geometric mean measured values.

**Table A 2.2.1-25: Measured concentrations of mesotrione in the test media**

| Nominal test concentration | Sampling | Measured concentration of mesotrione | | Mean fresh media | Mean aged media | Overall geometric mean | |
|---|---|---|---|---|---|---|---|
| [µg a.s./L] | [d] | [µg a.s./L] a) | [%] of nominal | [%] of nominal | [%] of nominal | [%] of nominal | [µg a.s./L] |
| Control | 0, 2, 5 (fresh), 2, 5, 7 (aged) | < LOD or LOQ | - | - | - | - | - |
| 0.238 | 0 (fresh) | 0.253 / 0.249 | 106 / 105 | 115 | 94 | 107 | 0.254 |
| | 2 (aged) | 0.212 / 0.211 | 89 / 88 | | | | |
| | 2 (fresh) | 0.321 / 0.321 | 135 / 135 | | | | |
| | 5 (aged) | 0.262 / 0.258 | 110 / 109 | | | | |
| | 5 (fresh) | 0.253 / 0.249 | 106 / 105 | | | | |
| | 7 (aged) | 0.200 / 0.205 | 84 / 86 | | | | |
| 0.763 | 0 (fresh) | 0.870 / 1.097 | 114 / 144 | 120 | 88 | 102 | 0.781 |
| | 2 (aged) | 0.664 / 0.655 | 87 / 86 | | | | |
| | 2 (fresh) | 0.914 / 0.871 | 120 / 114 | | | | |
| | 5 (aged) | 0.640 / 0.627 | 84 / 82 | | | | |
| | 5 (fresh) | 0.855 / 0.890 | 112 / 117 | | | | |
| | 7 (aged) | 0.681 / 0.745 | 89 / 98 | | | | |
| 2.44 | 0 (fresh) | 2.953 / 2.862 | 121 / 117 | 115 | 86 | 99 | 2.43 |
| | 2 (aged) | 2.035 / 1.999 | 83 / 82 | | | | |
| | 2 (fresh) | 2.806 / 2.715 | 115 / 111 | | | | |
| | 5 (aged) | 1.999 / 2.017 | 82 / 83 | | | | |
| | 5 (fresh) | 2.806 / 2.733 | 115 / 112 | | | | |
| | 7 (aged) | 2.256 / 2.292 | 92 / 94 | | | | |
| 7.81 | 0 (fresh) | 8.855 / 8.918 | 113 / 114 | 113 | 89 | 99 | 7.77 |
| | 2 (aged) | 7.013 / 6.855 | 90 / 88 | | | | |
| | 2 (fresh) | 8.633 / 8.506 | 111 / 109 | | | | |
| | 5 (aged) | 6.378 / 6.410 | 82 / 82 | | | | |
| | 5 (fresh) | 8.918 / 8.950 | 114 / 115 | | | | |
| | 7 (aged) | 7.490 / 7.458 | 96 / 95 | | | | |
| 25 | 0 (fresh) | 30.392 / 29.995 | 122 / 120 | 114 | 87 | 99 | 24.6 |
| | 2 (aged) | 23.169 / 23.566 | 93 / 94 | | | | |
| | 2 (fresh) | 27.614 / 28.249 | 110 / 113 | | | | |
| | 5 (aged) | 18.883 / 18.803 | 76 / 75 | | | | |
| | 5 (fresh) | 27.217 / 26.899 | 109 / 108 | | | | |
| | 7 (aged) | 22.851 / 23.248 | 91 / 93 | | | | |
| 80 | 0 (fresh) | 94.986 / 95.462 | 119 / 119 | 115 | 91 | 101 | 80.6 |
| | 2 (aged) | 78.079 / 77.365 | 98 / 97 | | | | |
| | 2 (fresh) | 92.604 / 88.080 | 116 / 110 | | | | |
| | 5 (aged) | 60.458 / 60.458 | 76 / 76 | | | | |

| Nominal test concentration | Sampling | Measured concentration of mesotrione | | Mean fresh media | Mean aged media | Overall geometric mean | |
|---|---|---|---|---|---|---|---|
| [µg a.s./L] | [d] | [µg a.s./L] [a)] | [%] of nominal | [%] of nominal | [%] of nominal | [%] of nominal | [µg a.s./L] |
| | 5 (fresh) | 87.842 / 90.699 | 110 / 113 | | | | |
| | 7 (aged) | 77.603 / 80.936 | 97 / 101 | | | | |

LOD = 0.004 µg a.s./L; LOQ = 0.18 µg a.s./L
- = not applicable
[a)] two samples were measured for each time point/concentration; calculated concentration considering measured concentration and dilution factor

*B. BIOLOGICAL RESULTS*

The mean frond numbers and dry weights together with the effects of mesotrione on the inhibition of growth rates and yield of *Wolffia arrhiza* during the 7-day test are presented in the tables below. After 7 days of exposure, significant inhibitory effects were determined for all parameters at all concentrations except the second lowest one (nominal 0.763 µg a.s./L). However, since inhibition at 0.763 µg a.s./L was negative and the CV of the respective control group was low, the lowest concentration of nominal 0.238 µg a.s./L was decided to be the LOEC. The endpoints are presented in the tables below.

On day 5 and 7 the tested plants showed chlorotic fronds at the nominal concentrations of 25.0 and 80.0 µg a.s./L. And at day 7 slight chlorosis was observed in addition at the nominal concentration of 7.81 µg a.s./L.

**Table A 2.2.1-26: Mean frond number and dry weights of *Wolffia arrhiza* during the 7-day test**

| Nominal concentration | Mean of frond numbers | | | | | Mean dry weight [mg] | | |
|---|---|---|---|---|---|---|---|---|
| [µg a.s./L] | 0 d | 2 d | 5 d | 7 d | 7 d – 0 d | 0 d | 7 d | 7 d – 0 d |
| Control | 50 | 106.0 | 247.5 | 416.2 | 366.2 | 1.5 | 12.2 | 10.7 |
| 0.238 | 50 | 97.2 | 225.6 | 345.0 | 295.0 | | 10.4 | 8.9 |
| 0.763 | 50 | 97.6 | 293.0 | 460.0 | 410.0 | | 13.3 | 11.8 |
| 2.44 | 50 | 96.0 | 207.6 | 329.8 | 279.8 | | 6.7 | 5.2 |
| 7.81 | 50 | 95.0 | 159.6 | 163.8 | 113.8 | | 3.4 | 1.9 |
| 25.0 | 50 | 80.8 | 132.6 | 155.2 | 105.2 | | 2.5 | 1.0 |
| 80.0 | 50 | 54.6 | 97.2 | 117.2 | 67.2 | | 1.6 | 0.1 |

**Table A 2.2.1-27: Effects of mesotrione on growth of *Wolffia arrhiza* during the 7-day test**

| Nominal concentrations | Based on frond number | | Based on dry weight | |
|---|---|---|---|---|
| [µg a.s./L] | Inhibition of yield [%] | Inhibition of growth rates [%] | Inhibition of yield [%] | Inhibition of growth rates [%] |
| Control | - | - | - | - |
| 0.238 | 19.4 * | 8.8 * | 16.7 * | 7.6 * |
| 0.763 | -12.0 | -4.7 | -10.2 | -4.1 |
| 2.44 | 23.6 * | 11.0 * | 51.1 * | 28.4 * |
| 7.81 | 68.9 * | 44.0 * | 82.4 * | 61.3 * |

| | | | | |
|---|---|---|---|---|
| 25.0 | 71.3 * | 46.6 * | 90.7 * | 75.7 * |
| 80.0 | 81.6 * | 59.8 * | 98.9 * | 96.3 * |

* Statistically significantly different from the control (for yield and growth rate of frond numbers and yield of dry weight according to Williams t-test, one-sided, α = 0.05 and for growth rate of dry weight according to Dunnetts t-test, one-sided, α = 0.05)
Negative values indicate an increase compared to the control.

**Table A 2.2.1-28: Toxicity of mesotrione to *Wolffia arrhiza* after 7 days of exposure**

| 7-day endpoints based on geometric mean concentrations [µg a.s./L] | | | | |
|---|---|---|---|---|
| | Based on frond numbers | | Based on dry weight | |
| | Growth rate (r) | Yield (y) | Growth rate (r) | Yield (y) |
| $EC_{50}$ (95% CL) | 28.9 (10.6 - 75.6) | 7.18 (2.70 - 19.0) | 6.28 (4.10 - 9.59) | 2.83 (1.72 - 4.59) |
| $EC_{20}$ (95% CL) | 3.10 (1.43 - 6.74) | 1.81 (0.822 - 4.06) | 1.84 (1.29 - 2.61) | 1.48 (0.997 - 2.21) |
| $EC_{10}$ (95% CL) | 0.967 (0.447 - 2.09) | 0.881 (0.385 - 2.02) | 0.966 (0.673 - 1.39) | 1.05 (0.703 - 1.58) |
| NOEC | 0.781 [a)] | | | |
| LOEC | 2.43 [a)] | | | |

[a)] significant differences were found in the 0.238 µg/L (nominal; 2.54 µg/L (actual)). However, as the inhibition of 0.763 µg/L was negative and the CV of the respective control group was low, 0.238 µg/L (nominal; 2.54 µg/L (actual)) was declined not to be the LOEC.

*C. VALIDITY CRITERIA*

The doubling time ($T_d$) of frond numbers in the control was calculated to be 2.3 days (required: < 2.5 d). Therefore, the study fulfilled the validity criterion of OECD 221 (2006).

**Conclusion**

The $E_rC_{50}$ values of meostrione for the freshwater aquatic macrophyte *Wolffia arrhiza* were determined to be 28.9 (95% CL: 10.6 - 75.6) µg a.s./L for frond numbers and 6.28 (95% CL: 4.10 - 9.59) µg a.s./L for dry weight. The validity criterion of OECD 221 (2006) was fulfilled.

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

### A 2.2.2.1 Study 1: Reproductive toxicity on *Daphnia magna*

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 211 and according to the principles of GLP. In the definitive test the validity criteria were met.<br>The study is considered to be reliable and suitable for the risk assessment. All results refer to nominal concentrations. |

| | |
|---|---|
| Reference: | KCP 10.2.2/01 |
| Report | SAE053H/01: Toxicity to the water flea *Daphnia magna* Straus under laboratory conditions (reproduction test), Lang née Zawadsky, C., 2016a, S16-03043 |
| Guideline(s): | OECD 211 (2012) |

| | |
|---|---|
| Deviations: | The spacing factor of maximum 3.2 between the test concentrations was exceeded, as the range-finding test indicated a flat dose response. This deviation is not considered to affect the validity and integrity of the study. |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | - |

**Material and Methods**

**A. MATERIALS**

| | |
|---|---|
| **1. Test material** | SAE053H/01<br>(Other names: Mesotrione/Nicosulfuron 80/30 OD) |
| **Description** | White to beige liquid/cream, OD (oil dispersion) |
| **Lot/Batch #** | 54606-101 |
| **Purity** | Nicosulfuron: 30 g/L nominal; 30.7 g/L analysed<br>Mesotrione: 80 g/L nominal; 81.7 g/L analysed<br>Density: 0.98 g/cm³ |
| **Stability of test material** | Stable under storage conditions (ambient temperature, 5°C – 30°C, in a dark and dry place)<br>Expiry date: 20 Mar 2018 |
| **2. Vehicle and/or positive control** | Vehicle control: test water |
| **3. Test organism** | |
| **Species** | Water flea (*Daphnia magna* Straus) |
| **Strain** | Clone V |
| **Source** | Bred at the test site and originally purchased from the Umweltbundesamt (Federal Environment Agency) in Berlin, Germany. |
| **Age** | At the start of the test, the test animals were less than 24 hours old. |
| **Acclimation period** | The *Daphnia* were bred in culture medium identical to the medium used for the test and under temperature and light conditions identical to those of the test. |
| **Feeding** | During the test, the daphnids were fed daily with green algae of the species *Desmodesmus subspicatus*. From day 1 to 7 at 0.1 mg carbon/daphnia/day, from day 8 to 14 at 0.15 mg carbon/daphnia/day and from day 15 to 21 at 0.2 mg carbon/daphnia/day. |
| **Test units** | The test was performed in 100-mL glass beakers containing 50 mL of test medium. The test vessels were covered with glass plates. |

**4. Environmental conditions**

**Test water**

The test was conducted in reconstituted water (Elendt M4 medium). Analytical grade salts and additives were dissolved in purified water to obtain the following concentrations:

| | |
|---|---|
| $NaHCO_3$ | 64.8 mg/L |
| $K_2HPO_4$ | 0.184 mg/L |
| $KH_2PO_4$ | 0.143 mg/L |
| $MgSO_4 \cdot 7\ H_2O$ | 123 mg/L |
| $Na_2SiO_3 \cdot 9\ H_2O$ | 10 mg/L |
| $CaCl_2 \cdot 2\ H_2O$ | 294 mg/L |
| $NaNO_3$ | 0.274 mg/L |
| KCl | 5.80 mg/L |
| $H_3BO_3$ | 2.86 mg/L |
| $MnCl_2 \cdot 4\ H_2O$ | 0.3605 mg/L |
| $ZnCl_2$ | 0.0130 mg/L |
| $CoCl_2 \cdot 6\ H_2O$ | 0.0100 mg/L |
| $CuCl_2 \cdot 2\ H_2O$ | 0.0118 mg/L |
| $Na_2MoO_4 \cdot 2\ H_2O$ | 0.0615 mg/L |
| $FeSO_4 \cdot 7\ H_2O$ | 0.996 mg/L |
| Titriplex III · 2 $H_2O$ | 2.50 mg/L |
| LiCl | 0.306 mg/L |
| RbCl | 0.071 mg/L |
| $SrCl_2 \cdot 6\ H_2O$ | 0.152 mg/L |
| NaBr | 0.0160 mg/L |
| KI | 0.00325 mg/L |
| $Na_2SeO_3$ | 0.00219 mg/L |
| $NH_4VO_3$ | 0.000575 mg/L |
| Thiamine HCl | 0.0750 mg/L |
| Cyanocobalamine (B12) | 0.00100 mg/L |
| Biotine | 0.0750 mg/L |

**Hardness** 12 – 13° dH, corresponding to 214 and 232 mg/L as $CaCO_3$

**Water temperature** Nominal: 18 – 22°C; actual: 19.7 – 21.7°C

**Lighting** 16 hour light (light intensity: nominal: 1000 – 1500 Lux; actual: 1250 – 1400 Lux) to 8 hour dark photoperiod

**Shaking** During the test, the test media were not aerated.

**B. STUDY DESIGN AND METHODS**

**1. In-life dates** 03 Jul 2016 to 12 Aug 2016

**2. Experimental conditions**

*Test design*

Neonates of *Daphnia magna* were exposed in a semi-static 21-day test to the test substance SAE053H/01 at five concentrations and a test water control. Toxic effects on survival and reproduction of the daphnids were assessed and the test animals were observed for visual abnormalities. The test media of all treatments were renewed each Monday, Wednesday and Friday of the study.

*Number of animals per treatment*

The study was started with ten daphnids per treatment. Each test animal was kept individually in one test unit.

*Test conditions*

The water temperature was maintained at 19.7 – 21.7°C and the test systems were illuminated at a 16 hour light to 8-hour dark photoperiod. The dissolved oxygen concentration in the test media and control was at least 8.7 mg/L. The pH values in the test media and control were between 7.53 and 8.88.

*Test concentrations*

SAE053H/01 was tested at the nominal concentrations of 0.00960, 0.0480, 0.240, 1.20 and 6.00 mg product/L. These concentrations correspond to nominal 0.000800, 0.00400, 0.0200, 0.100, 0.500 mg a.s./L mesotrione and 0.000300, 0.00150, 0.0075, 0.038, 0.188 mg a.s./L nicosulfuron based on the analysed content of active substances and the product density. The spacing factor exceeded the maximum factor given in the guideline as a very flat dose response was observed in the non-GLP range finding test. A control treatment was tested additionally.

*Treatment/Application*

A stock solution was prepared by dissolving 30.0 mg test item in test medium. This solution was slightly turbid. For the remaining test solutions, the stock solution was serially diluted with test medium and thoroughly mixed.

*Analytics*

Analytical samples were taken from all test concentrations and control at test start (fresh), after 2 days (aged), after 9 days (fresh) after 12 days (aged) after 16 days (fresh) and after 19 days (aged). Samples were analysed using HPLC-MS/MS. The analytical method is summarized in Part B, Section 5.

**3. Sampling and measurements**

The test replicates were observed for mortality (immobilisation) of adults daily and dead animals were removed. Observations like eggs in the brood pouch, males or winter eggs were recorded. Obvious differences in condition and size of the parental generation were reported. Offspring was counted and removed daily after appearance of first brood. Time of first production of offspring was also assessed.

Temperature, pH-value, oxygen concentration and total hardness of the test solutions (control, lowest and highest test item concentration, on day 0 in all test item concentrations) were measured after 0 days fresh, 7 days aged and fresh, 14 days aged and fresh and 21 days aged.

For verification of the test item concentrations and stability of the test item, samples from the freshly prepared test solutions and aged test solutions were taken from all concentration levels and the controls at one test solution renewal in the first week including test start, at one test solution renewal in the second week and at one test solution renewal during the third week.

From additional vessels stability control samples were taken, one of these stability control samples was last for 72 hours (weekend), the other for 48 hours, corresponding to the different test medium renewal periods.

**4. Calculation of toxicity**

Mean mortality was calculated for each test concentration and the control.

The mean reproduction rate was calculated for each test concentration and the control as well as the percentage deviation of the mean reproduction rate in the test substance treatments in relation to the control.

**5. Statistics**

No mortality of adult Daphnia above the allowed control mortality was observed in the control and up to and including the concentration level of 1.20 mg product/L. No clear dose-response relation was observed for this parameter therefore no statistical evaluation was performed for mortality data.

A test for normality of the data on reproductive output per parent animal from test start was performed by calculating the Shapiro-Wilk's statistic, a test for homogeneity of the data was performed according to Levene. The NOEC and LOEC were determined by using a multiple comparison method (Jonckheere-Terpstra, left sided). The $EC_{50}$ of reproduction (alive offspring per alive adult at test start) was determined using Probit analysis following the normal procedure.

A test for normality of the data on reproductive output per parent animal alive at test end was performed by calculating the Shapiro-Wilk's statistic, a test for homogeneity of the data was performed according to Levene. The NOEC and LOEC were determined by using a multiple comparison method (Bonferroni-Holms corrected Welch test, left sided). Since no inhibition of reproduction above 50 % was recorded for alive offspring per alive adult at the end of the test, no statistical analysis was performed regarding the $EC_{50}$.

**Results and Discussion**

The measured concentrations of mesotrione in the fresh test item solutions ranged from 82 to 107% of nominal with a mean initial concentration of 89% of nominal. The measured concentration in the aged solution was between 84 and 111% of nominal with a mean concentration of 91% of the nominal test concentration. The measured concentrations of nicosulfuron in the fresh test item solutions ranged from 104 and 151% of nominal with a mean concentration of 111% of nominal. The measured concentration in the aged solution was between 106 and 140% of nominal with a mean concentration of 113% of the nominal concentration (Table A 2.2.2-1). Toxicological endpoints were evaluated using nominal concentrations of the product.

In the control and up to and including the test item concentration of 1.20 mg product/L, no mortality above the allowed control mortality of 20% was observed. In the highest test concentration, 30% mortality was observed (Table A 2.2.2-2).

The mean number of alive offspring at test end per adult from test start was 166.6 ± 28.4 for the control and 72.0 ± 58.7 for the highest test item concentration. Statistically significant inhibitory effects were determined for sum of alive offspring at test end per adult from test start at test item concentrations of 0.240 mg product/L and 6.00 mg product/L. The inhibition to the control was 56.8 % in the highest item concentration of 6.00 mg product/L (Table A 2.2.2-2). The corresponding NOEC for alive offspring at test end per adult at test start was 0.0480 mg product/L, the $EC_{50}$ was extrapolated to 12.1 mg product/L.

The mean number of alive offspring at test end per adult alive at test end was 166.6 ± 28.4 for the control and 102.9 ± 38.2 for the highest test item concentration. Statistically significant inhibitory effects were determined for sum of alive offspring at test end per adult from test end at test item concentration of 6.00 mg product/L. The inhibition to the control was 38.2 % in the highest item concentration of 6.00 mg product/L

(Table A 2.2.2-2). The corresponding NOEC for alive offspring at test end per alive adult at test end was 1.20 mg product/L. No inhibition above 50% occurred and therefore the $EC_{50}$ was estimated to be > 6.00 mg product/L.

**Table A 2.2.2-1: Concentrations of mesotrione and nicosulfuron in the test media**

| **Nominal concentration** | | **Measured concentration of active substance** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **0 h fresh** | | **2 d aged** | | **9 d fresh** | | **12 d aged** | | **16 d fresh** | | **19 d aged** | |
| **[mg prod./L]** | **[mg a.s./L]** | [mg/L] | [%] nom | [mg/L] | [%] nom | [mg/L] | [%] nom | [mg/L] | [%] nom | [mg/L] | [%] nom | [mg/L] | [%] nom |
| Control | | n.d. | - | n.d. | - | n.d. | - | n.d. | - | n.d. | - | n.d. | - |
| **Active substance: mesotrione** | | | | | | | | | | | | | |
| 0.00960 | 0.000800 | 0.000688 | 86 | 0.000673 | 84 | 0.000854 | 107 | 0.000885 | 111 | 0.000712 | 89 | 0.000704 | 88 |
| 0.0480 | 0.00400 | 0.00341 | 85 | 0.00354 | 89 | 0.00364 | 91 | 0.00394 | 99 | 0.00368 | 92 | 0.00368 | 92 |
| 0.240 | 0.0200 | 0.01704 | 85 | 0.0179 | 90 | 0.0187 | 94 | 0.0181 | 91 | 0.0179 | 90 | 0.0192 | 96 |
| 1.20 | 0.100 | 0.0894 | 89 | 0.0853 | 85 | 0.0884 | 88 | 0.0932 | 93 | 0.0853 | 85 | 0.0870 | 87 |
| 6.00 | 0.500 | 0.437 | 87 | 0.429 | 86 | 0.439 | 88 | 0.441 | 88 | 0.411 | 82 | 0.431 | 86 |
| **Active substance: nicosulfuron** | | | | | | | | | | | | | |
| 0.00960 | 0.000300 | 0.000318 | 106 | 0.000322 | 107 | 0.000454 | 151 | 0.000419 | 140 | 0.000335 | 112 | 0.000332 | 111 |
| 0.0480 | 0.00150 | 0.00156 | 104 | 0.00161 | 107 | 0.00166 | 111 | 0.00180 | 120 | 0.00171 | 114 | 0.00167 | 111 |
| 0.240 | 0.0075 | 0.00788 | 105 | 0.00794 | 106 | 0.00819 | 109 | 0.00860 | 115 | 0.00807 | 108 | 0.00883 | 118 |
| 1.20 | 0.038 | 0.0400 | 105 | 0.0413 | 109 | 0.0420 | 111 | 0.0416 | 109 | 0.0402 | 106 | 0.0419 | 110 |
| 6.00 | 0.188 | 0.205 | 109 | 0.209 | 111 | 0.205 | 109 | 0.211 | 112 | 0.201 | 107 | 0.209 | 111 |

$LOQ_{mesotrione}$ = 0.000250 mg/L; $LOQ_{nicosulfuron}$ = 0.0000939 mg/L

- not applicable

n.d. not determined#

**Table A 2.2.2-2: Effects of SAE053H/01 on survival and reproduction of *Daphnia magna***

| Nominal concentration [mg product/L] | Mean mortality after 21 days [%] | Mean reproduction rate | | | |
|---|---|---|---|---|---|
| | | Alive offspring per adult from test start | | Alive offspring per surviving adult | |
| | | mean ± SD | [%] inhibition | mean ± SD | [%] inhibition |
| Control | 0 | 166.6 ± 28.4 | - | 166.6 ± 28.4 | - |
| 0.00960 | 10 | 155.7 ± 34.8 | 6.5 | 165.2 ± 18.5 | 0.8 |
| 0.0480 | 10 | 167.0 ± 18.5 | -0.2 | 168.0 ± 19.4 | -0.8 |
| 0.240 | 10 | 133.9* ± 34.3 | 19.6 | 143.0 ± 19.8 | 14.2 |
| 1.20 | 0 | 160.8 ± 15.0 | 3.5 | 160.8 ± 15.0 | 3.5 |
| 6.00 | 30 | 72.0* ± 58.7 | 56.8 | 102.9* ± 38.2 | 38.2 |
| **Endpoints [mg product/L]** | | | | | |
| 21-day $EC_{50}$ | > 6.00 | 12.1 | | > 6.00 | |
| 21-day NOEC | 1.20 | 0.0480 | | 1.20 | |
| 21-day LOEC | 6.00 | 0.240 | | 6.00 | |

* Statistically significantly different from control according to Jonckheere-Terpstra test, left sided or Bonferroni-Holms corrected Welch test, left sided

The validity criteria of the test were fulfilled: mortality in the control ≤ 20% (actual: 0%); mean reproduction rate in the control ≥ 60 living offspring per surviving adult at the end of the test (actual: 166.6 living offspring).

**Conclusion**

In this test on survival and reproduction of *Daphnia magna*, the 21-day overall NOEC and the extrapolated $EC_{50}$ (reproduction) of SAE053H/01 were determined to be 0.0480 mg product/L and 12.1 mg product/L, respectively.

## A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

No additional data submitted.

## A 2.3 KCP 10.3 Effects on arthropods

## A 2.3.1 KCP 10.3.1 Effects on bees

### A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

#### A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

## A 2.3.1.1.1.1 Study 1: Acute oral and contact toxicity to the honeybee

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 213, 214 and according to the principles of GLP.<br>The study is considered to be reliable and suitable for the risk assessment. |

| | |
|---|---|
| Reference: | KCP 10.3.1.1/01 |
| Report | SAE053H/01 – Acute oral and contact toxicity to the honey bee, *Apis mellifera* L. under laboratory conditions, Molitor, A.M., 2016a, S16-02516 |
| Guideline(s): | Yes, OECD 213 and 214 (1998) |
| Deviations: | Yes<br>For the contact toxicity test, a droplet of 2 µL was chosen in deviation to the OECD Guideline 214 recommendation of 1 µL, since a higher volume ensures a more reliable dispersion of the test item. However, no adverse effects were to be expected on the outcome of the study since experience of the test facility was proven that higher volumes are suitable.<br>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. This had no effect on the outcome of the study. |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | - |

**Materials and methods**

**A. MATERIALS**

| | |
|---|---|
| **1. Test material** | SAE053H/01<br>(Other name: Mesotrione/Nicosulfuron 80/30 OD) |
| **Description** | White to beige liquid/cream, OD (oil dispersion) |
| **Lot/Batch #** | 54606-101 |
| **Purity** | Nicosulfuron: 30 g/L nominal; 30.7 g/L analysed<br>Mesotrione: 80 g/L nominal; 81.7 g/L analysed<br>Density: 0.98 g/cm³ |
| **Stability of test material** | Stable under storage conditions (ambient temperature, 5 – 30°C, in a dark and dry place)<br>Expiry date: 20 Mar 2018 |
| **2. Vehicle and/or positive control** | Vehicle oral toxicity test: aqueous sucrose solution (50%, w/v)<br>Vehicle contact toxicity test: deionised water<br>Positive control: reference item |
| **Reference item** | Perfekthion (BAS 152 11 I) |
| **Description** | blue liquid |

| | |
|---|---|
| **Lot/Batch #** | FRE-001226 |
| **Purity** | 400.0 g/L dimethoate (nominal content)<br>420.3 g/L dimethoate (analysed content)<br>density: 1.072 g/cm³ |
| **Stability of reference item** | Stable under storage conditions (at room temperature, typically 25°C or cooler)<br>Expiry date: 10 Apr 2017 |

**3. Test organism**

| | |
|---|---|
| **Species** | Honeybee, *Apis mellifera* L. (Hymenoptera, Apidae),<br>Adult worker bees |
| **Source** | Healthy colony of the test facility's own stock, Eutinger Straße 24, 75223 Niefern-Öschelbronn, Germany<br>The hive used for honey bee collection for this test was adequately fed, healthy, as far as possible disease-free and queen-right. |
| **Acclimatisation** | One day prior to test start, the bees were randomly collected from the combs of the colony, introduced into the test units and kept under test conditions until the start of the test. During the acclimatisation period, they were fed ad libitum with untreated 50% (w/v) aqueous sucrose solution. |
| **Diet** | Oral toxicity test:<br>The bees were starved for approx. 2 hours prior to application start. Each unit was provided with the application solution for up to 6 hours, to ensure a sufficient uptake. The feeders were then removed and the bees were provided ad libitum with untreated 50% (w/v) aqueous sucrose solution.<br>Contact toxicity test:<br>During the test phase, the bees were supplied *ad libitum* with 50% (w/v) aqueous sucrose solution. |
| **Test units** | In both test procedures, the bees were kept in cages made of stainless steel (base: 8 cm x 4 cm; height: 6 cm). The front side of the cages was equipped with a transparent pane to enable observation. The bottom of the cages consisted of perforated steel, which guaranteed sufficient air supply. The cages were lined with filter paper. |

**4. Environmental conditions**

| | |
|---|---|
| **Temperature** | Nominal: 25 ± 2°C; actual: 24.5 – 25.2°C (oral toxicity test), 24.5 – 25.0°C (contact toxicity test) |
| **Relative humidity** | Nominal: 50 - 70%; actual: 56.3 – 61.6% (oral toxicity test), 58.0 – 65.7% (contact toxicity test) |
| **Photoperiod** | During the experimental phase, the bees were kept in darkness, except during application and assessments. |

## B. STUDY DESIGN AND METHODS

**1. In life dates** 06 Jul 2016 to 22 Jul 2016

**2. Experimental conditions**

*Test design*

Lethal effects of the test substance on the honeybee, *Apis mellifera* L., after oral and contact exposure were assessed at five doses of SAE053H/01 under laboratory conditions. In addition, one control and a reference item (four doses) were tested. For the oral treatment, the test substance was provided via feeding solution. For the contact treatment, the test substance was applied to the dorsal part of the thorax. Bee mortality and sublethal effects were assessed.

*Number of animals/treatment*

Ten bees/replicate; four replicates/test and reference substance treatment and control

*Doses tested*

Oral toxicity test
SAE053H/01 was tested at nominally 62.5, 125, 250, 500 and 1000 µg product/bee. The actual ingested doses were 68.71, 132.03, 262.22, 469.15 and 655.01 µg product/bee. A control group, receiving untreated 50% (w/v) aqueous sucrose solution, was tested in parallel.

A stock solution (1000 µg product/2 µL) was prepared for both, oral and contact toxicity test by filling up 5000 mg SAE053H/01 to a final volume of 10 mL with deionized water. For the oral test, the highest test dose solution was prepared by filling up 1 mL of this stock solution to 10 mL with a 50% (w/v) aqueous sucrose solution. All further test dose solutions were obtained by diluting the highest test dose solution with a 50% (w/w) aqueous sucrose solution.

Contact toxicity test
SAE053H/01 was tested at 62.5, 125, 250, 500 and 1000 µg product/bee. A control group was treated with deionised water.

The stock solution was prepared as described above and was equal to the highest tested dose. The remaining test doses were obtained by dilution of the stock solution with deionised water.

*Reference item*

In the oral toxicity test, Perfekthion was tested at nominally 0.06, 0.08, 0.11 and 0.14 µg dimethoate/bee. The actually ingested dose was 0.06, 0.09, 0.12 and 0.14 µg a.s./bee. For the contact toxicity test, Perfekthion was tested at 0.13, 0.17, 0.22 and 0.29 µg dimethoate/bee.

For both tests, a stock solution was prepared by weighing in 146.7 mg Perfekthion and filling it up to 20 mL with deionised water. From this stock solution a second stock solution was prepared with 1 mL of the first stock solution filled up with deionised water to 20 mL. This second stock solution was equal to the highest tested dose in the contact toxicity test. The remaining contact test dose solutions were prepared by diluting the second stock solution with deionised water. The oral test dose solutions were prepared by either diluting the second stock solution or by diluting the highest tested dose with 50% (w/w) aqueous sucrose solution.

*Treatment/Application*

Oral toxicity test
A quantity of 220 µL of test or reference substance application solution was offered to each cage of ten bees. Bees within a cage shared the test solution (trophallaxis) and therefore were assumed to have each received a similar dose. It was calculated that 20 µL of application solution contained the required nominal amount of test or reference substance per bee, even though 22 µL per bee were provided. A higher volume was chosen, since a certain amount of the solution stays in the feeder and cannot be reached by the bees.

Test facility experience has proven that higher volumes are suitable and no adverse effects on the outcome of the study are to be expected. The actual amount of test solution consumed by each replicate was determined by weighing the feeders (Eppendorf cups) before and after feeding. Each test unit was provided with the application solution for up to 6 hours, to ensure a sufficient uptake. During the subsequent observation period, the bees were supplied *ad libitum* with untreated 50% (w/v) aqueous sucrose solution. In the control group, the bees were fed with 220 µL of 50% (w/v) aqueous sucrose solution for up to 6 hours and thereafter were fed *ad libitum* with 50% (w/v) aqueous sucrose solution.

Contact toxicity test
After the bees had been anaesthetised with carbon dioxide, they were treated individually by applying 2 µL of deionised water (control), test or reference substance application solution dorsally to the thorax of the bee. Application was performed using a micro-applicator (Burkard Ltd.). Between every application, the outside of the micro-applicator needle was cleaned with a mixture of water and a water-wetting agent. This reduced the surface tension of the applied solution and ensured that the drop spread out immediately after application on each bee. After treatment, the bees were returned to the test cages and fed with a 50% (w/v) aqueous sucrose solution *ad libitum.*

**3. Observations and assessments**

Mortality of the bees was assessed at 4, 24 and 48 hours after test start (start of feeding or after contact application). At the same observation times, behavioural abnormalities such as symptoms of poisoning were assessed in the test substance and control group.

The consumption of application solution per replicate was determined by weighing the feeders at the start and at the end of the feeding application period. For each treatment group, the mean consumption of application solution per replicate was calculated by averaging the replicate values.

The test temperature and relative humidity were continuously recorded.

**4. Calculation of toxicity**

The percentage of mortality was calculated for each treatment group from the number of dead individuals in relation to the number of introduced test organisms. Mortality in the treatments was corrected for the mortality of the control group according to the formula of Abbott (1925), modified by Schneider-Orelli (1947).

**5. Statistics**

The $LD_{50}$ and its 95% confidence limits for the reference substance treatment were calculated by means of a Probit analysis using maximum likelihood regression. The statistical software program ToxRat Professional 3.2.1 was used for analysis. Since effects of the test substance on bee survival were < 50% up to the highest test dose, the $LD_{50}$ was directly estimated from the raw data.

**Results and Discussion**

## A. ORAL TOXICITY TEST

The actual consumed doses of SAE053H/01 in the treatments of nominal 62.5, 125, 250, 500 and 1000 µg product/bee were 68.71, 132.03, 262.22, 469.15 and 655.01 µg product/bee.

At the end of the test after 48 hours, mean mortality in the control and in the reference substance treatments was 2.5% and 0.0-94.9% (corrected), respectively. In the test substance treatments, corrected mortality was between -2.6 and 5.1% at study end (see following table). The 48-hour oral $LD_{50}$ for SAE053H/01 could not be calculated but was estimated to be > 655.01 µg product/bee.

Affected bees were recorded at the four highest test doses mostly 4 hours after test start. Single affected and moribund bees were observed after 48 hours in the two highest dose levels tested.

**Table 9.10.2.4-1: Oral toxicity of SAE053H/01 to honey bees (*Apis mellifera* L.)**

| Dose | | Mean mortality [%] | | | |
|---|---|---|---|---|---|
| Target [µg product/bee] | Actual uptake [µg product/bee] | 24 h | 48 h | 24 h corrected | 48 h corrected |
| Control | - | 2.5 | 2.5 | - | - |
| 62.5 | 68.71 | 0.0 | 2.5 | -2.6 | 0.0 |
| 125 | 132.03 | 0.0 | 0.0 | -2.6 | -2.6 |
| 250 | 262.22 | 0.0 | 2.5 | -2.6 | 0.0 |
| 500 | 469.15 | 0.0 | 7.5 | -2.6 | 5.1 |
| 1000 | 655.01 | 0.0 | 5.0 | -2.6 | 2.6 |
| **Reference substance: Perfekthion (active substance: dimethoate)** | | | | | |
| 0.06 µg a.s./bee | 0.06 µg a.s./bee | 0.0 [a)] | 2.5 [a)] | -2.6 [a)] | 0.0 [a)] |
| 0.08 µg a.s./bee | 0.09 µg a.s./bee | 0.0 [a)] | 22.5 [a)] | -2.6 [a)] | 20.5 [a)] |
| 0.11 µg a.s./bee | 0.12 µg a.s./bee | 55.0 [a)] | 70.0 [a)] | 53.8 [a)] | 69.2 [a)] |
| 0.14 µg a.s./bee | 0.14 µg a.s./bee | 90.0 [a)] | 95.0 [a)] | 89.7 [a)] | 94.9 [a)] |
| **Endpoint [µg product/bee]** | | | | | |
| 48-hour $LD_{50}$ (ingested) | > 655.01 | | | | |

[a)] Not provided in the report, calculated by the applicant based on individual results

The test is considered to be valid since mortality in the control was 2.5% (required ≤ 10%) and the 24-hour $LD_{50}$ of the reference item was 0.12 µg a.s./bee (required 0.10 - 0.35 µg a.s./bee).

## B. CONTACT TOXICITY TEST

At the end of the test after 48 hours, mean mortality in the control and in the reference substance treatments was 5.0% and 5.3-89.5% (corrected), respectively. In the test substance treatment, corrected mortality was between -5.3 and 13.2% at study end (see following table). The 48-hour contact $LD_{50}$ for SAE053H/01 could not be calculated but was estimated to be > 1000 µg product/bee.

Affected or moribund bees were recorded at the three highest test doses during all assessments.

**Table 9.10.2.4-2: Contact toxicity of SAE053H/01 to honey bees (*Apis mellifera* L.)**

| Actual dose [µg product/bee] | Mean mortality [%] | | | |
|---|---|---|---|---|
| | 24 h | 48 h | 24 h corrected | 48 h corrected |
| Control | 2.5 | 5.0 | - | - |
| 62.5 | 0.0 | 0.0 | -2.6 | -5.3 |
| 125 | 0.0 | 0.0 | -2.6 | -5.3 |
| 250 | 5.0 | 5.0 | 2.6 | 0.0 |
| 500 | 7.5 | 17.5 | 5.1 | 13.2 |
| 1000 | 5.0 | 10.0 | 2.6 | 5.3 |
| **Reference substance: Perfekthion (active substance: dimethoate)** | | | | |
| 0.13 µg a.s./bee | 10.0 a) | 10.0 a) | 7.7 a) | 5.3 a) |
| 0.17 µg a.s./bee | 35.0 a) | 52.5 a) | 33.3 a) | 50.0 a) |
| 0.22 µg a.s./bee | 57.5 a) | 65.0 a) | 56.4 a) | 63.2 a) |
| 0.29 µg a.s./bee | 85.0 a) | 90.0 a) | 84.6 a) | 89.5 a) |
| **Endpoint [µg product/bee]** | | | | |
| 48-hour $LD_{50}$ | > 1000 | | | |

a) Not provided in the report, calculated by the applicant based on individual results

The test is considered to be valid since mortality in the control was 5.0% (required ≤ 10%) and the 24-hour $LD_{50}$ of the toxic standard was 0.20 µg a.s./bee (required 0.10 - 0.30 µg a.s./bee).

**Conclusion**

In this acute toxicity test with the honeybee *Apis mellifera* L., the 48-hour oral and contact $LD_{50}$ of SAE053H/01 could not be calculated but were estimated to be above the highest tested dose (i.e. > 655.01 µg product/bee in the oral test and > 1000 µg product/bee in the contact test). All validity criteria were fulfilled.

## A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

Please refer to the combined toxicity study under A 2.3.1.1.1.

## A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

## A 2.3.1.2.1 Study 1: Chronic toxicity to the honeybee

| | |
|---|---|
| Comments of zRMS: | The study was conducted according to the methodologies available at the time and according to the principles of GLP. No deviations to the guideline were noted. The study is considered to be valid. |

| | |
|---|---|
| Reference: | KCP 10.3.1.2/01 |
| Report | SAE053H/01 – Assessment of effects on the adult honey bee, *Apis mellifera* L., in a 10 day chronic feeding test under laboratory conditions, Molitor, A.M., 2016b, S16-02518 |

| | |
|---|---|
| Guideline(s): | No, based on OECD Proposal for a new OECD Guideline (2016) and on the publication by Kling, A. and Schmitzer, S. (2015) |
| Deviations: | Not applicable |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | - |

**Materials and Methods**

**A. MATERIALS**

| | |
|---|---|
| **1. Test material** | SAE053H/01<br>(Other name: Mesotrione/Nicosulfuron 80/30 OD) |
| **Description** | White to beige liquid/cream, OD (oil dispersion) |
| **Lot/Batch #** | 54606-101 |
| **Purity** | Nicosulfuron: 30 g/L nominal; 30.7 g/L analysed<br>Mesotrione: 80 g/L nominal; 81.7 g/L analysed<br>Density: 0.98 g/cm³ |
| **Stability of test material** | Stable under storage conditions (ambient temperature, 5 – 30°C, in a dark and dry place)<br>Expiry date: 20 Mar 2018 |
| **2. Vehicle and/or positive control** | Vehicle: aqueous sucrose solution (50%, w/v)<br>Positive control: reference item |
| **Reference item** | Perfekthion (BAS 152 11 I) |
| **Description** | blue liquid |
| **Lot/Batch #** | FRE-001226 |
| **Purity** | 400.0 g/L dimethoate (nominal content)<br>420.3 g/L dimethoate (analysed content)<br>density: 1.072 g/cm³ |
| **Stability of reference item** | Stable under storage conditions (at room temperature, typically 25°C or cooler)<br>Expiry date: 10 Apr 2017 |
| **3. Test organism** | |
| **Species** | Honey bee, *Apis mellifera* L. (Hymenoptera, Apidea) |
| **Source** | Healthy colony of the test facility's own stock, Eutinger Straße 24, 75223 Niefern-Öschelbronn, Germany<br>The hive used for honey bee collection for this test was adequately fed, healthy, as far as possible disease-free and queen-right. |
| **Age** | Young adult worker bees (newly hatched; 1 to 2 days old) |
| **Pre-treatment culturing conditions** | Up to two days prior to test start, brood combs containing capped cells which are expected to hatch on the same day were taken |

| | |
|---|---|
| | out of a honey bee colony and transferred into the climatic chamber. The combs were kept under test conditions. One day prior to test start, the 0- to 1-day old bees were picked off the combs, transferred to the test cages and kept under test conditions until the start of the test. During acclimatisation, the bees were fed *ad libitum* with untreated 50% (w/v) aqueous sucrose solution. |
| **Diet** | The bees were fed with a 50% (w/v) sucrose solution containing either the test item or the reference item or pure 50% (w/v) sucrose solution (untreated control group). The treated and untreated food was offered using syringes which were replaced daily by a new one containing freshly treated or untreated food. |
| **Test units** | The bees were kept in cages made of stainless steel (base: 8 cm x 4 cm; height: 6 cm). The front side of the cages was equipped with a transparent pane to enable observation. The bottom of the cages consisted of perforated steel, which guaranteed sufficient air supply. The cages were lined with filter paper. |

**4. Environmental conditions**

| | |
|---|---|
| **Temperature** | nominal: 33 ± 2°C, actual: 31.7 – 33.2°C |
| **Relative humidity** | nominal: 50-70%, actual: 41.5* – 66.2%<br>* short-term deviation < 2 hours |
| **Photoperiod** | During the test, the bees were kept in darkness except during application and assessments. |

## B. STUDY DESIGN AND METHODS

**1. In-life dates** 28 Jun 2016 to 19 Jul 2016

**2. Experimental conditions**

*Test design*

In a 10-day chronic test, young adults of *Apis mellifera* L. were daily exposed to five doses of SAE053H/01 in 50% (w/v) aqueous sucrose solution. In parallel, an untreated control (50% (w/v) aqueous sucrose solution) and one dose of the reference item Perfekthion (active substance: dimethoate) were tested. Assessments of bee mortality and behavioural abnormalities were done daily during the study.

*Number of animals per treatment*

Four replicates per test and reference substance treatment and untreated control were used with 10 bees per replicate. Additionally, four test units without bees but with full food syringes containing pure 50% (w/v) aqueous sucrose solution were placed in the climatic chamber to evaluate evaporation.

*Test doses*

SAE053H/01 was tested at nominally 250, 500, 1000, 2000 and 4000 mg product/kg diet. A control group, receiving untreated 50% (w/v) aqueous sucrose solution, was tested in parallel.

*Reference item*

The reference item, Perfekthion (400 g/L dimethoate) was tested at a single dose concentration of 0.9 mg dimethoate/kg diet.

*Treatment/Application*

The 50% (w/v) sucrose solution was prepared with deionised water and stored under cool and dark conditions (refrigerator, ca. 6 ± 2°C) for a maximum period of 4 days. Three to four milliliters of treated/untreated diet were provided to the test organisms of each test unit in a plastic syringe with removed tip. Every morning during ten days, the syringes of all test cages were replaced by new syringes, filled with freshly prepared feeding solutions. The weight of the syringes was determined before and after feeding on the next day in order to determine the mean food consumption of the bees per replicate.

Test item: The feeding solutions were prepared freshly every day. A stock solution which served also as the feeding solution of the highest test concentration was prepared either by weighing in 0.476 g of the test item and filling up to 100 mL with 50% (w/v) aqueous sucrose solution or by weighing in 0.238 g of the test item and filling up to 50 mL with 50% (w/v) aqueous sucrose solution. Further dilutions of this feeding solution were prepared using 50% (w/v) aqueous sucrose solution to get the lower concentration levels of the feeding solutions.

Reference item: On day 1, 4 and 7, an amount of 91.0 or 91.1 mg of the reference item was diluted to 10 mL with deionised water to prepare a stock solution. From this stock solution, a second stock solution was prepared by dilution of 0.5 mL to 50 mL with deionised water. Every day, the feeding solution of the reference item was prepared by filling up 0.6 mL of the second stock solution to 20 mL with 50% (w/v) aqueous sucrose solution.

Controls: For the untreated control, a 50% (w/v) sucrose solution was used.

*Analytics*

Each day, analytical samples of the control and test item feeding solutions of all concentrations were taken for dose verification. No samples of the reference item feeding solutions were taken. The samples were analysed using HPLC-MS/MS. Details to the analytical method are summarized in Part B, Section 5.

**3. Observations and assessments**

Mortality and behavioural abnormalities were assessed every 24 hours (± 2 hours), ten days following start of exposure. In the reference item treatment group, behavioural assessments were not conducted as it was assumed that moribund and affected bees of the reference item treatment group would die by the end of the test.

The amount of feeding solution consumed was determined daily by weighing the feeders before and after feeding. The feeding syringes were replaced daily. Also, the syringes of the empty test units were weighed before and after insertion to determine evaporation.

Test temperature and humidity were recorded continuously with appropriate, calibrated equipment.

**4. Calculation of toxicity**

The percentage of cumulative mortality was calculated for each treatment group and assessment from the number of dead individuals in relation to the number of introduced test organisms. The cumulative mortality

of the test and reference item treatments was corrected for corresponding control mortality according to the formula of Abbott (1925), modified by Schneider-Orelli (1947).

The consumption of feeding solution per bee per day was calculated by dividing the total daily consumption per replicate by the number of living bees at the beginning of the respective feeding interval. For each treatment group, the mean consumption of feeding solution per bee per day was calculated by averaging the replicate values. The evaporation out of the food syringes was determined by daily weighing of the syringes in the respective, additional test cages. A mean value of evaporation per day was determined over the whole test period and the daily food consumption of the control, the test item and reference item treatments was corrected by the mean value of the corresponding day.

**5. Statistics**

The $LC_{50}$ and $LDD_{50}$ could not be calculated as the data did not fit any statistical model.
Step-down Cochran-Armitage Test Procedure (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there were statistically significant differences between the mortality data of the control and the test item treatment groups and to determine the NOEC and NOEDD based on mortality.

Statistical calculations were made by using the statistical program TOXRAT Professional 3.2.1.

**Results and Discussion**

The actual concentrations of mesotrione in the feeding solutions, determined directly before the first application, were in the range of 97% to 100% of the nominal concentrations. The actual concentrations of nicosulfuron in the feeding solutions determined directly before the first application were in the range of 88% to 97% of the nominal concentrations. No residues of mesotrione or nicosulfuron above the LOQ (2.08 and 0.783 mg a.s./L, respectively) were found in any of the control samples.

In this chronic toxicity feeding test, the mean uptake of SAE053H/01 by bees was 10.1, 20.1, 41.8, 84.2 and 138.21 µg product/bee/day for the test concentrations of 250, 500, 1000, 2000 and 4000 mg product/kg diet, respectively. For the reference item, the mean uptake by bees was 0.02 µg dimethoate/bee/day at the tested concentration of 0.9 mg dimethoate/kg diet.

Cumulative mortality in the control was 7.5% after ten days. The test item SAE053H/01 had no statistically significant effects on honeybee mortality at all tested concentrations after ten days when compared to the control. Cumulative mortalities ranged between 5.0% (-2.7% corrected) and 10.0% (2.7% corrected) (Table **Błąd! Użyj karty Narzędzia główne, aby zastosować Überschrift 4 do tekstu, który ma się tutaj pojawić.**-1). The reference item showed 100.0% (100.0% corrected) cumulative mortality at the end of the test.

No remarkable behavioural abnormalities were observed in all test item treatment groups.

The 10-day $LC_{50}$ could not be determined but was greater than 4000 mg product/kg diet, corresponding to a 10-day $LDD_{50}$ of greater than 138.21 µg product/bee/day. The 10-day NOEC was determined to be the highest test concentration of 4000 mg product/kg diet, corresponding to a 10-day NOEDD of 138.21 µg product/bee/day.

**Table** Błąd! Użyj karty Narzędzia główne, aby zastosować Überschrift 4 do tekstu, który ma się tutaj pojawić.**-1: Mortality of bees in the chronic toxicity feeding test after 10 days**

| Treatment group | Test concentration [mg product/kg diet] | Dose level consumed [µg product /bee/day] | Cumulative mortality after 10 days [%] | Corrected cumulative mortality after 10 days [%] |
|---|---|---|---|---|
| Control | 0.0 | 0.0 | 7.5 | - |
| Test item SAE053H/01 | 250 | 10.1 | 5.0 | -2.7 |
| | 500 | 20.1 | 7.5 | 0.0 |
| | 1000 | 41.8 | 5.0 | -2.7 |
| | 2000 | 84.2 | 10.0 | 2.7 |
| | 4000 | 138.21 | 10.0 | 2.7 |
| Reference item Perfekthion (a.s. dimethoate) | 0.9 mg a.s./kg diet | 0.02 µg a.s./bee/day | 100.0 | 100.0 |
| **10-day endpoints** | | | | |
| **10-day $LC_{50}$ (95% confidence limits)** | > 4000 mg product/kg diet (not determinable) | | | |
| **10-day $LDD_{50}$[1)] (95% confidence limits)** | > 138.21 µg product/bee/day (not determinable) | | | |
| **10-day NOEC** | 4000 mg product/kg diet | | | |
| **10-day NOEDD[1)]** | 138.21 µg product/bee/day | | | |

Note: No statistically significant differences in cumulative mortality of the test item treatment groups when compared to the control group were observed according to Step-down Cochran-Armitage Test Procedure (one-sided greater, $\alpha = 0.05$).
[1)] Based on consumed dose

The validity criteria were met (mean mortality in the control was below 15%, exact value: 7.5%), the average mortality in the reference item treatment was ≥ 50% at the end of the test (exact value: 100%).

**Conclusion**

In this 10-day chronic toxicity feeding study with SAE053H/01 in the honey bee, the 10-day $LDD_{50}$ could not be determined but was greater than 138.21 µg product/bee/day and the 10-day NOEDD was determined to be 138.21 µg product/bee/day (based on consumed dose). The validity criteria of the draft guidance document were fulfilled.

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

### A 2.3.1.3.1 Study 1: Toxicity to honey bee larvae

| | |
|---|---|
| Comments of zRMS: | The study was conducted according to the methodologies available at the time and according to the principles of GLP. No deviations to the guideline were noted. The study is considered to be valid. |

Reference: KCP 10.3.1.3/01

| | |
|---|---|
| Report | SAE053H/01 – Honey bee (*Apis mellifera* L.) larval toxicity test (repeated exposure), Vergé, E. & Wagner, J., 2016, S16-02503 |
| Guideline(s): | Yes, OECD draft guidance document for larval toxicity test, repeated exposure (July, 2015) |
| Deviations: | Yes, the relative humidity was lower than the preferred range of 95 ± 5% twice for more than two hours. This deviation is not considered to affect the validity and integrity of the study. |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | - |

**Materials and Methods**

**A. MATERIALS**

| | |
|---|---|
| **1. Test material** | SAE053H/01<br>(Other name: Mesotrione/Nicosulfuron 80/30 OD) |
| **Description** | White to beige liquid/cream, OD (oil dispersion) |
| **Lot/Batch #** | 54606-101 |
| **Purity** | Nicosulfuron: 30 g/L nominal; 30.7 g/L analysed<br>Mesotrione: 80 g/L nominal; 81.7 g/L analysed<br>Density: 0.98 g/cm³ |
| **Stability of test material** | Stable under storage conditions (ambient temperature, 5 – 30°C, in a dark and dry place)<br>Expiry date: 20 Mar 2018 |
| **2. Vehicle and/or positive control** | Vehicle: aqueous sucrose solution (50%, w/v)<br>Positive control: reference item |
| **Reference item** | Dimethoate tech. (BAS 152 I) |
| **Description** | White to grey solid; TC (technical compound) |
| **Lot/Batch #** | 35015A161 |
| **Purity** | 98.8% (w/w) |
| **Stability of reference item** | Stable under storage conditions (cool (below 10°C), dark and dry)<br>Expiry date: 20 Jan 2017 |
| **3. Test organism** | |
| **Species** | Honeybee, *Apis mellifera carnica* Pollmann (Hymenoptera, Apidea) |
| **Source** | Three healthy colonies maintained at test facility |
| **Age** | Synchronized first instar (L1) larvae |
| **Pre-treatment culturing conditions** | The hives used for larvae collection were adequately fed, healthy, disease-free and with known history and physiological status. No |

chemicals (antibiotics, anti-*Varroa* treatments, pesticides) had been used in the hives within 4 weeks preceding test start. The honeybee colonies were inspected periodically according to standard bee-keeping practices.

**Method of producing L1 larvae:**

Each of the three colonies used in the test was treated in parallel in the same way: On day -3, the queens of seven colonies were confined, each one in her own colony in an excluder cage containing an empty comb. The caging time was max. 30 hours. On day -2, the queen was released from the cage. The comb containing the eggs was left in the cage, near the brood during the incubation stage and until hatching (day 1). At day 1, combs were transferred to the laboratory using an insulated container.

**Diet**

The food was composed of three different artificial diets which were adapted to the needs of the larvae at different stages of development:

- Diet A (day 1): 50% royal jelly + 50% aqueous solution containing 2% yeast extract, 12% glucose and 12% fructose
- Diet B (day 3): 50% royal jelly + 50% aqueous solution containing 3% yeast extract, 15% glucose and 15% fructose
- Diet B (day 4-6): 50% royal jelly + 50% aqueous solution containing 4% yeast extract, 18% glucose and 18% fructose

**Test units**

Crystal polystyrene grafting cells (diameter 9 mm) were sterilized with ethanol and placed in 48 well plates. Plates were placed into a hermetic Plexiglass desiccator containing saturated potassium sulphate solution. Desiccators were placed into an incubator with forced air circulation.

**4. Environmental conditions**

**Temperature**

Preferably: 34 - 35°C, but not below 23°C or above 40°C; actual: 24.8 – 34.7°C

**Relative humidity**

Preferably: 95 ± 5%; actual: 47.3 – 100.0%

**Photoperiod**

During the test, the bees were kept in darkness except during observations.

**B. STUDY DESIGN AND METHODS**

**1. In-life dates**

06 Jun 2016 to 07 Jul 2016

**2. Experimental conditions**

*Test design*

The effects of the test substance SAE053H/01 to honey bee larvae (*Apis mellifera*) were assessed in an 8-d chronic toxicity test. Honey bee larvae were either treated with the test item at five concentrations, the reference item dimethoate tech. at a single concentration or remained untreated (control).

*Number of animals per treatment*

16 larvae/replicate; 3 replicates/test and reference substance treatment and control

*Test doses*

The toxicity of SAE053H/01 was determined at 230, 576, 1440, 3600 and 9000 mg product/kg diet, corresponding to nominal 19.2, 48.0, 120, 300 and 750 mg a.s./kg diet mesotrione and 7.20, 18.0, 45.1, 113 and 282 mg a.s./kg diet nicosulfuron based on the analysed content of active substances in the formulation and the product density. The test item concentrations were equivalent to cumulative doses of 35.4, 88.7, 222, 554 and 1390 µg product/larva. A control group, receiving untreated artificial diet, was tested in parallel.

*Reference item*

The reference item, dimethoate tech. was tested at a concentration of 7.39 µg a.s./larva (equivalent to 48.0 mg dimethoate tech./kg diet).

*Treatment/Application*

Stock solutions of the test and reference item were prepared by dissolving 990 mg and 133.6 mg, respectively, in autoclaved, deionized water and were used to prepare the treated diets via serial dilation. The treated diets were prepared daily and warmed in an incubator before use. The test item stock solution was prepared freshly at each application day.

At test start, 20 µL of diet A (without test item) was dropped into each cell, and then one larva was grafted from the comb to the cell, onto the surface of the diet, using a grafting tool. All larvae were fed once a day (except at day 2). At day 3, 20 µL of diet B (including test item) were administered to each larva. At day 4, 5 and 6, larvae were fed with 30, 40 and 50 µL of diet C (including test item), respectively.

**3. Observations and assessments**

From day 4 to day 8, dead larvae were counted with the help of a stereo microscope and then removed. Other observations (larval appearance and size) were recorded to aid the interpretation of mortality. On day 8, the presence of uneaten food was qualitatively recorded.

Analytical dose verification was performed on the treated larval diet from day 3 to day 6 and on the control diets of the same days. The concentrations of mesotrione and nicosulfuron were analysed by HPLC-MS/MS. Details to the analytical method are summarized in Part B, Section 5.

**4. Calculation of toxicity**

Mortality was evaluated on day 4, 5, 6, 7 and 8. The cumulative mortality [%] for each treatment group was calculated from the number of dead larvae in relation to the total number of larvae per treatment group across all replicates after re-grafting on day 3. The cumulative mortalities were corrected for control mortality according to the formula of Abbott (1925), modified by Schneider-Orelli (1947).

**5. Statistics**

Even though no clear dose-response relationship was observed the LOEC / LOED and NOEC / NOED were determined according to OECD Series on Testing and Assessment Number 54 (2006). Cochran-Armitage test (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there was a significant difference between the mortality data of the test item groups and the control group in order to determine the LOEC and the NOEC on day 8 (D8). The corresponding LOED and NOED were calculated by taking into account the density of the larval diet (1.1 g/cm³) and the cumulative feeding volume per larva (140 µL diet).

On day 8 (D8) the $LC_{10, 20}$ and the corresponding $LD_{10, 20}$ could not be determined. The $LC_{50}$ with 95% confidence limits were calculated with Trimmed Spearman-Karber analysis. The corresponding $LD_{50}$ was calculated by taking into account the density of the larval diet (1.1 g/cm³) and the cumulative feeding volume per larva (140 µL diet).
For the statistical evaluation the statistics program ToxRat professional, Version 3.2.1 was used.

**Results and Discussion**

The concentrations of mesotrione and nicosulfuron measured in the treated diets on day 3, 4, 5 and 6 were between 71 and 101% for mesotrione. In the nominal concentration of 1440 mg product/kg diet, the concentration of mesotrione on D4 was 71%. However, since the average concentration across the treatment group is within ± 20% (81 %) of the nominal this has no impact on the validity of the study. For nicosulfuron, the concentrations were between 85 and 110% of nominal (Table A 2.3.1.3-1). As required by the guideline the mean concentrations of the larval diets were within ± 20% of nominal. Thus the concentrations of the larval diet were confirmed and the endpoints are based on nominal concentrations.

At test end, the cumulative mortality in the control was 4.2%. In the test item concentrations up to and including 3600 mg product/kg diet, the cumulative mortality was between 0.0 and 6.3% (-4.4 and 2.2% corrected) and were not statistically significantly different from the control. At the highest concentration of 9000 mg product/kg diet, the mortality was 93.8% (93.5% corrected) and was statistically significantly different from the control. The reference item showed a cumulative mortality of 100% (100% corrected) at test end on day 8.

On day 8, uneaten food was observed in the two highest test item groups. Smaller larvae on day 7 were observed at these test item groups as well. At the highest test item group the larvae were additionally smaller on day 5 and 6 and the larvae on day 7 were black.

Based on these results, the $LC_{50}$ at day 8 was determined to be 5580 mg product/kg diet, corresponding to an $LD_{50}$ of 859 µg product/larva. The NOEC was determined at 3600 mg product/kg diet, corresponding to an NOED of 554 µg product/larva. The $LC_{10, 20}$ and $LD_{10,20}$ could not be determined. The results are shown in Table A 2.3.1.3-2.

**Table A 2.3.1.3-1: Concentrations of mesotrione and nicosulfuron in the treated diets during the exposure phase**

| Nominal concentration | | Measured concentration of active substance | | | | | | | | Mean concentrations |
|---|---|---|---|---|---|---|---|---|---|---|
| | | D3 | | D4 | | D5 | | D6 | | |
| [mg prod./ kg diet] | [mg a.s./ kg diet] | [mg/kg] | [%] nom | [mg/kg] | [%] nom | [mg/kg] | [%] nom | [mg/kg] | [%] nom | [%] |
| Control | | n.d. | - | n.d. | - | n.d. | - | n.d. | - | - |
| **Active substance: mesotrione** | | | | | | | | | | |
| 230 | 19.2 | 18.9 | 98 | 18.3 | 95 | 15.7 | 82 | 14.5 | 76 | 88 |
| 576 | 48.0 | 47.6 | 99 | 37.2 | 78 | 40.4 | 84 | 41.1 | 86 | 87 |
| 1440 | 120 | 109 | 91 | 84.8 | 71 | 99.3 | 83 | 92.0 | 77 | 81 |
| 3600 | 300 | 271 | 90 | 257 | 86 | 263 | 88 | 245 | 82 | 87 |
| 9000 | 750 | 650 | 87 | 628 | 84 | 715 | 95 | 758 | 101 | 92 |
| **Active substance: nicosulfuron** | | | | | | | | | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 230 | 7.20 | 7.93 | 110 | 6.73 | 963 | 6.92 | 96 | 7.50 | 104 | 101 |
| 576 | 18.0 | 19.3 | 107 | 15.9 | 88 | 16.9 | 94 | 16.7 | 93 | 96 |
| 1440 | 45.1 | 45.2 | 100 | 38.4 | 85 | 43.3 | 96 | 41.8 | 93 | 94 |
| 3600 | 113 | 119 | 105 | 103 | 91 | 105 | 93 | 97.0 | 86 | 94 |
| 9000 | 282 | 288 | 102 | 269 | 95 | 255 | 90 | 274 | 97 | 96 |

n.d. not detectable; - not determined
LOD (mesotrione) = 3000 µg/kg; LOD (nicosulfuron) = 1128 µg/kg

**Table -2: Cumulative mortality of larvae exposed to SAE053H/01 in a chronic toxicity test**

| **Test concentration [mg product/kg diet]** | **Cumulative dosage [µg product/larva]** | **Cumulative Mortality [a)]** | | | | | |
|---|---|---|---|---|---|---|---|
| | | **Day 4** | **Day 5** | **Day 6** | **Day 7** | **Day 8** | **Day 8, corrected** |
| Control | | 0.0 | 0.0 | 4.2 | 4.2 | 4.2 | - |
| 230 | 35.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | -4.4 |
| 576 | 88.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | -4.4 |
| 1440 | 222 | 0.0 | 2.1 | 2.1 | 4.2 | 6.3 | 2.2 |
| 3600 | 554 | 0.0 | 0.0 | 0.0 | 2.1 | 2.1 | -2.2 |
| 9000 | 1390 | 2.1 | 45.8* | 81.3* | 85.4* | 93.8* | 93.5 |
| **Reference item: Dimethoate, technical** | | | | | | | |
| 48.0 | 7.39 | 58.3 | 83.3 | 100 | 100 | 100 | 100 |
| **8-day endpoints** | | | | | | | |
| | | **[mg product/kg diet]** | | | **[µg product/larva]** | | |
| **LOEC / LOEDD** | | 9000 | | | 1390 | | |
| **NOEC / NOEDD** | | 3600 | | | 554 | | |
| **$LC_{50}$ / $LD_{50}$ (95% CL)** | | 5580 (5060 – 6160) | | | 859 (779 – 949) | | |

[a)] Results are averages based on 3 replicates, containing 16 larvae each
* Statistically significantly increased compared to control (Cochran-Armitage test, one-sided greater, $\alpha = 0.05$)

The test is considered valid since the cumulative larval mortality from day 4 to day 8 was 4.2% (required ≤ 15%) and larval mortality in the reference item was 100% (100% corrected; required ≥ 50%).

**Conclusion**

In this chronic larval toxicity study with SAE053H/01, the 8-day $LD_{50}$ was determined to be 859 µg product/larva, corresponding to an $LC_{50}$ of 5580 mg product/kg diet. The NOED was determined to be 554 µg product/larva, corresponding to an NOEC of 3600 mg product/kg diet. The validity criteria of the guideline were fulfilled.

### A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

No additional data submitted.

### A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

No additional data submitted.

### A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

No additional data submitted.

## A 2.3.2 KCP 10.3.2 Effects on arthropods other than bees

### A 2.3.2.1 KCP 10.3.2.1 Standard laboratory testing

## A 2.3.2.1.1 Study 1: Toxicity to *Aphidius rhopalosiphi*

| | |
|---|---|
| Comments of zRMS: | The study follows the guideline specified by Mead Briggs *et al.* and according to the principles of GLP. No deviations to the guideline were noted.<br>The study is considered to be reliable and suitable for the risk assessment. |

| | |
|---|---|
| Reference: | KCP 10.3.2.1/01 |
| Report | SAE053H/01: Toxicity to the aphid parasitoid *Aphidius rhopalosiphi* de Stefani Perez (Hymenoptera, Braconidae) under laboratory conditions, Walter, C., 2016a, S16-01607 |
| Guideline(s): | Yes, IOBC (Mead-Briggs et al., 2000) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication<br>(if vertebrate study) | - |

**Materials and Methods**

**A. MATERIALS**

| | |
|---|---|
| **1. Test material** | SAE053H/01<br>(Other name: Mesotrione/Nicosulfuron 80/30 OD) |
| **Description** | White to beige liquid/cream, OD (oil dispersion) |
| **Lot/Batch #** | 54606-101 |
| **Purity** | Nicosulfuron: 30 g/L nominal; 30.7 g/L analysed<br>Mesotrione: 80 g/L nominal; 81.7 g/L analysed<br>Density: 0.98 g/cm³ |
| **Stability of test material** | Stable under storage conditions (ambient temperature, 5 – 30°C, in a dark and dry place)<br>Expiry date: 20 Mar 2018 |
| **2. Vehicle and/or positive control** | Vehicle control: 200 L/ha purified water<br>Positive control: reference substance |

| | |
|---|---|
| **Reference substance** | Perfekthion (BAS 152 11 I) |
| **Description** | Blue liquid, EC (emulsifiable concentrate) |
| **Lot/Batch #** | FRE-001226 |
| **Purity** | 400.0 g/L dimethoate (nominal content)<br>420.3 g/L dimethoate (analysed content)<br>density: 1.072 g/cm³ |
| **Stability of reference substance** | Stable under storage conditions (cool (1-10°C), dark, dry)<br>Expiry date: 10 Apr 2017 |

**3. Test organism**

| | |
|---|---|
| **Species** | *Aphidius rhopalosiphi* DeStefani-Perez (Hymenoptera: Braconidae) |
| **Source** | Katz Biotech AG, An der Birkenpfuhlheide 10, D-15837 Baruth, Germany |
| **Age** | Adults within 48 hours of emergence |
| **Acclimatisation** | In hatching containers stored in a room with 25.1-26.5°C and 63.5-74.2% RH and a 16 hour photoperiod of ~1500 Lux, fed with honey water gelatine solution (100 g honey, 50 g dest. water, 1.5 g gelatine) |
| **Diet** | 20% aqueous sucrose solution ad libitum during exposure, no food during parasitisation |
| **Test units** | Mortality test:<br>Two treated square glass plates (length: 13 cm, serving as upper and lower covers with treated surface inwards) were assembled with an aluminium frame (length: 13 cm, height: 1.5 cm, thickness: 1 cm) to an exposure unit. Three sides of the aluminium frame contained six screened ventilation holes (diameter: 1 cm). The fourth side of the frame contained a single opening (diameter: 1 cm) for the introduction of the test organisms and subsequent feeding. Test units were covered with black cards, ventilation of units was provided via aquarium pumps connected by flexible tubes to one ventilation hole.<br>Reproduction test:<br>A plexiglas tube (diameter: ~10 cm; length: ~25 cm) was placed upon a pot containing aphid infested barley seedlings. The soil was covered with sand. The top of the tube was covered with gauze. |

**4. Environmental conditions** The study was performed in a climatic chamber.

| | |
|---|---|
| **Temperature** | Nominal: 18 - 22°C; actual: 18.0 – 20.8°C |
| **Relative humidity** | Nominal: 60 - 90%; actual: 63.4 – 80.9% |
| **Photoperiod** | 16-hour light (light intensity nominal: 400-3000 Lux, in the mortality test and 4000-20000 Lux in the reproduction test; actual: ~1700 Lux during mortality test, ~9000 Lux in the reproduction test) to 8-hour dark photoperiod |

**B. STUDY DESIGN AND METHODS**

**1. In-life dates** 02 May 2016 to 17 May 2016

**2. Experimental conditions**

*Test design*

Lethal and sub-lethal effects of the test substance on the parasitoid wasp *Aphidius rhopalosiphi* were assessed in a multiple rate test under worst-case laboratory conditions. A control and a reference substance were tested in parallel. The test organisms were exposed via contact to dry residues on glass plates (artificial substrate) for 48 hours. Mortality and condition of the wasps were assessed after 2, 24 and 48 hours. After exposure, the reproductive capacity of surviving females from the test groups with a corrected mortality of ≤ 50% was assessed in a reproduction test. Sixteen to seventeen surviving healthy females per test group were individually confined over untreated aphid-infested barley plants. After a 24-hour parasitisation period, the females were removed and the plants were kept for 12 days before the number of aphid mummies was assessed.

*Number of animals per treatment*

Mortality test:
Ten wasps (2 males, 8 females) per replicate; four replicates per test and reference substance treatment and control

Reproduction test:
16 or 17 surviving healthy females per test group (individually confined in the reproduction test units)

*Test doses*

SAE053H/01 was tested at nominally 250, 465, 866, 1612 and 3000 mL product/ha, corresponding to 20.43, 37.99, 70.75, 131.7 and 245.1 g a.s./ha mesotrione and 7.68, 14.28, 26.59, 49.49 and 92.1 g a.s./ha nicosulfuron (based on analysed content of active substances). A control group was exposed to residues of deionised water.

*Reference substance*

Perfekthion was tested at nominally 0.3 mL product/ha, corresponding to 0.126 g a.s./ha dimethoate based on analysed formulation content.

*Treatment/Application*

Prior to test start, a stock solution (equal to the highest test concentration) of SAE053H/01 was prepared by dispersing 7.936 g product in deionized water. The remaining test solutions were prepared by dilution of the stock solution with deionized water. The application solution of the reference substance was prepared by first producing a 0.2% stock solution (0.1 g product in 50 g deionized water) and diluting it accordingly. The control test units were sprayed with deionized water only.

Appropriate volumes of the application solutions were sprayed onto the glass plates of each replicate by means of laboratory-spraying equipment (Schachtner, 71640 Ludwigsburg, Germany) with the spray nozzle type TeeJet 80015 EVS and a spraying pressure of 1.8 bar. Prior to application, the sprayer had been calibrated to deliver a target of 2.0 ± 0.2 mg/cm$^2$ spray solution (equivalent to 200 L ± 10% /ha). After application and after the spray deposits on the glass plates had dried (within one hour), the test units were assembled with the treated sides of the glass plates facing inwards. Then, the wasps were introduced into the test units using an aspirator (= start of the test).

**3. Observations and assessments**

Mortality and abnormal behaviour of the wasps were assessed after 2, 24 and 48 hours.

After the 48-hour mortality assessment the surviving females were removed from the exposure units and transferred individually to the reproduction units. After a 24-hour parasitisation period females were removed from the reproduction units and their condition (alive, dead or not recovered) were recorded. The number of parasitised aphids was counted in each replicate 12 days after the end of the parasitisation period.

The temperature and the relative humidity were recorded continuously during the test with a calibrated data logger. Light intensity was measured once during each phase with a luxmeter.

**4. Calculation of toxicity**

The percentage of mortality after 48 hours was calculated for each replicate from the combined number of dead and moribund individuals in correlation to the number of introduced test organisms. A mean value and the standard deviation were calculated for each treatment group. The corrected mortality was obtained by comparing the value observed in each treatment group with that in the control group, according to the formula of ABBOTT (1925), modified by SCHNEIDER-ORELLI (1947).

The number of aphid mummies obtained for up to 17 replicates within the 24-hour parasitisation period was used to calculate the mean value (± standard deviation) for each test item group and the control group. Only results for the females found alive at the end of the 24-hour parasitisation period were used for calculation of reproduction. The reduction in reproduction rate in the test item groups compared to the control group was calculated.

**5. Statistics**

Fisher's Exact Binomial Test (one-sided greater) was used to detect significant differences between Bonferroni-Holms corrected mortality data of the test item treatment groups and the control.
Reproduction data met normality (Shapiro-Wilk's Test) and homoscedasticity (Levene Test). Thus, statistical analysis was conducted using Dunnett's Multiple t-Test (one-sided smaller). The significance level was set to $\alpha = 0.05$ for all tests.
$LR_{50}$ was determined by Probit analysis. The $ER_{50}$ could not be determined because no clear rate/response relationship was found.
For evaluation the statistical program ToxRat Professional 3.2.1 was used.

**Results and Discussion**

After 48 hours of exposure, mean mortality in the control and in the reference substance treatment was 5.0% and 100%, respectively. In lowest test substance treatment of 250 mL product/ha, mortality was 7.5%, and not statistically significantly different from the control (see following table). For the remaining test substance treatments of 465, 866, 1612 and 3000 mL product/ha, mortality was statistically significantly different from the control. The 48-hour $LR_{50}$ was determined to be 871.2 (95% CL: 723.4 – 1049.6) mL product/ha.

Test organisms tried to avoid contact with the treated areas during the 24- and 48-hour assessments at rates of 465 mL product and above.

Reproduction was assessed for the control and the three lowest test substance treatment groups of 250, 465 and 866 mL product/ha. At the end of the reproduction test, the mean parasitisation rate was 40.9 mummies per surviving female in the control. In the three test substance treatments, the mean parasitisation rate was statistically significantly lower compared to the control (see following table). The $ER_{50}$ could not be calculated but was estimated at > 250 mL product/ha.

**Table A 9.10.2.4-1: Effects of SAE053H/01 on *Aphidius rhopalosiphi***

| Application rate [mL product/ha] | Mortality after 48 hours | | Parasitisation rate | |
|---|---|---|---|---|
| | [%] ± SD | [%] corrected | mummies/female [mean ± SD] | Reduction in par. Rate [%] |
| Control | 5.0 ± 5.8 | - | 40.9 ± 15.3 | - |
| 250 | 7.5 ± 15.0 | 2.6 | 27.7* ± 11.4 | 32.2 |
| 465 | 32.5* ± 23.6 | 28.9 | 18.9* ± 12.4 | 53.8 |
| 866 | 40.0* ± 40.8 | 36.8 | 20.6* ± 12.9 | 49.6 |
| 1612 | 70.0* ± 18.3 | 68.4 | - | - |
| 3000 | 97.5* ± 5.0 | 97.4 | - | - |
| **Reference substance: Perfekthion (active substance: dimethoate)** | | | | |
| 0.3 mL product/ha | 100* ± 0.0 | 100.0 | n.a. | n.a. |
| **Endpoint [mL product/ha]** | | | | |
| 48-hour $LR_{50}$ (95% CL) | | 871.2 (723.4 – 1049.6) | | |
| $ER_{50}$ reproduction | | estimated at > 250 | | |

* Value statistically significantly different from control (mortality: based on Fisher's Exact Binomial Test with Bonferroni Correction, one-sided greater, α = 0.05; reproduction: Dunnett's Multiple t-Test, one sided smaller, α = 0.05)

Mortality in the control and toxic reference group was 5.0% and 100%, respectively (required ≤ 13% and 50-100%). Furthermore, mean control parasitisation rate was 40.9 aphid mummies per female (required > 5 aphid mummies per female) and no control female failed to produce mummies (required not more than two). Therefore, the test is considered to be valid.

**Conclusion**

Under worst-case laboratory conditions (artificial substrate), the 48-hour $LR_{50}$ and $ER_{50}$ (reproduction) of SAE053H/01 for the parasitoid wasp *Aphidius rhopalosiphi* were 871.2 (95% CL: 723.4 – 1049.6) mL product/ha and > 250 mL product/ha. All validity criteria were fulfilled.

## A 2.3.2.1.2 Study 2: Toxicity to *Typhlodromus pyri*

| | |
|---|---|
| Comments of zRMS: | The study follows the guideline specified by Blümel *et al.* and according to the principles of GLP. No deviations to the guideline were noted.<br>The study is considered to be reliable and suitable for the risk assessment. |

| | |
|---|---|
| Reference: | KCP 10.3.2.1/02 |
| Report | SAE053H/01: Toxicity to the predatory mite, *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) under laboratory conditions, Walter, 2016b, S16-01608 |
| Guideline(s): | Yes, IOBC (Blümel et al., 2000) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | - |

## Materials and Methods

### A. MATERIALS

| | |
|---|---|
| **1. Test material** | SAE053H/01<br>(Other name: Mesotrione/Nicosulfuron 80/30 OD) |
| **Description** | White to beige liquid/cream, OD (oil dispersion) |
| **Lot/Batch #** | 54606-101 |
| **Purity** | Nicosulfuron: 30 g/L nominal; 30.7 g/L analysed<br>Mesotrione: 80 g/L nominal; 81.7 g/L analysed<br>Density: 0.98 g/cm³ |
| **Stability of test material** | Stable under storage conditions (ambient temperature, 5 – 30°C, in a dark and dry place)<br>Expiry date: 20 Mar 2018 |
| **2. Vehicle and/or positive control** | Vehicle control: deionised water<br>Positive control: reference substance |
| **Reference substance** | Perfekthion (BAS 152 11 I) |
| **Description** | Blue liquid |
| **Lot/Batch #** | FRE-001226 |
| **Purity** | 400.0 g/L dimethoate (nominal content)<br>420.3 g/L dimethoate (analysed content)<br>density: 1.072 g/cm³ |
| **Stability of reference substance** | Stable under storage conditions (cool, 1-10°C, dark, dry).<br>Expiry date: 10 Apr 2017 |
| **3. Test organism** | |
| **Species** | Predatory mite (*Typhlodromus pyri* Scheuten; Acari: Phytoseiidae) |
| **Source** | The test organisms were bred at the test facility according to OVERMEER (1985) with species confirmation once a year. |
| **Age** | Protonymphs ≤ 24 hours old |
| **Acclimatisation** | Four days prior to test start, all eggs were removed from the stock and the eggs that were laid in a period of 24 hours were collected at the following day and kept under test conditions (24.5 – 25.1°C, 64.3 – 79.6% RH and ~ 3000 Lux). |
| **Diet** | The mites were fed *ad libitum* with bean (*Vicia faba*) and birch (*Betula pendula*) pollen. Each type of pollen was supplied separately at test initiation and replenished at each assessment date (except day 14). |
| **Test units** | For exposure: two 24 x 50 mm glass cover slides (thickness: 0.13-0.17 mm) were fixed together by two cover glasses, glued on the upper surface. A non-drying glue gel (prevent escaping) was applied on the glass slides before application, bounding a 10-13 cm² exposure arena.<br>For assembling: water-saturated foam with a glass plate (same size) placed on the top. The glass plate was covered with wet filter paper, which was constantly provided with water (mixture of tap water and deionized water, 1:2). After application and drying of the deposits |

| | |
|---|---|
| | the glass cover slides were placed on the top of the wet filter paper. The thin gap between the two cover slides was filled with water by capillary forces, which serves as drinking water for the test organisms. |
| **4. Environmental conditions** | The study was performed in a ventilated climatic chamber. |
| **Temperature** | Nominal: 23 - 27°C; actual: 24.8 – 26.3°C |
| **Relative humidity** | Nominal: 60 - 90%; actual: 60.2 – 73.9% |
| **Photoperiod** | 16-hour light (light intensity: ~ 5000 Lux) to 8-hour dark photoperiod |

**B. STUDY DESIGN AND METHODS**

| | |
|---|---|
| **1. In-life dates** | 09 May 2016 to 23 May 2016 |

**2. Experimental conditions**

*Test design*

Lethal and sub-lethal effects of the test substance on the predatory mite *Typhlodromus pyri* were assessed in a multiple rate test under standard laboratory conditions. A control and a reference substance were tested in parallel. The test organisms were exposure via contact to dry residues on glass plates (artificial substrate) for 14 days. Mortality and behavioural effects were assessed after 3 and 7 days. From day 7 to day 14, cumulative reproduction was recorded by counting the number of eggs per female on day 10, 11 and 14. The reproduction phase was carried out for the treatment groups with ≤ 50% mortality.

*Number of animals per treatment*

20 protonymphs/replicate; four replicates/test and reference substance treatment and control

*Test doses*

SAE053H/01 was tested at nominally 75, 143, 274, 523 and 1000 mL product/ha, corresponding to 6.13, 11.68, 22.39, 42.73 and 81.7 g a.s./ha mesotrione and 2.30, 4.39, 8.41, 16.06 and 30.7 g a.s./ha nicosulfuron based on analysed content of active substance. The test item solutions were sprayed with a volume of 200 L/ha.

A control group was exposed to residues of deionised water.

*Reference substance*

Perfekthion was tested at nominally 12 mL product/ha, corresponding to 5.04 g a.s./ha dimethoate based on analysed formulation content.

*Treatment/Application*

Prior to test start, a stock solution of SAE053H/01 (equal to the highest test concentration solution) was prepared by dispersing 2.45 g of test item in deionized water. The lower application rates were prepared by dilution of the corresponding amount of stock solution with deionized water. The application solution of the reference substance was prepared by using a 0.2% stock solution (0.1 g test item in deionized water) and diluting it accordingly. The control test units were sprayed with deionized water only.

Appropriate volumes of the application solutions were sprayed onto the glass plates of each replicate by means of laboratory-spraying equipment (Schachtner, 71640 Ludwigsburg, Germany) with the spray nozzle type TeeJet 80015 EVS and a spraying pressure of 1.8 bar. Prior to application, the sprayer had been calibrated to deliver a target of 2.0 ± 0.2 mg/cm$^2$ spray solution (equivalent to 200 L ± 10% /ha). Treatment residues of the test item could be observed after spraying the glass surfaces of the test concentrations 274, 523 and 1000 mL product/ha with increasing intensity.
After application and after the spray deposits on the glass plates had dried (within one hour), the test units were assembled and the mites were introduced into the test arenas using a fine-bristled brush under a stereomicroscope (= start of the test).

**3. Observations and assessments**

The number of living, dead and escaped mites was counted on days 3 and 7 after application using a stereomicroscope. Dead mites were removed, escaped mites were considered as dead.

Reproduction was assessed only for treatment groups with a corrected mortality ≤ 50 %. On day seven of exposure the sex of the test organisms was determined by the shape and size of the body. The sex-ratio was adapted for the control by transferring males within the treatment group. The number of offspring per female was determined by counting the number of females and eggs/larvae on day 10, 11 and 14. Eggs laid until day seven inclusive were removed from the test arena and were not counted. Males and females were counted and the number of eggs and larvae was determined. Dead animals, eggs and larvae were removed after counting. All assessments were conducted using a stereomicroscope.

The temperature and the relative humidity were recorded continuously during the test.

**4. Calculation of toxicity**

The cumulative juvenile mortality and escaping rate was calculated for each mortality assessment day. The study endpoint was the cumulative juvenile mortality at day 7. The percentage of mortality was calculated for each replicate from the combined number of dead and escaped individuals in correlation to the number of introduced test organisms. The escape rate was calculated as well. A mean value and the standard deviation were calculated for each treatment group and assessment day.

The corrected mortality and escape rate was obtained by comparing the value observed in each treatment group with that in the control group, according to the formula of ABBOTT (1925), modified by SCHNEIDER-ORELLI (1947).

The cumulative reproduction per female (from day 7 to day 14) was evaluated at the end of the reproduction period. The cumulative number of eggs laid per female during the reproduction period was calculated for each replicate. For each treatment group the cumulative mean reproduction value and the standard deviation were calculated. Evaluation of reproduction data was based only on replicates containing females over the whole reproduction phase. The percentage reduction in the reproduction rate was calculated as well.

**5. Statistics**

Fisher's Exact Binomial Test (one-sided greater) was used to detect significant differences between Bonferroni-Holms corrected mortality data / escaping data of the test item treatment groups and the control.

Reproduction data met normality (Shapiro-Wilk's Test) but not homoscedasticity (Levene Test). Thus, statistical analysis was conducted using Welch t-Test after Bonferroni Holm (one-sided smaller). The $LR_{50}$ and the $ER_{50}$ could not be determined.

The significance level was set to $\alpha = 0.05$ for all tests. For evaluation the statistical program ToxRat Professional 3.2.1 was used.

**Results and Discussion**

After 7 days of exposure, mean mortality in the control and in the reference substance treatment was 0.0% and 75.0%, respectively. In the two lowest test substance treatments of 75 and 143 mL product/ha, mean mortality was 3.8 and 2.5%, respectively, and was not statistically significantly different from the control. In the three highest test substance treatments of 274, 523 and 1000 mL product/ha, mean mortality ranged between 7.5 and 10.0% and was statistically significantly different from the control (see following table). The rate of escapees was not statistically significantly different compared to the control at any test item concentration. The 7-day $LR_{50}$ of SAE053H/01 could not be calculated as the observed effects were below 50% but was estimated to be > 1000 mL product/ha.

Reproduction was assessed for the control and all test substance treatment groups. Mean egg production from day 7 to day 14 in the control was 5.2 eggs per female. In the test substance treatments mean egg production ranged between 3.7 and 6.5 eggs per female (corresponding to a reduction in reproduction between -25.0 and 28.8%) and was not statistically significantly different from the control (see following table). The $ER_{50}$ (reproduction) of SAE053H/01 could not be calculated as the observed effects were below 50% but was estimated to be > 1000 mL product/ha.

**Table 9.10.2.4-2: Effects of SAE053H/01 on *Typhlodromus pyri* after 14 days**

| Application rate [mL product/ha] | Mortality after 7 days [a] mean ± SD [%] | Escapees mean ± SD [%] | Cumulative reproduction from day 7 to day 14 [mean number of eggs per female ± SD] | Effect on reproduction [%] [b] |
|---|---|---|---|---|
| Control | 0.0 ± 0.0 | 0.0 ± 0.0 | 5.2 ± 1.6 | - |
| 75 | 3.8 ± 4.8 | 0.0 ± 0.0 | 6.5 ± 0.2 | -25.0 |
| 143 | 2.5 ± 5.0 | 1.3 ± 2.5 | 3.7 ± 0.8 | 28.8 |
| 274 | 10.0* ± 4.1 | 7.5 ± 6.5 | 5.5 ± 1.4 | -5.8 |
| 523 | 8.8* ± 4.8 | 6.3 ± 4.8 | 5.6 ± 2.4 | -7.7 |
| 1000 | 7.5* ± 6.5 | 3.8 ± 4.8 | 4.3 ± 1.5 | 17.3 |
| **Reference substance: Perfekthion (active substance: dimethoate)** | | | | |
| 12.0 | 75.0 ± 7.1 | 20.0 ± 4.1 | - | - |
| **Endpoints [mL product/ha]** | | | | |
| 7 d $LR_{50}$ | | estimated at > 1000 | | |
| 14 d $ER_{50}$ | | estimated at > 1000 | | |

Note: There were no statistically significant differences between the control escapees and the treatment escapees (result of a Fisher's Exact test, α = 0.05, one-sided greater) and between the control reproduction and the treatment reproduction (result of a Welch t-test, α = 0.05, one-sided smaller)

a) Mortality based on the sum of dead and escaped organisms

b) Negative values indicate lower mortality in the test item group compared to the control group

* Value statistically significantly different from control (mortality: results of a Fisher's Exact test, α = 0.05, one-sided greater)

Mortality in the control and toxic reference group was 0.0 % and 75.0%, respectively (required ≤ 20% and 50-100%). Furthermore, mean reproduction in the control was 5.2 eggs per female (required > 4 eggs per female). Therefore, the test is considered to be valid.

**Conclusion**

Under worst-case laboratory conditions (artificial substrate), the 7-day $LR_{50}$ and the 14 d $ER_{50}$ of SAE053H/01 for the predatory mite *Typhlodromus pyri* could not be calculated but were estimated to be > 1000 mL product/ha. The validity criteria were fulfilled.

## A 2.3.2.2 KCP 10.3.2.2 Extended laboratory testing, aged residue studies

### A 2.3.2.2.1 Study 1: Toxicity to *Aphidius rhopalosiphi* – aged residues

| | |
|---|---|
| Comments of zRMS: | The study follows the guideline specified by Mead Briggs *et al.* and according to the principles of GLP. No deviations to the guideline were noted.<br>The study is considered to be reliable and suitable for the risk assessment. |

| | |
|---|---|
| Reference: | KCP 10.3.2.2/01 |
| Report | Effects of SAE053H/01 on the parasitic wasp *Aphidius rhopalosiphi* DeStefani-Perez in an extended laboratory test (under semi-field conditions |

| | |
|---|---|
| | aged residues on potted maize plants), Röhlig, 2017a, 17 48 NAR 0001 |
| Guideline(s): | Yes, IOBC (Mead-Briggs et al., 2009), modified for an aged residue test |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | - |

**Materials and Methods**

**A. MATERIALS**

| | |
|---|---|
| **1. Test material** | SAE053H/01<br>(Other name: Mesotrione/Nicosulfuron 80/30 OD) |
| **Description** | White to beige liquid/cream, OD (oil dispersion) |
| **Lot/Batch #** | 54606-101 |
| **Purity** | Nicosulfuron: 30 g/L nominal; 30.2 g/L analysed<br>Mesotrione: 80 g/L nominal; 80.6 g/L analysed<br>Density: 0.984 g/cm³ |
| **Stability of test material** | Stable under storage conditions (ambient temperature, 5 – 30°C, in a dark and dry place)<br>Expiry date: 20 Mar 2018 |
| **2. Vehicle and/or positive control** | Vehicle control: 400 L/ha deionised water<br>Positive control: reference substance |
| **Reference substance** | Perfekthion (BAS 152 11 I) |
| **Description** | Blue liquid |
| **Lot/Batch #** | FRE-001302 |
| **Purity** | 400.0 g/L dimethoate (nominal content)<br>405.2 g/L dimethoate (analysed content)<br>density: 1.074 g/cm³ |
| **Stability of reference substance** | Stable under storage conditions (cool, < 10°C).<br>Expiry date: 31 Dec 2017 |
| **3. Test organism** | |
| **Species** | Parasitic wasp (*Aphidius rhopalosiphi* DeStefani-Perez; Hymenoptera: Bracconidae) |
| **Source** | Katz Biotech AG, 15837 Baruth, Germany in the stage of mummies, rearing at test facility |
| **Age** | Protonymphs < 48 hours old |
| **Acclimatisation** | Rearing at test conditions (20 – 25°C, 60 – 80% humidity and > 3000 Lux) |

| | |
|---|---|
| **Diet** | During holding, pupae were fed with aqueous fructose solution. Ermeging adults were fed with a 25% w/w aqueous fructose solution. |
| **Test units** | Exposure: Acrylic cylinder (diameter: 11 cm, height: 20 cm) with one maize plant segment with two leaves in a glass bottle filled with tap water, fixed in a plastic beaker (diameter: 12 cm, height: 9 cm) filled with quartz sand. The test unit was ventilated with a small pump connected to a hole in the side of the cylinder.<br>Reproduction: Acrylic cylinder as above with 20 wheat seedlings, 8 days old, infested with > 100 adult and nymphal cereals aphids and covered with gauze. |
| **Test substrate** | Potted maize plants (variety "Milkstar") supplied by Nordsaat Saatzucht GmbH, 38895 Langenstein, Germany, which had not been treated with any plant protection product. Plants were grown in soil near the test facility placed under a UV-permeable roof with gauze walls. Application started at BBCH 13, height of the plants was approximately 0.20 – 0.25 m. |

**4. Environmental conditions**

| | |
|---|---|
| **Temperature** | Semi-field conditions: 1.7 – 23.5 °C<br>Laboratory conditions:<br>Nominal: 18 – 22 °C; actual: 19 – 22 °C (DAT 0 + 7) |
| **Relative humidity** | Semi-field conditions: 56 – 84 %<br>Laboratory conditions:<br>Nominal: 60 – 90 %; actual: 67 – 73 % (DAT 0 + 7) |
| **Photoperiod** | 16-hour light to 8-hour dark photoperiod;<br>Light intensity during exposure:<br>Nominal: 400 – 3000 Lux; actual: 1110 Lux (DAT 0), 1090 Lux (DAT 7)<br>Light intensity during parasitisation:<br>Nominal: 400 – 3000 Lux; actual: 2520 Lux (DAT 0), 2430 Lux (DAT 7)<br>Light intensity during reproduction:<br>Nominal: 4000 – 20000 Lux; actual: 6530 Lux (DAT 0), 6450 Lux (DAT 7) |

## B. STUDY DESIGN AND METHODS

**1. In-life dates** 08 May 2017 to 29 May 2017

**2. Experimental conditions**

*Test design*

Lethal and sub-lethal effects on the parasitic wasp *Aphidius rhopalosiphi* were assessed at three rates of SAE053H/01 (fresh and aged residues) under extended laboratory conditions. A control and a reference substance were tested in parallel. Extended laboratory bioassays were carried out after 0 and 7 days of aging. Mortality and reproduction as well as behavior were assessed.

*Number of animals per treatment*

Mortality test:
Five wasps (females) per replicate; six replicates per test and reference substance treatment and control

Reproduction test:
15 surviving healthy females per test group (individually confined in the reproduction test units)

*Test doses*

SAE053H/01 was tested at nominally 1000, 1500 and 1850 mL product/ha. A control group was exposed to residues of deionised water.

*Reference substance*

Perfekthion was tested at nominally 30 mL product/ha at DAT 0 (outdoor application), and 10 mL product/ha at DAT 7 (laboratory application).

*Treatment/Application*

For preparing the application solutions of SAE053H/01, 4.551, 6.827 and 8.419 g test item were diluted with deionised water. For the reference substance, 0.149 and 0.269 g reference item were diluted in deionised water. The control test units were sprayed with deionised water only.

The application of the test solutions (DAT 0) was carried out under semi-field (outdoor) conditions. The test solutions were sprayed onto potted maize plants using spray equipment for small plot applications (plot-sprayer PL 2 with Lechler ES 90-015 nozzles, agrotop GmbH, Obertraubling, Germany). Prior to spraying the potted maize plants of each treatment group were set up in a 25 m² application plot. The spray volume was calibrated before the application corresponding to 400 L/ha of water ± 10 %. After the application, the applied maize plants were stored under semi-field conditions (rain protection under a UV-permeable roof).

All plants were sprayed with 10 % w/w aqueous fructose solution, 1-2 hours prior to each exposure and left to dry. The soil in the pots was then covered with dry light-coloured sand to create a uniform surface before the plants were treated. Afterwards, 5 impartially selected females of *Aphidius* were confined to each test unit. The test units were closed with fine gauze and then placed in a controlled-environment test room.

**3. Observations and assessments**

At approximately 2, 24 and 48 hours after start of exposure of the wasps, the number of surviving wasps and the condition of the wasps were determined.
To evaluate the possible repellent effects of the test item to the wasps, assessments of the position of the individual insects within the test arenas were carried out during the initial 3 hours after their release. Five separate sets of observations were made (at 30-minute intervals, starting approximately 15-30 minutes after the introduction of the wasps to all of the test arenas had been completed).
For bioassays started on DAT 0 and DAT 7, five sequential assessments provided 150 observations for each treatment, i.e. 30 wasps on 5 occasions. Since the test item did not appear to be repellent to the wasps, further assessments were not necessary.

The reproduction of wasps was determined by the number of parasitised aphids.

The temperature and the relative humidity were recorded continuously during the test. Light intensity was measured at the start of assessments.

**4. Calculation of toxicity**

Mortality (total number of moribund and dead insects) in percent for each treatment group was calculated. Observations in the treatment groups were expressed relative to the water control group. The corrected mortality in the treatment groups was calculated according to Abbott (1925).

The mean number of mummies produced per surviving female was calculated for each treatment (including standard deviation). Observations in the treatment groups were expressed relative to the water control group. For the assessments of reproductive capacity, it was noted in which replicate arenas the female wasp was dead or not found alive when insects were removed after the 24 h parasitisation period. Only results for the wasps found alive when insects were removed after the 24-h parasitisation period were used for the analysis. For sub-lethal endpoints relative performance was expressed as percent reduction.

The percentage of observations of wasps settled on the plants over the whole assessment period was calculated for each treatment. As a measurement of repellency of insects from the treated foliage during the initial 3 hours of the definitive bioassay, the percentage of wasps settled on the plants in each replicate was calculated for each of the five assessment occasions and then a mean value was obtained for each replicate. These values were angularly transformed (square root arcsine) prior to comparison by one-way analysis of variance.

**5. Statistics**

Mortality was analysed for statistical significance using the Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm (test item) or Fisher's Exact Binomial test (reference item) as distribution-free tests which do not require testing for normality or homoscedasticity prior to analysis. The accepted significance level was $\alpha = 0.05$.

The repellence (position) was analysed for statistical significance using Dunnet's t-test following Shapiro-Wilk's test on normal distribution and Levene's test on variance homogeneity.

The reproductive capacity was analysed for statistical significance using the William's t-test following Shapiro-Wilk's test on normal distribution and Levene's test on variance homogeneity.

**Results and Discussion**

Mortality of *Aphidius rhopalosiphi* in the control group was 6.7 and 3.3% after 48 h of exposure to freshly applied and 7-day old aged spray deposits, respectively. In the test substance treatments, mortality rates were 6.7% (DAT 0 and 7) for 1.0 L product/ha, 6.7% (DAT 0) and 0 % (DAT 7) for 1.5 L product/ha and 6.7 % (DAT 0) and 3.3 % (DAT 7) for 1.85 L product/ha which was not statistically significantly different from the control for any of the test item rates. In the reference substance treatment, mortality was 93.3% after exposure to freshly applied and 7-day old spray deposits (see following table).

Reproduction in the control group was 22.1 and 21.5 mummies per female after exposure to freshly applied and 7-days old aged spray deposits, respectively. In the test substance treatments, reproduction was 21.7 mummies (DAT 0) and 22.4 mummies per female (DAT 7) for 1.0 L product/ha, 22.3 mummies (DAT 0) and 20.9 (DAT 7) for 1.5 L product/ha and 22.6 mummies (DAT 0) and 21.9 mummies per female (DAT 7) for 1.85 L product/ha which was not statistically significantly different from the control for any of the test item rates.

Wasps settled on the plants were 55.3 % (DAT 0) and 56.7 % (DAT 7) in the control, 47.3 % (DAT 0) and 50.7 % (DAT 7) at 1.0 L product/ha, 55.3 % (DAT 0) and 40.7 % (DAT 7) at 1.5 L product/ha and 51.3 %

(DAT 0) and 48.7 % (DAT 7) at 1.85 L product/ha. None of the test item rates was statistically significantly different from the control.

Based on these results, SAE053H/01 had no unacceptable effects (effects < 50%) on either the survival or the subsequent reproductive capacity of the wasps at 1.0, 1.5 and 1.85 L product/ha after 0 and 7 days of aging.

**Table** Błąd! Użyj karty Narzędzia główne, aby zastosować Überschrift 3 do tekstu, który ma się tutaj pojawić.**-1: Mortality and reproduction of *Aphidius rhopalosiphi* exposed to SAE053H/01**

| **Bioassay** | **Application rate** | **Mean mortality after 48 h** | | **Wasps settled on the plants** | **Reproduction after 11 days** | |
|---|---|---|---|---|---|---|
| | | **[%]** | **corrected [%]** | **[%]** | **[mean number of mummies per female]** | **[% effect rel. to control]** |
| 1 (0 DAT) | Control | 6.7 | - | 55.3 | 22.1 | - |
| | 1.0 L test item/ha | 6.7 | 0 | 47.3 | 21.7 | 1.8 |
| | 1.5 L test item/ha | 6.7 | 0 | 55.3 | 22.3 | -0.9 |
| | 1.85 L test item/ha | 6.7 | 0 | 51.3 | 22.6 | -2.3 |
| | 30 mL reference item/ha | 93.3 * | 92.9 | - | - | - |
| 2 (7 DAT) | Control | 3.3 | - | 56.7 | 21.5 | - |
| | 1.0 L test item/ha | 6.7 | 3.4 | 50.7 | 22.4 | -4.2 |
| | 1.5 L test item/ha | 0 | -3.4 | 40.7 | 20.9 | 2.8 |
| | 1.85 L test item/ha | 3.3 | 0 | 48.7 | 21.9 | -1.9 |
| | 10 mL reference item/ha | 93.3 * | 93.1 | - | - | - |

* Statistically significantly different from the control (mortality: Fisher's Exact Binomial Test, $\alpha = 0.05$)
DAT days after treatment

The test is considered to be valid since mortality in the control and toxic reference group was ≤ 10% (actual: 6.7) and > 50% (actual: 93.3), respectively. Furthermore, mean reproduction in the control was at least 5 mummies per female (actual: > 21.5) and not more than 2 replicates in the control had zero values (actual: 1).

**Conclusion**

After exposure to freshly applied and aged residues applied to maize plants, SAE053H/01 had no unacceptable effects (effects < 50%) on either the survival or the subsequent reproductive capacity of *Aphidius rhopalosiphi* at application rates of 1.0, 1.5 and 1.85 L product/ha after 0 and 7 days of aging. The validity criteria were fulfilled.

## A 2.3.2.2.2 Study 2: Toxicity to *Aleochara bilineata*

| | |
|---|---|
| Comments of zRMS: | The study follows the guideline specified by Grimm *et al.* and according to the principles of GLP. No deviations to the guideline were noted.<br>The study is considered to be reliable and suitable for the risk assessment. |

| | |
|---|---|
| Reference: | KCP 10.3.2.2/02 |
| Report | Effects of SAE053H/01 on the rove beetle *Aleochara bilineata* Gyll. in an extended laboratory test, Röhlig, 2017b, 17 48 NKE 0002 |
| Guideline(s): | Yes, IOBC (Grimm et al., 2000) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | - |

**Materials and Methods**

**A. MATERIALS**

| | |
|---|---|
| **1. Test material** | SAE053H/01<br>(Other name: Mesotrione/Nicosulfuron 80/30 OD) |
| **Description** | White to beige liquid/cream, OD (oil dispersion) |
| **Lot/Batch #** | 5406-101 |
| **Purity** | Nicosulfuron: 30 g/L nominal; 30.2 g/L analysed<br>Mesotrione: 80 g/L nominal; 80.6 g/L analysed<br>Density: 0.984 g/cm³ |
| **Stability of test material** | Stable under storage conditions (ambient temperature, 5 – 30°C, in a dark and dry place)<br>Expiry date: 20 Mar 2018 |
| **2. Vehicle and/or positive control** | Vehicle control: Deionised water<br>Positive control: reference item |
| **Reference item** | Dimethoate EC 400 |
| **Description** | EC (emulsifiable concentrate), further appearance not reported |
| **Lot/Batch #** | FRE-001226 |
| **Purity** | 400 g/L dimethoate (nominal content)<br>420.3 g/L dimethoate (analysed content)<br>density: 1.072 g/cm³ |
| **Stability of reference item** | Stable under storage conditions (storage at a cool, dry, well-ventilated place).<br>Expiry date: 10 Apr 2017 |
| **3. Test organism** | |
| **Species** | Rove beetle *Aleochara bilineata* Gyll. (Coleoptera, Staphylinidae) |
| **Source** | The test organisms are reared at the test facility. The original source is Bayerische Landesanstalt, München, Germany. |
| **Age** | 1-7 days old adults |

| | |
|---|---|
| **Acclimation period** | Parasitised pupae of the onion fly and adult beetles emerging from them were kept under the same conditions as used for the exposure period.<br>One day before application, beetles were placed individually into containers. Prior to test start the beetles were grouped together for copulation. Pairs were afterwards transferred to new vessels with moist sand. |
| **Host organisms** | Pupae of the onion fly, *Delia antiqua*, served as hosts for the *Aleochara* larvae. They were obtained from a commercial supplier (De Groene Vlieg, Nieuwe Tonge, The Netherlands). |
| **Diet** | Thawed *Chironomus* larvae (obtained from Sosnowiec polska, Poland) were used as food. Approximately one hour after application and thereafter every two to three days until the termination of the exposure phase, the beetles were fed depending on food consumption. |
| **Test units** | *Exposure phase:*<br>Plastic containers (height: 8 cm, 14 cm in diameter) filled up to 5 cm with standard soil LUFA 2.1 and covered with a lid of nylon gauze were used as test units. The side walls of the vessels were treated with Fluon.<br>*Hatching phase:*<br>One plastic vessel with a sieve bottom (mesh size of approx. 2 x 2 mm) and a second vessel below were used as test units during the hatching phase. |

**4. Environmental conditions**

| | |
|---|---|
| **Soil** | Standard soil LUFA 2.1 was used with the following parameters (acc. to DIN classification):<br><br>Soil type: Silty sand<br>$C_{org.}$: 0.71 %<br>pH as $CaCl_2$: 4.9<br>Cation exchange capacity: 4.3 meq/100 g<br>Max. WHC: 32.1 g/100 g dry weight<br>Weight per volume: 1437 g/1000 mL<br><br>1106 g dry soil (770 cm³ soil) was moistened with deionised water yielding 1230 g moist soil with a final nominal water content of approximately 35 % of WHC, the test unit containing moist soil was weighed just after treatment and the weight was recorded; the test units were then weighed every 2-3 days until the drying period and the soil was adjusted to the moisture level at the start, if necessary, using deionised water. |
| **Temperature** | Nominal: 18 – 22°C; actual: 19 – 21°C |
| **Relative humidity** | Nominal: 60 – 90%; actual: 61 – 82 % |
| **Photoperiod** | 16-hour light to 8-hour dark photoperiod<br>Light intensity during exposure and hatching phase: nominal < 2000 Lux, actual 1820 Lux |

**B. STUDY DESIGN AND METHODS**

**1. In-life dates** 05 Jan 2017 to 24 May 2017

**2. Experimental conditions**

*Test design*

Effects of the test substance on reproduction of the rove beetle *Aleochara bilineata* were assessed in a multiple rate test under extended laboratory conditions. Adult beetles (1-7 days old) were exposed to dried spray deposits applied onto sandy soil (LUFA 2.1). During the first three weeks of exposure, the beetles were offered pupae of the onion fly *Delia antiqua* as host organisms for parasitisation. After 28 days after start of exposure, adult beetles were removed from the test substrate and, after drying of the test substrate for one week, the *Delia* pupae were transferred to hatching test units. Reproductive capacity was measured as the number of second generation adult beetles emerging from the onion fly pupae during the hatching phase of 35 days.

*Number of animals per treatment*

Ten pairs (ten females and ten males) of *Aleochara bilineata*/replicate; four replicates/test and reference substance treatment and control

*Test doses*

SAE053H/01 was tested at nominally 0.0938, 0.188, 0.375, 0.75 and 1.5 L product/ha, corresponding to 0.003, 0.006, 0.011, 0.023 and 0.045 g a.s./ha nicosulfuron and 0.008, 0.015, 0.030, 0.060 and 0.121 g a.s./ha mesotrione, respectively, based on analysed active substance contents.
A control group was exposed to deionised water.

*Reference item*

Dimethoate 400 EC was tested at nominally 1.5 L product/ha, corresponding to 630.5 g a.s./ha dimethoate based on analysed active substance content.

*Treatment/Application*

Prior to test start, a stock solution of SAE053H/01 (equal to the highest test concentration) was prepared by filling up 0.369 g of the test substance with deionised water. The remaining test item solutions were prepared by serial dilution. The application solution of the reference substance was prepared by diluting 0.402 g of the reference substance with deionised water. The control test units were sprayed with deionised water only.

Appropriate volumes of the application solutions were sprayed onto the test units containing the moist sand by means of an adequate spraying apparatus (i.e. laboratory track-sprayer from Schachtner, Ludwigsburg, Germany). Prior to application, the sprayer had been calibrated to deliver 4.0 ± 0.4 mg spray solution/$cm^2$ (equivalent to 400 L/ha). The inner walls of the test units were protected from contamination with the spray liquid by removable aluminium collars to make sure that only the substrate surface was sprayed.

Immediately after application, the test organisms were released into the test units.

Approximately 500 *Delia* pupae were offered three times to each test unit with intervals of one week (i.e. at 7, 14 and 21 days after application). The *Delia* pupae were carefully incorporated into substrate.

At the end of exposure after 28 days, all beetles (dead or alive) were counted and removed from the test substrate. The substrate with the parasitised onion fly pupae was kept under test conditions during one further week for drying. After 35 days after start of exposure, the fly pupae were removed from the substrate and carefully placed into the hatching test units.

**3. Observations and assessments**

After test termination, the total number of emerged *Aleochara* beetles was determined for each test replicate.

Test temperature and relative humidity were continuously recorded. Light intensity was measured once during the experimental phase.

After release of the test organisms into the test units, the weight of each test unit was determined. Every two to three days, the quantity of evaporated water was determined by weighing the exposure units and water loss was replenished with deionised water. The weight of the added *Delia* pupae was taken into consideration when weighing the test units.

**4. Calculation of toxicity**

The reproductive capacity of the beetles was calculated as the mean number of offspring per replicate with standard deviation. Furthermore, the percentage reduction of reproductive capacity in the test and reference substance treatments in relation to the control was calculated.

**5. Statistics**

Reproductive performance was analysed for statistical significance using the Dunnett's-t-test (test item) or Student-t-test (reference item). The accepted significance level was $\alpha = 0.05$.

Since there were only low effects on reproductive capacity in the test item treatment groups, a calculation of the $ER_{50}$ (median effect rate) was not possible.

For evaluation the statistical program ToxRat Professional 3.2.1 was used.

**Results and Discussion**

In the control group, the mean reproductive capacity at the end of the hatching phase was 707 beetles per replicate. For the treatments withSAE053H/01, the mean reproductive capacity ranged between 663 and 703 beetles per replicate and was not statistically significantly different from the control. The reduction of reproduction capacity in comparison with the control was between 0.6 and 6.2% (Table A 2.3.2.2-1). Based on these findings and since reduction of reproductive capacity was < 50% up to the highest application rate of 1500 mL product/ha, the $EC_{50}$ for reproduction was determined as > 1500 mL product/ha.

**Table A 2.3.2.2-1: Reproduction of *A. bilineata* exposed to SAE053H/01**

| Application rate [L product/ha] | Reproductive capacity [mean number of offspring per replicate ± SD] | [% reduction of control] |
|---|---|---|
| **Test substance: SAE053H/01** | | |
| Control | 707 ± 24.7 | - |
| 0.0938 | 703 ± 21.5 | 0.6 |
| 0.188 | 687 ± 40.1 | 2.9 |
| 0.375 | 663 ± 18.0 | 6.2 |
| 0.75 | 675 ± 20.5 | 4.6 |
| 1.5 | 669 ± 30.9 | 5.3 |
| **Reference substance: Dimethoate 400 EC** | | |
| 1.5 | 41 ± 45.3 | 94.2 |
| **Endpoint (95% CL) [mL SAE053H/01/ha]** | | |
| $ER_{50}$ (reproduction) | > 1500 | |

SD standard deviation

The test is considered to be valid since the reproductive capacity was ≥ 400 beetles per test unit in the control group (actual: ~~682~~ 707 beetles). Furthermore, mean reduction of reproductive capacity in the reference group was ≥ 50% when compared with the control (actual: 94.2%).

**Conclusion**

Under extended laboratory conditions (standard soil LUFA 2.1), the $ER_{50}$ of SAE053H/01 for the rove beetle *Aleochara bilineata* was determined to be > 1500 mL product/ha. The validity criteria were fulfilled.

### A 2.3.2.3 KCP 10.3.2.3 Semi-field studies

No additional data submitted.

### A 2.3.2.4 KCP 10.3.2.4 Field studies

No additional data submitted.

## 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

#### A 2.4.1.1.1 Study 1: Toxicity to *Eisenia fetida*

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 222 and according to the principles of GLP. In the definitive test all validity criteria were met.<br>The study is considered to be reliable and suitable for the risk assessment. |

| | |
|---|---|
| Reference: | KCP 10.4.1.1/01 |
| Report | SAE053H/01: Sublethal toxicity to the earthworm *Eisenia fetida* (Annelida, Lumbricidae) in artificial soil with 10 % peat, Wagenhoff, E., 2016a, S16-01484 |
| Guideline(s): | Yes, OECD 222 (2004), ISO 11268-2 (2012) |
| Deviations: | Yes, since a combined approach was followed to determine both the NOEC and $EC_x$, a spacing factor not exceeding 1.8 should have been used. In this test, the spacing factor was 2. The deviation is not considered to affect the validity and integrity of the study, as no $EC_x$ (reproduction) could be determined due to the lack of a clear dose-response relationship up to the highest test concentration of 100 mg product/kg dry soil. |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | - |

**Materials and Methods**

**A. MATERIALS**

**1. Test material** — SAE053H/01
(Other name: Mesotrione/Nicosulfuron 80/30 OD)

| | |
|---|---|
| **Description** | White to beige liquid/cream, OD (oil dispersion) |
| **Lot/Batch #** | 54606-101 |
| **Purity** | Nicosulfuron: 30 g/L nominal; 30.7 g/L analysed<br>Mesotrione: 80 g/L nominal; 81.7 g/L analysed<br>Density: 0.98 g/cm³ |
| **Stability of test material** | Stable under storage conditions (ambient temperature, 5°C – 30°C, in a dark and dry place)<br>Expiry date: 20 Mar 2018 |

**2. Vehicle and/or positive control** — Vehicle control: Deionised water
Positive control: The reference item carbendazim (formulation 'Twist WP 60 % w/w') was tested in a separate study in March 2016. During this study, carbendazim showed statistically significant effects on reproduction at 2.40 mg a.s./kg dry soil (required: between 1 and 5 mg a.s./kg dry soil) and hence, acceptable sensitivity of the test system was assured.

**3. Test organism**

| | |
|---|---|
| **Species** | Earthworm *Eisenia fetida* |
| **Source** | Obtained from a healthy laboratory rearing stock maintained in the testing facility. |

| | |
|---|---|
| **Age** | Adults with clitellum at least two months old, but not older than one year (difference in age of earthworms: ≤ 4 weeks); body weight at test start: 300 - 598 mg |
| **Acclimatisation** | One day before exposure, the adult earthworms were selected and transferred from the rearing medium into moist, untreated artificial soil for acclimatisation. |
| **Diet** | Finely ground cow manure was used as food. On day 1 after application, 4 g food were uniformly distributed on the soil surface of each test vessel and moistened with 4 g deionised water sprayed onto the soil surface. On day 7, 14 and 21 after test start, food was strewn onto the soil surface and moistened depending on the feeding activity of the earthworms. On the 28-day assessment, after adult worms were removed, 4 g of food were added per test vessel for the reproduction test. Offspring were not fed further during the remaining 28 days of the study. |
| **Test units** | White plastic vessels (approximately 17 cm x 12.5 cm x 6 cm; 1000 $cm^3$), filled with artificial soil to a height of approximately 5 cm, corresponding to 500 g dry soil, were used as test vessels. The test vessels were covered by plastic lids with holes to prevent worms from escaping and to allow for gaseous exchange, whilst limiting evaporation. |

**4. Environmental conditions**

**Soil**

Artificial soil was prepared with the following constituents:

| | |
|---|---|
| Sphagnum peat | 10% |
| Kaolin clay | 20% |
| Fine industrial sand | approximately 70% |
| Calcium carbonate ($CaCO_3$) | < 1% (for adjustment of pH to 6.0 ± 0.5 at test start) |

The maximum water holding capacity (WHC) of the soil was determined as 53.65%.
One day prior to test start, the artificial soil was pre-moistened with deionised water to obtain approximately half of the final water content. Final moistening to approximately 55% WHC was achieved during the application.

| | |
|---|---|
| **Temperature** | nominal: 18 - 22°C; actual: 20.6 – 21.7°C |
| **Photoperiod** | 16-hour light (light intensity: 500-800 Lux) to 8-hour dark photoperiod |

**B. STUDY DESIGN AND METHODS**

**1. In-life dates** 21 Jul 2016 to 16 Sep 2016

**2. Experimental conditions**

*Test design*

Adult earthworms were exposed to soil treated with the test substance at eight concentrations or remaining untreated (control) for a period of four weeks. After this period, the adults were removed from the test

vessels and their mortality, growth (body weight change) and food consumption were determined. The cocoons and juvenile earthworms remained in the test vessels for additional four weeks. The reproduction rate was determined by counting the number of offspring hatched from the cocoons after this additional test period of four weeks (56 days after application).

*Number of animals per treatment*

Ten earthworms/replicate; four replicates/test substance treatment and eight replicates/control

*Test conditions*

After application, the soil moisture content in each test vessel was adjusted to 30.3 – 31.5% by addition of deionized water. The soil moisture content at study end was 29.6 – 31.8%. The pH value in the test substance treatments and control was 6.0 – 6.1 at the start of the test and 6.0 – 6.3 at the end of the test. During the test period, the test temperature was 20.6 – 21.7°C.

*Test concentrations*

SAE053H/01 was tested at 0.79, 1.58, 3.16, 6.25, 12.5, 25.0, 50.0 and 100 mg product/kg dry soil corresponding to 0.0247, 0.0495, 0.0990, 0.196, 0.392, 0.783, 1.57 and 3.13 mg a.s./kg dry soil nicosulfuron and 0.0659, 0.132, 0.263, 0.521, 1.04, 2.08, 4.17 and 8.34 mg a.s./kg dry soil mesotrione (based on analysed content of active substances and product density). A control (receiving deionised water only) was tested in parallel.

*Treatment/Application*

The highest test concentration was prepared by dissolving 1.005 g of the test item with deionised water up to a weight of 502.5 g. It served as a stock solution for the lower test concentrations and was therefore diluted with deionised water accordingly.

For each test item group, 110 g of the respective test item solution was mixed into 2739 g of pre-moistened soil substrate (equivalent to 2200 g dry soil substrate). For the control, 210 g of deionized water (containing no test item) were mixed into 5229 g of pre-moistened soil substrate (equivalent to 4200 g dry soil substrate). Thus, the desired soil moisture content of 55% WHC was obtained. Immediately after mixing, the test substrate of each treatment group was split and 648 g (corresponding to 500 g dry soil substrate) were placed into the test units.

All worms were washed, weighed individually and the ones meeting the selection criteria were randomly assigned to batches of 10 worms. Then, the groups were assigned to the test container replicates using a procedure to obtain a homogeneous distribution of the worm groups throughout all treatment groups regarding their mean body weight. The earthworms were placed on the surface of the artificial soil after application.

After 4 weeks, the artificial soil was transferred to a tray and adult worms were assessed for mortality, growth (body weight change) and food consumption. The remaining soil (without the adult worms) was then returned to the respective test containers for reproduction assessment.

On day 56, juveniles were removed by placing the test units in a water bath at 55°C and counting the emerging worms after approximately 30 minutes of heating. This method was validated by the test facility once a year.

**3. Sampling and measurements**

After four weeks of exposure, adult test organisms were sorted from the soil and their reaction to a gentle mechanical stimulus at the anterior end was tested. Individuals showing no reaction or missing were counted as dead. If observed, any behavioural or pathological symptoms were reported.

Adult earthworms were weighed individually at the beginning of the test. After 28 days of exposure, the total weight of all surviving earthworms per replicate was determined after washing them shortly before weighing.

On day 7, 14 and 21 after test start, food consumption of the adult worms was estimated for each replicate using an evaluation system with five categories.

At test termination after eight weeks, the number of living juveniles per test vessel was determined.

At the start of the test, soil moisture was checked for all treatment groups and the control. The soil moisture was adjusted weekly during the first 28 days of the test period by reweighing the test units taking into consideration the respective amount of added food. From day 28 to day 56, no moisture adjustment was performed since the water loss was on a low level and thus regarded as tolerable. At the end of the test, the soil moisture was checked again for all treatment groups and the control.

The pH of the soil was checked for all treatment groups and the control at the start and at the end of the test.

The temperature was recorded continuously with appropriate, calibrated equipment. Lighting intensity was measured once during the test.

**4. Calculation of toxicity**

The percentage of adult mortality was calculated for each replicate from the number of dead individuals in relation to the number of introduced test organisms. In addition, the mean mortality for each treatment group was determined.

The body weight change per earthworm was determined for each replicate and the mean body weight change per earthworm was calculated for each treatment group and given as absolute (in mg) and relative (in percent) value.

The amount of food consumed within the first 28 days was estimated for each treatment group.

The number of juveniles per replicate was counted after eight weeks. The reduction of mean number of juveniles per replicate was determined for each test item treatment in comparison to the control.

**5. Statistics**

Multiple Fisher's exact test with Bonferroni-Holm adjustment (one-sided greater) was performed for analysis of mortality data.

Data on body weight change (weight change per replicate in mg) were tested for normality and homoscedasticity using Shapiro-Wilk test and Levene’s test, respectively, followed by Dunnett’s t-test (two-sided).

Data on reproduction was tested for normality and homoscedasticity using Shapiro-Wilk test and Levene's test, respectively. A trend analysis by contrasts was performed and since no linear trend was found, Dunnett's t-test (one-sided smaller) was conducted to compare the test item groups with the control group.

For data evaluation, the statistical programme ToxRat Professional 3.2.1 was used. The level of significance was set to $\alpha = 0.05$ for all statistical tests.

The $EC_{10}$, $EC_{20}$ and $EC_{50}$ for reproductive output could not be calculated since no clear dose-response relationship was observed up to the highest test concentration of 100 mg product/kg dry soil.

**Results and Discussion**

After four weeks of exposure, mean mortality of adult test organisms in the control and in the test item treatments was 5.0% and 0.0-12.5%, respectively (see following table). No statistically significant difference in mortality of the test item treatments was observed in comparison to the control. Thus, the survival rate of adult earthworms after four weeks of exposure to SAE053H/01 was not affected up to and including the highest test concentration, i.e. the NOEC for mortality is ≥ 100 mg product/kg dry soil. Moreover, no behavioural abnormalities or any pathological symptoms were observed in adult test organisms at any test concentration.

After four weeks of exposure, the mean change in body weight of adult earthworms in the control was 24.3%. In the test item treatments, the mean change in body weight of adult earthworms ranged between -4.8% and 28.7% with a statistically significant decrease in body weight change determined at 25.0, 50.0 and 100 mg test item/kg soil dry weight when compared to the control group (see following table). Thus, the NOEC for body weight change was determined to be 12.5 mg product/kg dry soil and the corresponding LOEC was determined to be 25.0 mg product/kg dry soil.

No difference in food consumption was determined between the control and all test item treatments.

In the control, a mean reproduction rate of 157.4 juveniles per replicate was found. In all test item treatments, the mean reduction in reproduction was between -17.4% and 16.1% of the control value and was not statistically significantly different from the control (see following table). Therefore, reproduction of adult earthworms after exposure to SAE053H/01 was not affected up to and including the highest test concentration, i.e. the NOEC for reproduction is ≥ 100 mg product/kg dry soil. The $EC_{10}$, $EC_{20}$ and $EC_{50}$ for reproduction could not be determined due to a lack of a clear dose-response relationship up to the highest test concentration of 100 mg product/kg dry soil.

**Table 9.10.2.4-1: Effects of SAE053H/01 on earthworm mortality, body weight change and reproduction**

| Treatment [mg product/kg dry soil] | Mean mortality after 4 weeks of exposure [%] | Mean change in body weight after 4 weeks of exposure [%] | Reproduction rate after 8 weeks | | |
|---|---|---|---|---|---|
| | | | [mean number of juveniles/replicate] | Coefficient of variation [%] | Reduction compared to control [%] |
| Control | 5.0 | 24.3 | 157.4 | 20.9 | - |
| 0.790 | 2.5 | 20.7 | 147.0 | 33.5 | 6.6 |
| 1.58 | 2.5 | 28.7 | 174.5 | 5.6 | -10.9 |
| 3.16 | 2.5 | 20.8 | 158.3 | 37.3 | -0.6 |
| 6.25 | 12.5 | 22.7 | 160.0 | 16.4 | -1.7 |
| 12.5 | 12.5 | 15.8 | 173.5 | 37.2 | -10.2 |
| 25.0 | 0.0 | 7.8* | 184.8 | 10.1 | -17.4 |
| 50.0 | 0.0 | 5.4* | 162.0 | 21.8 | -2.9 |
| 100 | 2.5 | -4.8* | 132.0 | 13.4 | 16.1 |
| **Endpoints [mg product/kg dry soil]** | | | | | |
| $EC_{10}$, $EC_{20}$, $EC_{50}$ (reproduction) | | Not determinable (no clear dose-response relationship) | | | |
| NOEC (body weight change) | | 12.5 | | | |
| LOEC (body weight change) | | 25.0 | | | |
| NOEC (mortality, reproduction) | | ≥ 100 | | | |
| LOEC (mortality, reproduction) | | > 100 | | | |

Note: Test substance treatments were not statistically significantly different from the control for mortality (Multiple Fisher's exact test with Bonferroni-Holm adjustment, one-sided greater, α = 0.05) and reproduction (Dunnett's t-test, one-sided smaller, α = 0.05).
Negative values indicate an increase compared to the control.
* Statistically significantly different from the control, according to Dunnett's t-test (two-sided, α = 0.05)

The validity of the test was fulfilled since each control replicate produced ≥ 118 juveniles (required ≥ 30 juveniles) and the coefficient of variance of the reproduction rate per test vessel in the control was 20.9% (required ≤ 30%). Furthermore, mean mortality of adults in the control was 5.0% (required ≤ 10%).

**Conclusion**

The NOEC for mortality and reproduction was ≥ 100 mg product/kg dry soil, the highest concentration tested. Based on a reduction of body weight change of adult earthworms, the NOEC for body weight change was determined to be 12.5 mg product/kg dry soil with a corresponding LOEC of 25.0 mg product/kg dry soil. The $EC_{10}$, $EC_{20}$ and $EC_{50}$ for reproduction could not be determined due to a lack of a clear dose-response relationship up to the highest test concentration of 100 mg product/kg dry soil. All validity criteria were fulfilled.

### A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

No additional data submitted.

## A 2.4.2 KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other

**than earthworms)**

### A 2.4.2.1 KCP 10.4.2.1 Species level testing

### A 2.4.2.1.1 Study 1: Toxicity to *Folsomia candida*

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 232 and according to the principles of GLP. In the test all validity criteria were met.<br>The study is considered to be reliable and suitable for the risk assessment. |

| | |
|---|---|
| Reference: | KCP 10.4.2.1/01 |
| Report | SAE053H/01 – Effects on the reproductive output of the springtail *Folsomia candida* Willem (Collembola, Isotomidae) in artificial soil, Häuser, R., 2016, S16-01485 |
| Guideline(s): | Yes, OECD 232 (2009) |
| Deviations: | Yes, in five replicates 11 instead of 10 test organisms were introduced due to a handling error. As the evaluation of results was corrected accordingly, this deviation is considered to have no impact on the validity and integrity of the study. |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication<br>(if vertebrate study) | - |

**Material and Methods**

**A. MATERIALS**

| | |
|---|---|
| **1. Test material** | SAE053H/01<br>(Other name: Mesotrione/Nicosulfuron 80/30 OD) |
| **Description** | White to beige liquid/cream, OD (oil dispersion) |
| **Lot/Batch #** | 54606-101 |
| **Purity** | Nicosulfuron: 30 g/L nominal; 30.7 g/L analysed<br>Mesotrione: 80 g/L nominal; 81.7 g/L analysed<br>Density: 0.98 g/cm³ |
| **Stability of test material** | Stable under storage conditions (ambient temperature, 5°C – 30°C, in a dark and dry place)<br>Expiry date: 20 Mar 2018 |
| **2. Vehicle and/or positive control** | Vehicle control: purified water<br>Positive control: The reference item boric acid was tested in a separate study in February 2016. The $EC_{50}$ for reproduction was determined as 105.2 mg/kg dry soil (required: ~ 100 mg/kg dry soil) and hence, acceptable sensitivity of the test system was assured. |
| **3. Test organism** | |

| | |
|---|---|
| **Species** | Springtail (*Folsomia candida* Willem; Collembola: Isotomidae) |
| **Source** | In-house culture at the test facility, identity of the species is confirmed once a year |
| **Age** | Juvenile springtails (9-11 days old), obtained from a synchronised breeding culture, were used in the test. |
| **Acclimatisation** | For 11 days, juveniles were maintained in a controlled-environment cabinet (19.5 – 21.4°C) before being used for the test. |
| **Diet** | At the beginning of the test and after a period of 14 days, 3 mg of granulated yeast was added to each test vessel. |
| **Test units** | The test units were glass vessels (6.5 cm in diameter and approximately 250 mL volume) secured with a screw lid containing a ventilation hole. Each test unit was filled with 30 g wet weight of artificial soil. |

**4. Environmental conditions**

| | |
|---|---|
| **Soil** | Artificial soil was prepared with the following constituents (percentage distribution on dry weight basis): |

| | |
|---|---|
| Sphagnum peat | 5% |
| Kaolin clay | 20% |
| Industrial sand | 75% |
| Calcium carbonate | < 1% (for pH adjustment) |

The dry components were blended and mixed thoroughly. Then, the maximum water holding capacity (WHC) was determined as 41.27% and the pH was determined to be 5.9.
Two days prior to test start, deionised water was added to the artificial soil to achieve approximately half of the final water content.

| | |
|---|---|
| **Temperature** | Nominal: 20 ± 2°C; actual: 19.5 – 21.0°C |
| **Photoperiod** | 16 hour light (light intensity: nominal: 400 - 800 Lux, actual ~500 Lux) to 8 hour dark photoperiod |

## B. STUDY DESIGN AND METHODS

**1. In-life dates** 20 Jul 2016 to 22 Aug 2016

**2. Experimental conditions**

*Test design*

Juvenile springtails were exposed to soil treated with the test substance at five concentrations for a period of four weeks. A water control (deionised water) was tested in parallel. After four weeks of exposure, the number of adults was counted and mortality was determined. The reproduction output was determined by counting the number of offspring (F1) that emerged from the eggs laid by the test animals.

*Number of animals per treatment*

Four replicates per test substance treatment and eight replicates each for the control were used with ten springtails per replicate (except for five replicates to which 11 animals had been introduced by accident). One additional vessel per treatment group without test organisms was set up for pH and water content determination.

*Test conditions*

After application, the soil moisture content in each test vessel was adjusted to 20.8 – 23.3% by addition of water. The soil moisture content at study end was 19.4 – 20.1%. The pH value in the test substance treatments and control was 5.6 – 5.7 at the start of the test and 5.4 – 5.7 at the end of the test. During the test period, the test temperature was 19.5 – 21.0°C and the light intensity approximately 500 Lux.

*Test concentrations*

SAE053H/01 was tested at 62.5, 125, 250, 500 and 1000 mg product/kg dry soil corresponding to 5.210, 10.42, 20.84, 41.68 and 83.37 mg a.s./kg dry soil mesotrione and 1.958, 3.916, 7.832, 15.66 and 31.33 mg a.s./kg dry soil nicosulfuron (based on analysed formulation contents and product density).

Additionally, a water control (deionised water) was tested in parallel. The reference item boric acid was tested in a separate study.

*Treatment/Application*

For the preparation of test item solutions, a stock solution (= highest test concentration) was prepared by dispersing 0.978 g of test item in deionised water. The remaining test item solutions were prepared by dilution of the stock solution. The treatment solutions were mixed with the soil and then 30 g of treated test substrate was added to each test unit.

**3. Sampling and measurements**

After 28 days of exposure the test soil of each exposure unit was poured into a 500 mL plastic container with a diameter of approximately 11 cm. Tap water was added to a height of approximately 2 cm (approx. 150 mL). After adding some drops of black ink and gently stirring the watered soil for approximately 30 seconds with a small spoon, further 150 mL tap water were carefully added to avoid the development of a wet edge, where springtails could climb up and avoid being photographed.
The number of parental and juvenile springtails, which floated to the surface, was determined. Adult springtails which were not found on the surface after the extraction were considered as dead. To facilitate counting, photos of the water surface were made with a digital camera (Pentax K3). Adult and juvenile springtails were counted on a screen of a pen tablet (Genius Mouse Pen 8 x 6) in combination with a mousotron software program (mousotron 5.0) and with the Folsomia counter software (each picture was individually corrected by hand).

The efficiency of the extraction method is validated within the toxic reference item study once a year. Overall efficiency of extraction (adults and juveniles) of the latest validation was 96.4 % (required: > 95%).

Any pathological symptoms or distinct changes in behaviour observed during test were recorded.

The soil moisture was checked at the beginning, after 14 days and after 28 days by weighing the test units. Water losses were compensated for by addition of water.

At the start and end of the test, the pH of the artificial soil was measured. The test temperature and relative humidity were recorded continuously. The light intensity was measured once during the test.

**4. Calculation of toxicity**

The percentage of mortality after 28 days was calculated for each replicate from the number of dead adult individuals (not present after extraction) in correlation to the number of 10 introduced springtails. In case 11 instead of 10 test organisms had been introduced (see deviations) the percentage of mortality was calculated from the number of dead adult individuals (not present after extraction) in correlation to the number of 11 introduced springtails. From the percentage values per replicate the mean value and standard deviation were calculated for each treatment group.

For each treatment group the mean value of the number of juveniles, standard deviation and coefficient of variation were calculated. Replicate values were corrected in case 11 juveniles had been introduced at test start by accident. Therefore the number of determined juveniles at test end was corrected per 10 adults.
The effect on reproductive output was determined by calculating the reduction in reproduction rate compared to control according to ABBOTT (1925).

**5. Statistics**

The level of significance for all statistical procedures was set to $\alpha = 0.05$.

For all statistical tests the normalised data for mortality and reproductive output was applied. Mortality data (number introduces females and dead adult females per treatment group) of the test item groups were analysed for significant differences compared to the control group using multiple Fisher's exact test with Bonferroni-Holm adjustment (one-sided greater, $\alpha = 0.05$).
Concerning reproductive output Shapiro-Wilk test rejected the correspondence with normal distribution. The hypothesis of homogeneity was rejected by Levene´s test. Accordingly, multiple Mann-Whitney U-test with Bonferroni-Holm adjustment (one-sided smaller, $\alpha = 0.05$) was used for statistical analysis to detect significant differences between the test item groups and the control group.
$EC_{10}$ and $EC_{20}$ for reproductive output could not be calculated reliably since a clear dose-response relationship was absent in the tested concentration range and therefore are not reported. $EC_{50}$ for reproductive output could not be calculated.
For data evaluation the statistical programme ToxRat Professional 3.2.1 was used.

**Results and Discussion**

In the control, mean mortality of adult test organisms after four weeks was 1.3%. Mortality in the test substance concentrations was between 2.5 and 10.0% and not significantly different from the control. The NOEC of SAE053H/01 for mortality was therefore determined at ≥ 1000 mg product/kg dry soil. The results are presented in the following table.

No physiological symptoms or abnormal behaviour of springtails were observed.

In the control, 808.6 juvenile springtails per replicate were found on average. The mean reproduction rates in the test substance treatments up to and including 125 mg product/kg dry soil were reduced by 5.8 - 7.0% compared to the control and not statistically significantly different from the control. In the three highest treatments of 250, 500 and 1000 mg product/kg dry soil, reproduction was reduced by 12.0 – 29.9%, which was statistically significantly different from the control. The NOEC for reproduction was therefore determined to be 125 mg product/kg dry soil. As no effects above 50% were observed, the $EC_{50}$ could not be determined. The $EC_{20}$ and $EC_{10}$ could also not be determined as no clear dose-response relationship was observed. The results are presented in the following table.

**Table 9.10.2.4-1: Effects of SAE053H/01 on survival and reproduction of *Folsomia candida***

| Treatment [mg product/kg dry soil] | Mortality after 4 weeks [%] | Reproduction output after 4 weeks | |
|---|---|---|---|
| | | [mean juveniles/test vessel ± SD] | Reduction in reproductive output [%] |
| Control | 1.3 | 808.6 ± 71.0 | - |
| 62.5 | 10.0 | 751.8 ± 141.1 | 7.0 |
| 125 | 7.5 | 761.5 ± 27.9 | 5.8 |
| 250 | 10.0 | 702.3* ± 36.5 | 13.1 |
| 500 | 2.5 | 711.5* ± 39.1 | 12.0 |
| 1000 | 7.5 | 566.5* ± 20.1 | 29.9 |
| **Endpoints [mg product/kg dry soil]** | | | |
| NOEC | ≥ 1000 (mortality) and 125 (reproduction) | | |

* Value statistically significantly different compared to the control (mortality: no statistical significance according to multiple Fisher's exact test with Bonferroni-Holm adjustment, α = 0.05, one-sided greater; reproduction: multiple Mann-Whitney U-test with Bonferroni-Holm adjustment, one-sided smaller, α= 0.05)

The validity of the test was fulfilled since mean mortality of adults in the control was 1.3% (required ≤ 20%) at the end of the test, the reproduction rate was at least 808.6 juveniles per control replicate (required ≥ 100) and the coefficient of variation of reproduction was 8.8% in the control (required ≤ 30%).

**Conclusion**

In this study the NOEC of SAE053H/01 for *Folsomia candida* was determined to be 125 mg product/kg dry soil (based on reproduction). The $EC_{50}$ could not be determined. All validity criteria were fulfilled.

## A 2.4.2.1.2 Study 2: Toxicity to *Hypoaspis aculeifer*

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 226 and according to the principles of GLP. In the test all validity criteria were met.<br>The study is considered to be reliable and suitable for the risk assessment. |

| | |
|---|---|
| Reference: | KCP 10.4.2.1/02 |
| Report | SAE053H/01 – Effects on the reproductive output of the predatory mite *Hypoaspis aculeifer* Canestrini (Acari: Laelapidae), Wagenhoff, E., 2016b, S16-01486 |
| Guideline(s): | Yes, OECD 226 (2008) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | - |

## Materials and Methods

### A. MATERIALS

**1. Test material**

SAE053H/01
(Other name: Mesotrione/Nicosulfuron 80/30 OD)

| | |
|---|---|
| **Description** | White to beige liquid/cream, OD (oil dispersion) |
| **Lot/Batch #** | 54606-101 |
| **Purity** | Nicosulfuron: 30 g/L nominal; 30.7 g/L analysed<br>Mesotrione: 80 g/L nominal; 81.7 g/L analysed<br>Density: 0.98 g/cm³ |
| **Stability of test material** | Stable under storage conditions (ambient temperature, 5°C – 30°C, in a dark and dry place)<br>Expiry date: 20 Mar 2018 |

**2. Vehicle and/or positive control**

Vehicle control: purified water
Positive control: The reference item Perfekthion (a.s. dimethoate) was tested in a separate study in January 2016. The $EC_{50}$ for reproduction was determined as 5.5 mg a.s./kg dry soil (required: 3.0 – 7.0 mg a.s./kg dry soil) and hence, acceptable sensitivity of the test system was assured.

**3. Test organism**

| | |
|---|---|
| **Species** | Predatory mite *Hypoaspis aculeifer* Canestrini (Acari: Laelapidae) |
| **Source** | From a healthy colony, cultured at the test facility, identity of test species is confirmed once a year |
| **Age** | Adults (32 days after starting of the egg laying for synchronisation) |
| **Acclimatisation** | Synchronisation took place in a controlled environment room at 19.8 – 21.1°C and darkness. |
| **Diet** | During their development the mites were fed with cheese mites (*T. putrescentiae*) 2 to 3 times a week. |
| **Test units** | Glass vessels (5.5 cm height, 5.5 cm diameter, volume ~ 100 mL) with screw lid containing ventilation hole closed with mite-impermeable gauze (mesh size 100 µm). Units were filled with 20 g artificial soil (dry mass). |

**4. Environmental conditions**

**Soil**

Artificial soil was prepared with the following constituents:

| | |
|---|---|
| Sphagnum peat | 5% |
| Kaolin clay | 20% |
| Industrial sand | ~ 75% |
| Calcium carbonate | < 1% (to adjust pH) |

After blending and mixing the dry constituents, the maximum water holding capacity (WHC) of the soil was determined to be 41.73%. The artificial soil was moistened to approximately half of the final water content four days before application. The additional water

| | |
|---|---|
| | required to achieve the final nominal water content of 50% WHC was added when applying the test substance. |
| **Temperature** | Nominal: 20 ± 2°C; actual: 19.6 – 21.0°C |
| **Photoperiod** | 16 hour light (light intensity: nominal 400 - 800 Lux; actual ~ 490 Lux) to 8 hour dark photoperiod |

## B. STUDY DESIGN AND METHODS

**1. In-life dates** 26 Jul 2016 to 16 Aug 2016

**2. Experimental conditions**

*Test design*

Adult female mites were exposed to soil treated with the test substance at five concentrations for a period of 14 days. Deionised water was used as a control treatment. The reference item Perfekthion (a.s. dimethoate) was tested in a separate study. At the end of the exposure period, the surviving individuals were extracted from the test units. The number of juveniles per test unit and additionally the number of surviving females were determined. The reproductive output and the mortality in the test item group were compared to that of the control group.

*Number of animals per treatment*

Ten female mites per replicate; four replicates per test substance treatment and eight replicates per control

An additional replicate without mites was prepared for each treatment group to determine pH and water content.

*Test conditions*

The definite water content of the test substrate at test initiation and at test termination was determined to be 21.2 – 21.9% and 20.5 – 21.6%, respectively. The final moisture content did not differ by more than 10% from the start value (actual: 5.9%). Soil pH values at test initiation were recorded between 5.7 and 5.8, at test termination pH was between 5.6 and 5.7. The test temperature was 19.6 – 21.0°C. A photoperiod of 16 hour light (light intensity: nominal 400-800 Lux, actual ~ 490 Lux) to 8 hour dark photoperiod was maintained.

*Test concentrations*

SAE053H/01 was tested at 10.0, 20.0, 40.0, 80.0 and 160 mg product/kg dry soil corresponding to 0.313, 0.627, 1.253, 2.506 and 5.012 mg a.s./kg dry soil nicosulfuron and 0.834, 1.667, 3.335, 6.669 and 13.339 mg a.s./kg dry soil mesotrione (based on analysed formulation contents and product density). A control was tested in parallel. The reference item Perfekthion (a.s. dimethoate) was tested in a separate study.

*Treatment/Application*

The test solution for the highest test concentration was prepared by dispersing 0.109 g of the test item in deionised water. The remaining test item solutions were prepared by diluting the highest test concentration with deionised water.
The substrate was adjusted to a water content of 50 % of the total water-holding capacity by adding the remaining quantity of deionized water (control treatments) or test item solution (test item treatments) to the

pre-moistened substrate. Once treated, 20 g (dry weight equivalent) of the treated soil was transferred into each test unit. The mites were introduced into the test units within 60 minutes after preparation of the final test soil by using a fine brush.

**3. Sampling and measurements**

On day 14 of the test the surviving adult females and juveniles were extracted from the soil using a high temperature gradient extractor (Macfadyen, 1961), a modified Berlese-Tullgren funnel. This apparatus consists of a light source mounted over a funnel containing a wire mesh that fits the circumference of the funnel. Mites move away from the heat and light, fall through the mesh and are funnelled into a jar, filled with 70% ethanol.
The test container with the test soil and the organisms were emptied into a funnel with a wire mesh barrier. The mesh allows the organisms to pass but prevents test soil from trickling into the liquid. Each replicate was extracted over a separate jar. The jars were covered with fabric to prevent the organisms from escaping. The emptied test containers were checked for remaining organisms (adult females and juveniles). After extraction, the organisms in the liquid were stored in the refrigerator until counting. The duration of the extraction was 48 hours in total with a temperature gradient between 23.7° C and 47.9° C. The efficiency of the extraction method is tested regularly once a year.

The soil water content was measured at test start and end for each treatment group. The water content of the soil substrate in the test vessels was maintained throughout the test by weighing and if necessary re-watering the test vessels 2 times a week. Losses were replenished as necessary.

At the beginning and end of the test the soil pH was determined. Temperature was recorded continuously; light intensity was measured once at the start of the test.

**4. Calculation of toxicity**

The percentage of total mortality after 14 days was calculated for each replicate from the number of not recovered adult females compared to the number of initially introduced adult females. The mean value and standard deviation were calculated for each treatment group. Any physical or pathological symptoms or distinct changes in the behavior of the mites were recorded as adverse effects.

The mean number of juveniles in each treatment group was calculated by averaging the replicate values. For each treatment group the mean value, standard deviation and coefficient of variation was calculated.
The effect on reproductive output was determined by calculating the reduction of reproduction rate compared to control according to ABBOTT (1925).

**5. Statistics**

The level of significance was set to $\alpha = 0.05$ for all statistical tests.

Multiple Fisher's exact test with Bonferroni-Holm adjustment (one-sided greater) was used for comparison of mortality data of the test item treatments and the control group.

Reproductive output data were tested for normality and homoscedasticity using Shapiro-Wilk's test and Levene's test followed by Williams' test (one-sided smaller) to detect significant differences between reproductive output data of the test item groups and the control group.

For data evaluation the statistical programme ToxRat Professional 3.2.1 was used.

## Results and Discussion

In the control group, mean mortality of adult test organisms after 14 days was 11.3%. Mean mortality at all test substance concentrations ranged between 2.5 and 20.0% and was not statistically significantly different from the control (see following table).

No behavioural abnormalities or pathological symptoms were observed.

In the control group, the mean number of juveniles per replicate was 283.6. The reduction in reproduction compared to the control in the test item treated groups up to and including 80.0 mg product/kg dry soil group was between -1.7% (increase in reproduction) and 12.2% and was not statistically significant different from the control. In the highest test item treatment of 160 mg product/kg dry soil the reduction was 20.5% and statistically significantly different from the control (see following table).

**Table 9.10.2.41-2: Effects of SAE053H/01 on survival and reproduction of *Hypoaspis aculeifer***

| **Treatment** [mg product/kg dry soil] | **Mortality after 14 days** [%] | **Reproduction output after 14 days** | |
|---|---|---|---|
| | | **[mean juveniles/ test vessel ± SD]** | **Reduction in reproduction [a)] [%]** |
| Control | 11.3 | 283.6 ± 42.0 | - |
| 10.0 | 12.5 | 286.8 ± 31.4 | -1.1 |
| 20.0 | 2.5 | 288.3 ± 25.4 | -1.7 |
| 40.0 | 10.0 | 270.8 ± 45.9 | 4.5 |
| 80.0 | 20.0 | 249.0 ± 51.7 | 12.2 |
| 160 | 12.5 | 225.5* ± 31.9 | 20.5 |
| **14-d endpoints [mg product/kg dry soil]** | | | |
| $EC_{20}$ & $EC_{10}$ (reproduction and mortality) | n.d. | | |
| NOEC (reproduction) | 80.0 | | |
| NOEC (mortality) | ≥ 160 | | |

Note: There were no statistically significant differences between mortality in the test item treatments and the control (results of a multiple Fisher's Exact Test with Bonferroni-Holm adjustment, one-sided greater, $\alpha = 0.05$)
n.d. not determined
* Statistically significantly different from the control (result of a Williams' test, one-sided smaller, $\alpha = 0.05$)
a) Negative values indicate and increased reproduction compared to the control.

The validity of the test was fulfilled since the mortality of female adults in the control was 11.3% (required ≤ 20%) at the end of the test, the mean reproduction rate was 283.6 juveniles per control replicate (required ≥ 50) and the coefficient of variation of reproduction in the control was 14.8% (required ≤ 30%).

## Conclusion

In this test on chronic toxicity to *Hypoaspis aculeifer*, the NOECs for mortality and reproduction of SAE053H/01 were determined to be ≥ 160 and 80.0 mg product/kg dry soil, respectively. All validity criteria were fulfilled in the study.

Remark on endpoints determined: The test was designed to obtain an NOEC as endpoint but was not appropriate to determine $EC_x$. However, based on the results of the study, no $EC_x$ could have been determined anyway due to effects being observed in the highest treatment level, only.

### A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

No additional data submitted.

## A 2.5 KCP 10.5 Effects on soil nitrogen transformation

### A 2.5.1.1 Study 1: Toxicity to the soil microflora

| | |
|---|---|
| Comments of zRMS: | Effects on the activity of soil microflora (nitrogen transformation test) was performed in line with requirements of OECD 216.<br>The soil nitrate formation rates were below the 15% trigger value given by the guideline. Since validity criteria were met the study is acceptable for risk assessment purposes. |

| | |
|---|---|
| Reference: | KCP 10.5/01 |
| Report | SAE053H/01: Effects on the activity of soil microflora under laboratory conditions (nitrogen transformation), Duffner, A., 2016, S16-01487 |
| Guideline(s): | Yes, OECD 216 (2000) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | - |

**Material and Methods**

**A. MATERIALS**

| | |
|---|---|
| **1. Test material** | SAE053H/01<br>(Other name: Mesotrione/Nicosulfuron 80/30 OD) |
| **Description** | White to beige liquid/cream, OD (oil dispersion) |
| **Lot/Batch #** | 54606-101 |
| **Purity** | Nicosulfuron: 30 g/L nominal; 30.7 g/L analysed<br>Mesotrione: 80 g/L nominal; 81.7 g/L analysed<br>Density: 0.98 g/cm³ |
| **Stability of test material** | Stable under storage conditions (ambient temperature, 5°C – 30°C, in a dark and dry place)<br>Expiry date: 20 Mar 2018 |
| **2. Vehicle and/or positive control** | Vehicle control: deionised water<br>Positive control: The method is validated by routinely testing the inhibition of nitrogen transformation using sodium chloride as a positive control. The results of the latest positive control test performed in Dec 2015 confirmed the sensitivity of the test system, i.e. a significant impact (i.e. deviation from control > 25%) of sodium chloride on nitrogen turnover (after 28 days) and short-term |

respiration activities of soil microflora (after 28 days) when applied at 20 g/kg dry soil.

**3. Test soil**

| | | |
|---|---|---|
| Soil type | Medium silty sand (DIN 11277)<br>Sandy loam (USDA) | |
| Batch # | F2.3 0316 | |
| Organic C | 0.69% | |
| $NO_3^-$N content | 18.8 mg/kg dry soil | |
| Total nitrogen | 0.08% | |
| pH | 7.0 | |
| Cation exchange capacity | 7.5 meq/100 g | |
| Max. water holding cap. (WHC) | 38.77% | |
| Dry weight | 93.0% | |
| Microbial biomass | 41.4mg C/100 g dry soil<br>(6.00% of organic C) | |
| Particle size analyses | USDA | DIN 11277 |
| Clay | 7.9% | 7.9% |
| Silt | 32.6% | 34.4% |
| Sand | 59.5% | 57.7% |

**Source** Offenbach, Germany
UTM Zone 32: E 439713, N 5449850

**Soil history** The soil had not been cultivated since 2012. For at least four years prior to soil sampling, no plant protection products had been applied to the sampling site. No organic fertilizer had been applied to the site for at least six months prior to soil sampling. To increase the soil pH calcium oxide and magnesium oxide had been applied to the site in two years prior to soil sampling.

**Soil sampling** 21 Jan 2016
The soil was sampled at a depth of ~20 cm and 2-mm sieved.

**Soil preparation** Seven days prior application the soil was pre-moistened to approx. 42 % of the $WHC_{max}$. Afterwards pre-moistened soil was incubated under conditions as for the exposure phase. On the day of application the pre-moistened soil was complemented with 0.5 % w/w of lucerne meal (per soil dry weight).

**Test units** Glass bottles (1 L) loosely closed with screw caps. Approx. 550 g of treated soil was placed in each bottle.

**4. Environmental conditions** The test was performed in the dark.

**Temperature** nominal: 20 ± 2°C; actual: 18.6 – 21.4°C

**Soil moisture** ~42% of the soil WHC

## B. STUDY DESIGN AND METHODS

**1. In-life dates** 27 Apr 2016 to 24 Jun 2016

**2. Experimental conditions**

*Test design*

Soil aliquots of a natural standard soil were treated once with the test substance at two concentrations (low and high dose) mixed into the soil or remaining untreated (control receiving deionized water treated quartz sand without test item). Effects on nitrogen transformation activity of the soil microflora were assessed by determination of ammonium, nitrite and nitrate contents at day 0, 7, 14, 28 and 42. Based on the analysed nitrate contents, nitrate formation rates were calculated.

*Number of replicates per treatment*

For each treatment/control, triplicate test units were set up.

*Test conditions*

At the time of application, the soil moisture content was adjusted to ~42% of the soil max. WHC. At test start the water content was between 15.2 and 15.6%, at test end the content was between 15.7 and 16.5%. The actual test temperature was 18.6 – 21.4 °C. The pH ranged between 6.9 and 7.3.

*Test concentrations*

SAE053H/01 was tested at 1.96 mg product/kg dry soil (low dose) and 9.80 mg product/kg dry soil (high dose), corresponding to 0.061 and 0.307 mg a.s./kg dry soil nicosulfuron and 0.163 and 0.817 mg a.s./kg dry soil mesotrione (based on the analysed content of active substances and the product density). Additionally, a control (deionised water) was tested in parallel.

*Treatment/Application*

The high dose test item solution was prepared by dispersing 0.0357 g of test item in deionised water. The lower dose test item solution was prepared by diluting an aliquot of the higher dose solution. The solutions were thoroughly mixed. The respective amount of control or test item solution to achieve ~42% of the maximum WHC of the soil was added to the treatment group. Each treatment group (soil with the test item added at two different concentrations or untreated control) was prepared from 1650 g pre-moistened test soil.

An amount of 0.5% (w/w) lucerne meal (related to soil dry weight) was added to the soil.

**3. Sampling and measurements**

At day 0, 7, 14, 28, and 42 after treatment, sub-samples were withdrawn from the soil bulk batches and subjected to analysis.

Soil nitrate was determined by measuring the $NO_3^-$ contents of aqueous soil extracts by means of Segmented Flow Analysis (SFA) using photometric measurement. The $NO_3^-$ concentrations of the soil samples were calculated from the measured values.
Each 10 g soil sample that had been taken from each treatment replicate was mixed with 40 mL of a 0.0125 M $CaCl_2$ solution. The samples were vigorously shaken for approx. one hour. Afterwards the samples were centrifuged for 5 minutes at 3800 rpm and an aliquot of the supernatant was used for nitrate-N determination.
Calibration was performed with eight freshly prepared standard dilutions (1.0, 2.0, 5.0, 10.0, 20.0, 50.0, 100, 200 mg $NO_3^-$/L) derived from a nitrate standard solution (10 g/L $NO_3^-$).

The test temperature was recorded continuously. The initial weight of the test units was recorded and used for remoistening. Losses of water were replenished as necessary with deionised water.

### 4. Calculation of toxicity

The $NO_3^-$contents of the soil samples expressed as mg $NO_3^-$N/ kg soil weight and the Nitrate-N formation rates were calculated. The means of Nitrate-N formation rates were calculated based on means of nitrogen-N contents.

### 5. Statistics

For statistical analysis of nitrogen turnover, the nitrate-N formation rates of each replicate were used, but reported nitrate-N formation rates are presented as mean values. The results of nitrate-N contents were not evaluated statistically since this is not required for agrochemicals in accordance with OECD guideline 216. The significance level was set to $\alpha = 0.05$ for all tests.
Nitrate-N formation rate data of the whole study period (day 0 to 42) were tested for normality using Shapiro-Wilk's test which confirmed normal distribution of both datasets. The hypothesis of homogeneity was confirmed by Levene´s test. Accordingly, Multiple Sequentially-rejective t-test with Bonferroni-Holm adjustment (two-sided) was applied for statistical analysis to detect significant differences between the test item groups and the control group.
For data evaluation the statistical programme ToxRat Professional 3.2.1 was used.

## Results and Discussion

The study was terminated after 42 days, since by this time the deviations of the nitrate-N formation rates of the overall study period (0 to 42 days after application) were ≤ 25 % for both test item groups compared to the control.
After 42 days the soil nitrate-N content deviated from the control by +3.61 % at 1.96 mg product/kg soil dry weight and +3.89 % at 9.80 mg product/kg soil dry weight. The nitrate-N formation rate deviations from the control for the overall study period (0 to 42 days after application) were determined as +2.20% at 1.96 mg product/kg soil dry weight and +5.61% at 9.80 mg product/kg soil dry weight. No statistically significant differences were determined for both test item groups compared to the control group (see following table).
The reference item sodium chloride, which was tested at 20 g/kg soil dry weight in a separate in a separate study from Nov to Dec 2015, showed statistically significantly reduced nitrate formation rates (after 28 days) and soil respiration rates (after 28 days) compared to the control.

**Table A 2.5-1: Effects of SAE053H/01 on nitrogen transformation in soil**

| **Mean $NO_3^-$-N content [mg/kg dry soil]** | | | | | | |
|---|---|---|---|---|---|---|
| | **Control** | | **1.96 mg product/kg dry soil** | | **9.80 mg product/kg dry soil** | |
| **Sampling** | **$NO_3^-$-N content** | **CV [a)]** | **$NO_3^-$-N content** | **Deviation [b)]** | **$NO_3^-$-N content** | **Deviation [b)]** |
| Day 0 | 18.8 | 1.54 | 19.7 | 4.79 | 19.2 | 2.13 |
| Day 7 | 1.12 | 8.30 | 1.43 | 27.7 | 1.00 | -10.7 |
| Day 14 | 5.38 | 8.79 | 6.95 | 29.2 | 7.29 | 35.5 |
| Day 28 | 21.7 | 6.26 | 24.2 | 11.5 | 24.8 | 14.3 |
| Day 42 | 36.0 | 2.43 | 37.3 | 3.61 | 37.4 | 3.89 |
| **Mean $NO_3^-$N formation rate [mg/kg dry soil per day] at different time intervals** | | | | | | |
| | **Control** | | **1.96 mg product/kg dry soil** | | **9.80 mg product/kg dry soil** | |
| **Interval** | **$NO_3^-$-N formation** | | **$NO_3^-$-N formation** | **Deviation [b)]** | **$NO_3^-$-N formation** | **Deviation [b)]** |

| | | | | | |
|---|---|---|---|---|---|
| Day 0-7 | -2.53 | -2.61 | -3.16 | -2.60 | -2.77 |
| Day 0-14 | -0.959 | -0.911 | 5.01 | -0.851 | 11.26 |
| Day 0-28 | 0.104 | 0.161 | 54.81 | 0.200 | 92.3 |
| Day 0-42 | 0.410 | 0.419 | 2.20 | 0.433 | 5.61 |

Note: The values are means of triplicates. No statistically significant differences compared to the control were observed according to Multiple Sequentially-rejective t-test with Bonferroni-Holm adjustment (two-sided, $\alpha = 0.05$).
a) % variation within control replicates (coefficient of variation, calculated as standard deviation/mean value*100)
b) % deviation from the control
positive values = stimulating effect
negative values = inhibitory effect

The variation between replicate control samples (CV) was less than ± 15% for all test parameters and sampling times (actual: 1.54 – 8.79%). Therefore, the validity criterion of the test was fulfilled.

**Conclusion**

Based on the results of this study, SAE053H/01 has no long-term effects on soil nitrate content and soil nitrate formation rates of soil microflora at both test concentrations of 1.96 and 9.80 mg product/kg dry soil. The validity criterion was fulfilled.

## 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 additional data submitted.

### A 2.6.2 KCP 10.6.2 Testing on non-target plants

#### A 2.6.2.1 Study 1: Effects on Seedling emergence

| | |
|---|---|
| Comments of zRMS: | The study on the Effects on the seedling emergence and growth on non-target terrestrial plant species was performed in line with requirements of OECD 208 and according to the principles of GLP.<br>All the validity criteria were fulfilled.<br>The study is reliable and suitable for the risk assessment. |

| | |
|---|---|
| Reference: | KCP 10.6.2/01 |
| Report | SAE053H/01: Effects on the seedling emergence of ten non-target plant species under greenhouse conditions, Gröning, C., 2017a, S16-02421 |
| Guideline(s): | Yes, OECD 208 (2006) |
| Deviations: | Yes, temperature and relative humidity were outside of the nominal range recommended in OECD 208 on some days during the study for more than two hours. However, as all validity criteria were fulfilled and no effects were observed in any control group, the deviations are not considered to affect the validity and integrity of the study. |

| | |
|---|---|
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | - |

**Materials and Methods**

**A. MATERIALS**

**1. Test material**

SAE053H/01
(Other name: Mesotrione/Nicosulfuron 80/30 OD)

**Description**: White to beige liquid/cream, OD (oil dispersion)
**Lot/Batch #**: 54606-101
**Purity**: Nicosulfuron: 30 g/L nominal; 30.7 g/L analysed
Mesotrione: 80 g/L nominal; 81.7 g/L analysed
Density: 0.98 g/cm³
**Stability of test material**: Stable under storage conditions (ambient temperature, 5°C – 30°C, in a dark and dry place)
Expiry date: 20 Mar 2018

**2. Vehicle and/or positive control**

Vehicle control: deionised water
No positive control required

**3. Test plants**

Six dicotyledonous species:

| | |
|---|---|
| Sugar beet | *Beta vulgaris* (Amaranthaceae) |
| White cabbage | *Brassica oleracea* (Brassicaceae) |
| Soy bean | *Glycine max* (Fabaceae) |
| Lettuce | *Latuca sativa* (Asteraceae) |
| Turnip | *Brassica rapa* (Brassicaceae) |
| Cucumber | *Cucumis sativus* (Cucurbitaceae) |

Four monocotyledonous species:

| | |
|---|---|
| Onion | *Allium cepa* (Amaryllidaceae) |
| Oat | *Avena sativa* (Poaceae) |
| Ryegrass | *Lolium perenne* (Poaceae) |
| Maize | *Zea mays* (Poaceae) |

**Source**: White cabbage, cucumber, lettuce, onion and maize: Hild; soybean: BayWa; oat: sugar beet: Strube; turnip: Dürr; oat and ryegrass: Partnerbio
**Test containers**: Plant pots with a diameter of 15 cm

**4. Environmental conditions**

Test plants were grown under controlled climatic conditions in a greenhouse.
**Temperature**: Nominal: 22 ± 10°C; actual: 20.7 – 45.7°C
**Relative humidity**: Nominal: 70 ± 25%; actual: 27.3 – 90.4%
**Photoperiod**: Natural light was complemented by high pressure sodium lamps to maintain a minimum light intensity of 10000 Lux (actual: 14882 - 21063 Lux); photoperiod of 16 hours light and 8 hours dark.

## B. STUDY DESIGN AND METHODS

**1. In-life dates** 01 Aug 2016 to 05 Sep 2016

**2. Experimental conditions**

*Test design*

The inhibitory effect of SAE053H/01 on seedling emergence and seedling early growth of ten crop species, six dicotyledons (white cabbage, turnip, soy bean, lettuce, cucumber and sugar beet) and four monocotyledons (onion, oat, ryegrass and maize) was investigated in a multiple rate study over a test period of 21 days following 50% emergence in the control. SAE053H/01 was applied to the soil substrate where seeds of the test species had been sown shortly before application. The test plants were assessed for seedling emergence, post emergence mortality and phytotoxicity symptoms on days 7, 14 and 21 of observation. Furthermore, the plant shoot dry weight was determined at test termination.

*Number of replicates per treatment*

Each treatment group consisted of a total of 20 plants, divided into ten pots containing each two plants for the dicotyledons and maize, and into five pots containing each four seeds for the monocotyledons except maize.

*Test conditions*

Air temperature and relative humidity in the greenhouse were 20.7 – 45.7°C and 27.3 – 90.4%. Natural light was complemented by high pressure sodium lamps to maintain a minimum light intensity of 10000 Lux (actual: 14882 - 21063 Lux) and a photoperiod of 16 hours light and 8 hours dark. Watering was done from the bottom to provide the plants with sufficient water. The water supply was controlled and replenished regularly. Plants were fertilised with a nutrient solution of 0.2% “Wuxal Flüssigdünger”. A volume of 100 mL was added to every plant saucer.

*Test concentrations*

The test plants were treated with SAE053H/01 at application rates of 4.69, 9.38, 23.44, 46.88 and 93.75 mL product/ha for turnip and onion. For ryegrass, the application rates were 23.44, 46.88, 93.75, 187.5 and 375.0 mL product/ha and for the remaining test species the application rates were 93.75, 187.5, 375.0, 750.0 and 1500 mL product/ha. These, in total, nine application rates correspond to 0.147, 0.294, 0.734, 1.469, 2.937, 5.874, 11.75, 23.50 and 46.99 mL a.s./ha nicosulfuron and 0.391, 0.782, 1.954, 3.908, 7.816, 15.63, 31.26, 62.53 and 125.05 mL a.s./ha mesotrione (based on analysed content of active substances and product density). For each test species, a control receiving deionised water was tested in addition.

*Treatment/Application*

Prior to experimental start, the seeds of the test plants were sown in pots containing the soil substrate (soil type: silty sand), which consisted of 84.9% sand, 4.3% clay and 10.8% silt with a pH of 7.4 and a total organic matter content of < 0.3%. The pots were kept under the same climatic conditions as in the test.

The test substance was applied in a water volume of 200 L/ha. The controls received deionised water at 200 L/ha. Application was performed with a laboratory track-sprayer (Company Schachtner, Ludwigsburg, Germany) with Teejet 80015 EVS nozzles and a spraying pressure of 2.5 bar. Prior to application, the apparatus was calibrated to provide the required application volume.

The highest test item solution served as stock solution and was prepared by dispersing 14.700 g of SAE053H/01 in deionised water. The remaining test item solutions were prepared by diluting aliquots of the highest test item solution with deionised water. The control received deionised water only.

**3. Sampling and measurements**

The actual application rates of the test substance were determined by HPLC/PDA analysis of the active substances nicosulfuron and mesotrione in samples of the application solutions of the highest application rate (1500 mL product/ha) and the control. Details to the analytical method are summarized in Part B, Section 5.

Plant pots for all species were observed 7, 14 and 21 days after 50% of the control seeds had emerged in order to determine the number of emerged seeds, the cumulative number of dead young plants and to visually assess any phytotoxic symptoms. The condition of test plants was assessed during the in-life phase utilizing a rating system based upon EPPO guideline 1/135(4) (2014) taking into account necrosis, chlorosis and other characteristics that might be treatment related.

At the final assessment after 21 days, the shoot dry weight was determined for all plants of one replicate as a pooled sample. Plants were clipped at soil level and dried at 60°C until constant weight was reached.

Test temperature and air humidity were recorded continuously (every hour) throughout the test. Light conditions were regulated automatically and measured once a week at plant level.

**4. Calculation of toxicity**

The sum of emerged plants and the seedling emergence in percent was calculated for each treatment group for the final assessment date. The inhibition of emergence was calculated compared to the control group.
The cumulative mean mortality of emerged plants was calculated for each treatment group from the number of dead seedlings in relation to the number of emerged plants for the final assessment.
Phytotoxicity was reported as the median value per treatment group for the final assessment day.
The total shoot dry weight per replicate was divided by the number of plants survived in the respective replicate. The average shoot dry weight per plant was determined for each replicate.
The mean value and standard deviation were determined per treatment group based on the replicate values.
The inhibition on shoot dry weight compared to the control was calculated in percent for each test item group.

**5. Statistics**

A statistical evaluation was performed for the data of seedling emergence, post-emergence mortality and shoot dry weight. For determination of significant difference to the control the significance level was set to $\alpha = 0.05$ for all tests.
The data on shoot dry weight was tested for normality and homoscedasticity using Shapiro-Wilk`s Test and Levene-Test followed by a Multiple Sequentially-rejective Welch t-test after Bonferroni-Holm in case the data were non-homogenous. The William`s Multiple Sequential t-test procedure was conducted in case that both requirements were fulfilled. In case the data were not normal distribution the Step-down Jonckheere-Terpstra test procedure was used.
Statistical analyses of the shoot dry weight also included the determination of effect rates ($ER_{50}$) and their 95% confidence limits by Probit analysis using linear max. likelihood regression, where possible.
The data on seedling emergence and post-emergence mortality was analysed by the Fisher`s Exact Binomial Test with Bonferrroni Correction.
Statistical analysis was performed using the program ToxRatPro Version 3.2.1.

**Results and Discussion**

## A. VERIFICATION OF APPLICATION RATE

HPLC/PDA analysis of the active substances nicosulfuron and mesotrione in the application solution of the highest application rate resulted in a mean recovery of 97% and 93%, respectively. Therefore, the test results were based on nominal application rates.

## B. SEEDLING EMERGENCE

Seedling emergence was not statistically significantly reduced compared to the control for any test species. The highest effect on seedling emergence was observed for white cabbage at 9.38 mL product/ha with 26.3%.
The LOER and $ER_{50}$ could therefore not be determined but the $ER_{50}$ was estimated to be above the highest tested individual rate of 93.75 mL product/ha for white cabbage and onion, above 375.0 mL product/ha for ryegrass and above 1500 mL product/ha for the remaining species. The NOER were determined to be ≥ 93.75, 375.0 and 1500 mL product/ha, respectively (see following table).

**Table 9.10.3-1: Inhibitory effect of SAE053H/01 on seedling emergence**

| Plant species | NOER [mL product/ha] | LOER [mL product/ha] | $ER_{50}$ [mL product/ha] |
|---|---|---|---|
| **White cabbage (*Brassica oleracea)*** | ≥ 93.75 | n.d. [a)] | > 93.75 [b)] |
| **Sugar beet (*Beta vulgaris)*** | ≥ 1500 | n.d. [a)] | > 1500 [b)] |
| **Soy bean (*Glycine max)*** | ≥ 1500 | n.d. [a)] | > 1500 [b)] |
| **Lettuce (*Lactuca sativa)*** | ≥ 1500 | n.d. [a)] | > 1500 [b)] |
| **Cucumber *(Cucumis sativus)*** | ≥ 1500 | n.d. [a)] | > 1500 [b)] |
| **Turnip (*Brassica rapa)*** | ≥ 1500 | n.d. [a)] | > 1500 [b)] |
| **Onion (*Allium cepa)*** | ≥ 93.75 | n.d. [a)] | > 93.75 [b)] |
| **Oat *(Avena sativa )*** | ≥ 1500 | n.d. [a)] | > 1500 [b)] |
| **Ryegrass (*Lolium perenne)*** | ≥ 375.0 | n.d. [a)] | > 375.0 [b)] |
| **Maize (*Zea mays)*** | ≥ 1500 | n.d. [a)] | > 1500 [b)] |

n.d. not determined
[a)] LOER could not be determined due to a lack of statistically significant difference
[b)] $ER_{50}$ could not be calculated due to a lack of inhibition ≥ 50% but can be regarded as above the highest rate tested

## C. BIOMASS (SHOOT DRY WEIGHT)

Biomass was statistically significantly different from the control for all test species except onion, oat, maize and soy bean. Inhibition above 50% was observed for white cabbage, turnip, cucumber and lettuce. The resulting endpoints are shown in the following table.

**Table 9.10.3-2: Inhibitory effect of SAE053H/01 on shoot dry weight**

| Plant species | NOER [mL product/ha] | LOER [mL product/ha] | $ER_{50}$ (95% CL) [mL product/ha] |
|---|---|---|---|
| **White cabbage (*Brassica oleracea)*** | 9.38 | 23.44 | 57.7 (33.8 – 127.2) |
| **Sugar beet (*Beta vulgaris)*** | < 93.75 | 93.75 | n.d. [c)] |
| **Soy bean (*Glycine max)*** | ≥ 1500 | n.d. [a)] | > 1500 [b)] |
| **Lettuce (*Lactuca sativa)*** | 93.75 | 187.5 | 99.4 (0.1 – 2102.5) |
| **Cucumber *(Cucumis sativus)*** | < 93.75 | 93.75 | 583.8 (417.8 – 876.4) |
| **Turnip (*Brassica rapa)*** | 93.75 | 187.5 | 160.5 (116.6 – 221.2) |
| **Onion (*Allium cepa)*** | ≥ 93.75 | n.d. [a)] | n.d. [c)] |
| **Oat *(Avena sativa )*** | ≥ 1500 | n.d. [a)] | > 1500 [b)] |
| **Ryegrass (*Lolium perenne)*** | ≥ 375.0 | n.d. [a)] | > 375.0 [b)] |
| **Maize (*Zea mays)*** | ≥ 1500 | n.d. [a)] | > 1500 [b)] |

n.d. not determined
[a)] LOER could not be determined due to a lack of statistically significant difference
[b)] $ER_{50}$ could not be calculated due to a lack of inhibition ≥ 50% but can be regarded as above the highest rate tested
[c)] $ER_{50}$ could not be calculated due to missing dose-response relationship

## D. MORTALITY

Statistically significant post-emergence mortality was observed for sugar beet, turnip, cucumber and lettuce. The highest mortality was found for sugar beet with 100% at the test item rates of 375.0, 750.0 and 1500 mL product/ha on day 21. The resulting endpoints are shown in the following table.

**Table 9.10.3-3: Effects of SAE053H/01 on post-emergence mortality**

| Plant species | NOER [mL product/ha] | LOER [mL product/ha] | $ER_{50}$ (95% CL) [mL product/ha] |
|---|---|---|---|
| **White cabbage (*Brassica oleracea)*** | ≥ 93.75 | n.d. [a)] | > 93,75 [b)] |
| **Sugar beet (*Beta vulgaris)*** | < 93.75 | 93.75 * | n.d. [c)] |
| **Soy bean (*Glycine max)*** | ≥ 1500 | n.d. [a)] | > 1500 [b)] |
| **Lettuce (*Lactuca sativa)*** | 187.5 | 375.0* | 411.4 (286.1 – 602.3) |
| **Cucumber *(Cucumis sativus)*** | 750.0 | 1500 * | > 1500 [b)] |
| **Turnip (*Brassica rapa)*** | 93.75 | 187.5 * | 508.7 (325.3 – 890.7) |
| **Onion (*Allium cepa)*** | ≥ 93.75 | n.d. [a)] | > 93.75 [b)] |
| **Oat *(Avena sativa )*** | ≥ 1500 | n.d. [a)] | > 1500 [b)] |
| **Ryegrass (*Lolium perenne)*** | ≥ 375.0 | n.d. [a)] | > 375.0 [b)] |
| **Maize (*Zea mays)*** | ≥ 1500 | n.d. [a)] | > 1500 [b)] |

n.d. not determined
[a)] LOER could not be determined due to a lack of statistically significant difference
[b)] $ER_{50}$ could not be calculated due to a lack of inhibition ≥ 50% but can be regarded as above the highest rate tested
[c)] The calculated $ER_{50}$ was not considered reliable as the value was extrapolated
* Statistically significantly different from the control according to Fisher's exact test (one-sided greater, $\alpha = 0.05$)

## E. PHYTOTOXICITY

Phytotoxic effects (including stunted growth, necrosis, chlorosis, leaf deformation and wilting) were observed for dicotyledonous species, only. The most affected species (median 4) were lettuce at 750.0 mL product/ha and turnip at 750.0 and 1500 mL product/ha. A summary of observed effects is presented in the following table.

**Table 9.10.3-4: Phytotoxic effects of SAE053H/01 on non-target plants (21 days after application)**

| Plant species | Rates of observation [mL product/ha] | | | | | Maximum median rating [a)] |
|---|---|---|---|---|---|---|
| | Chlorosis | Necrosis | Stunted growth | Leaf deforma-tion | Wilting | |
| **White cabbage (*Brassica oleracea)*** | ≥ 23.44 | ≥ 23.44 | ≥ 23.44 | - | - | 2 |
| **Sugar beet (*Beta vulgaris)*** | 187.5 | 93.75 and 187.5 | 93.75 | - | - | 3 |
| **Soy bean (*Glycine max)*** | ≥ 93.75 | - | ≥ 375.0 | ≥ 750.0 | - | 2 |
| **Lettuce (*Lactuca sativa)*** | ≥ 187.5 | ≥ 187.5 | ≥ 187.5 | - | - | 4 |
| **Cucumber *(Cucumis sativus)*** | ≥ 93.75 | ≥ 93.75 | ≥ 93.75 | - | - | 3 |
| **Turnip (*Brassica rapa)*** | ≥ 93.75 | ≥ 187.5 | ≥ 93.75 | ≥ 750.0 | - | 4 |
| **Onion (*Allium cepa)*** | - | - | - | - | - | 1 |
| **Oat *(Avena sativa )*** | - | - | 375.0 and 750.0 | - | 375.0 and 750.0 | 1 |
| **Ryegrass (*Lolium perenne)*** | - | - | - | - | - | 1 |
| **Maize (*Zea mays)*** | - | - | - | - | - | 1 |

- not observed

[a)] Rating according to EPPO 1/135(3) (2006) including all phytotoxic effects; 1 = normal plant appearance, 2 = slight symptoms, 3 = moderate symptoms, 4 = strong symptoms, 5 = plants totally affected

## F. VALIDITY CRITERIA

The seedling emergence was ≥ 70% (actual: 75 – 100%). Control plants did not exhibit visible phytotoxic effects and mean plant survival was ≥ 90% (actual: 100%). Furthermore, environmental conditions and growing media for a particular species were identical. Therefore, all validity criteria were fulfilled.

### Conclusion

In this study on seedling emergence of plants treated with SAE053H/01, no statistically significant effects on seedling emergence were observed. Statistically significant effects on post-emergence mortality occurred for sugar beet, turnip, cucumber and lettuce. The lowest $ER_{50}$ (mortality) was determined as 411.4 (95% CL: 286.4 – 602.3) mL product/ha for lettuce.
Phytotoxicity was observed for dicotyledonous species, only.
Statistically significant effects on biomass (shoot dry weight) were observed for all species except soy bean, onion, oat and maize. White cabbage was the most sensitive species for biomass with an $ER_{50}$ for shoot dry weight of 57.7 (95% CL: 33.8 – 127.2) mL product/ha. The validity criteria in the study were fulfilled.

### A 2.6.2.2 Study 2: Effects on Vegetative vigour

| | |
|---|---|
| Comments of zRMS: | The study on effects on the vegetative vigour of ten non-target terrestrial plant species was performed in line with requirements of OECD 227 and according to the principles of GLP.<br>All the validity criteria were fulfilled. The study is reliable and suitable for the risk assessment. |

| | |
|---|---|
| Reference: | KCP 10.6.2/02 |
| Report | SAE053H/01: Effects on the vegetative vigour of ten non-target plant species under greenhouse conditions, Gröning, C. 2017b, S16-02422 |
| Guideline(s): | Yes, OECD 227 (2006) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication<br>(if vertebrate study) | - |

**Materials and Methods**

**A. MATERIALS**

**1. Test material**

SAE053H/01
(Other name: Mesotrione/Nicosulfuron 80/30 OD)

| | |
|---|---|
| **Description** | White to beige liquid/cream, OD (oil dispersion) |
| **Lot/Batch #** | 54606-101 |
| **Purity** | Nicosulfuron: 30 g/L nominal; 30.7 g/L analysed<br>Mesotrione: 80 g/L nominal; 81.7 g/L analysed<br>Density: 0.98 g/cm³ |
| **Stability of test material** | Stable under storage conditions (ambient temperature, 5°C – 30°C, in a dark and dry place)<br>Expiry date: 20 Mar 2018 |

**2. Vehicle and/or positive control**

Vehicle control: deionised water
No positive control required

**3. Test plants**

Six dicotyledonous species:

| | |
|---|---|
| Sugar beet | *Beta vulgaris* (Amaranthaceae) |
| White cabbage | *Brassica oleracea* (Brassicaceae) |
| Soy bean | *Glycine max* (Fabaceae) |
| Lettuce | *Latuca sativa* (Asteraceae) |
| Turnip | *Brassica rapa* (Brassicaceae) |
| Cucumber | *Cucumis sativus* (Cucurbitaceae) |

Four monocotyledonous species:

| | |
|---|---|
| Onion | *Allium cepa* (Amaryllidaceae) |
| Oat | *Avena sativa* (Poaceae) |
| Ryegrass | *Lolium perenne* (Poaceae) |

| | |
|---|---|
| | Maize *Zea mays* (Poaceae) |
| **Source** | White cabbage, cucumber, lettuce, onion and maize: Hild; soybean: BayWa; oat: sugar beet: Strube; turnip: Dürr; oat and ryegrass: Partnerbio |
| **Test containers** | Plant pots with a diameter of 15 cm |
| **4. Environmental conditions** | Test plants were grown under controlled climatic conditions in a greenhouse. |
| **Temperature** | Nominal: 22 ± 10°C; actual: 16.3 – 34.4°C (trial L3, lettuce); 14.7 – 34.1°C (trial L5, all species except lettuce and sugar beet); 15.1 – 32.7°C (trial L6, sugar beet) |
| **Relative humidity** | Nominal: 70 ± 25%; actual: 27.5 – 89.7% (trial L3, lettuce); 12.5 – 80.4% (trial L5, all species except lettuce and sugar beet); 29.3 – 74.9% (trial L6, sugar beet) |
| **Photoperiod** | Natural light was complemented by high pressure sodium lamps to maintain a minimum light intensity of 10000 Lux (actual: 13327 - 18496 Lux (trial L3); 13579 – 16802 Lux (trial L5); 13250 – 15573 Lux (trial L6) and a photoperiod of 16 hours light and 8 hours dark. |

## B. STUDY DESIGN AND METHODS

**1. In-life dates** 07 Sep 2016 to 28 Dec 2016

**2. Experimental conditions**

*Test design*

The inhibitory effect of SAE053H/01 on vegetative vigour of six dicotyledons (sugar beet, white cabbage, turnip, cucumber, soy bean and lettuce) and four monocotyledons (onion, oat, ryegrass and maize), was investigated in a multiple rate study during 21 days. SAE053H/01 was applied when the plants had reached BBCH 12-13. The test plants were assessed for mortality and phytotoxicity symptoms on days 7, 14 and 21. Furthermore, the plant shoot dry weight was determined at test termination.

*Number of replicates per treatment*

Each treatment group consisted of a total of 20 plants, divided into ten pots containing each two plants for all dicotyledons and maize, and into five pots containing each four seeds for all monocotyledons except maize.

*Test conditions*

Air temperature and relative humidity in the greenhouse were outside of the nominal range (see above), but no effects on the study are expected as validity criteria were fulfilled. Natural light was complemented by high pressure sodium lamps to maintain a minimum light intensity of at least 10000 Lux and a photoperiod of 16 hours light and 8 hours dark. Watering was done to the plant saucer of each pot to provide the plant roots with water. The water supply was controlled and replenished regularly. Plants were fertilised two to three times in the different trials (L3, L5 and L6) with a nutrient solution of 0.2% “Wuxal Flüssigdünger”. A volume of 100 mL was added to every plant saucer.

*Test concentrations*

The test plants were treated with SAE053H/01 at different application rates for the different trials. Lettuce was treated with 4.69, 9.38, 22.43, 46.88 and 93.75 mL product/ha (trial L3). White cabbage, turnip and cucumber were treated with 9.38, 23.43, 46.88, 93.75 and 187.5 mL product/ha (trial L5). Soy bean, onion, oat, ryegrass and maize were treated with 93.75, 187.5, 375, 750 and 1500 mL product/ha (trial L5). Sugar beet was treated with 2.34, 4.69, 9.38, 23.44 and 46.88 mL product/ha (trial L6). These, in total ten, product application rates correspond to 0.073, 0.147, 0.294, 0.734, 1.469, 2.937, 5.874, 11.75, 23.50 and 46.99 mL a.s./ha nicosulfuron and 0.195, 0.391, 0.782, 1.954, 3.908, 7.816, 15.63, 31.26, 62.53 and 125.05 mL a.s./ha mesotrione (based on analysed content of active substances and product density). For each test species, a control receiving deionised water was tested in addition.

*Treatment/Application*

Prior to experimental start, the seeds of the test plants were sown in pots containing the soil substrate (soil type: silty sand), which consisted of 84.9% sand, 4.3% clay and 10.8% silt with a pH of 7.4 and a total organic matter content of < 0.3%. The test substance was applied when the plants had reached BBCH 12-13 (test start).

The test substance was applied in a water volume of 200 L/ha. The controls received deionised water at 200 L/ha. Application was performed with a laboratory track-sprayer (Company Schachtner, Ludwigsburg, Germany) with Teejet 80015 EVS nozzles and a spraying pressure of 2.5 bar. Prior to application, the apparatus was calibrated to provide the required application volume.

In each trial, the respective highest test item solution served as stock solution and was prepared by dispersing 0.230, 14.7 and 0.115 g of SAE053H/01 for trial L3, L5 and L6 in deionised water, respectively. The remaining test item solutions were prepared by diluting aliquots of the highest test item solution with deionised water. The control received deionised water only.

**3. Sampling and measurements**

The actual application rates of the test substance were determined by HPLC/PDA analysis of the active substances nicosulfuron and mesotrione in samples of the application solutions of the highest application rate for all three trials (93.75, 1500 and 46.88 mL product/ha, respectively) and the control. The analytical method is summarized in Part B, Section 5.

The test plants were assessed for mortality and phytotoxicity symptoms on days 7, 14 and 21. The condition of test plants was assessed during the in-life phase utilizing a rating system based upon EPPO guideline 1/135(4) (2014).

At the end of the observation period, the surviving plants were clipped at soil level for determination of shoot dry weight. The weight of the above-ground shoot portion of all surviving plants per replicate was measured after drying at 60 °C until constant weight was reached.

Test temperature and air humidity were recorded continuously (once per hour) throughout the test. Light intensity was regulated automatically and measured once a week.

**4. Calculation of toxicity**

The cumulative mean mortality (%) for each treatment group was calculated per treatment group for the final assessment day (21 DAA).

Phytotoxicity was assessed per plant and was reported as the median value per treatment group for the final assessment day.

The total shoot dry weight per replicate was divided by the number of plants survived in the respective replicate to determine the average shoot dry weight per plant. The mean value and standard deviation was determined per treatment group based on the replicate values. The inhibition on shoot dry weight compared to the control group was calculated in percent for each test item group.

**5. Statistics**

A statistical evaluation was performed on the data of mortality and shoot dry weight for the last assessment day (day 21). For determination of significant difference to the control the significance level was set to $\alpha = 0.05$ for all tests.
The data of mortality were tested with the Multiple Fisher's exact test with Bonferroni-Holm adjustment.
The data of shoot dry weight were tested for normality and homoscedasticity using Shapiro-Wilk`s Test and Levene-Test followed by William`s t-test in case that both requirements were fulfilled. The Multiple Welch t-test with Bonferroni-Holm adjustment was conducted in case that the data were normal distributed but non-homogenous. If the data were neither normal distributed nor homogenous and the trend analysis by contrast was significant, the Jonckheere-Terpstra test was conducted. If the trend analysis by contrast did not reveal a linear trend, Multiple Mann-Whitney U-test with Bonferroni-Holm adjustment was used.
Statistical analyses of the shoot dry weight also included the determination of effect rates ($ER_{50}$) and their 95% confidence limits by Probit analysis using linear max. likelihood regression, where possible.
Statistical analysis was performed using the program ToxRat Pro Version 3.2.1.

**Results and Discussion**

**A. VERIFICATION OF APPLICATION RATE**

Analysis of the active substances nicosulfuron and mesotrione in the application solutions of the highest test rate resulted in a mean recovery of nicosulfuron of 104, 105 and 92% of nominal for trial L3, L5 and L6, respectively. For mesotrione the recovery was 96, 107 and 91% of nominal for trial L3, L5 and L6, respectively. Therefore, the test results were based on nominal application rates.

**B. BIOMASS (SHOOT DRY WEIGHT)**

Statistically significant effects on shoot dry weight could be detected for all plant species. The highest inhibition rate at the end of the test was found in lettuce (92.3%) at 46.88 mL product/ha followed by white cabbage (86.2%) at 187.5 mL product/ha. No statistically significant difference was determined for maize at 750 mL product/ha but the LOER was seen at 187.5 mL product/ha as 375 and 1500 mL product/ha caused statistically significant effects on shoot dry weight. The resulting endpoints are presented in the following table.

**Table 9.10.3-5: Inhibitory effect of SAE053H/01 on shoot dry weight**

| Plant species | NOER [mL product/ha] | LOER [mL product/ha] | $ER_{50}$ (95% CL) [mL product/ha] |
|---|---|---|---|
| **Sugar beet (*Beta vulgaris)*** | 4.69 | 9.38 | > 9.38 [a)] |
| **White cabbage (*Brassica oleracea)*** | < 9.38 | ≤ 9.38 | 32.21 (27.48 – 35.76) |
| **Turnip (*Brassica rapa)*** | 9.38 | 23.43 | 29.13 (24.27 – 33.82) |
| **Cucumber *(Cucumis sativus)*** | 9.38 | ≤ 9.38 | 43.74 (33.86 – 49.37) |
| **Soy bean (*Glycine max)*** | < 93.75 | ≤ 93.75 | 227.18 (69.73 – 324.22) |
| **Lettuce (*Lactuca sativa)*** | 4.69 | 9.38 | 8.47 (7.33 – 9.84) |
| **Onion (*Allium cepa)*** | 93.75 | 187.5 | 837.57 (598.21 – 1278.82) |
| **Oat *(Avena sativa )*** | 93.75 | 187.5 | 646.71 (422.52 – 817.92) |
| **Ryegrass (*Lolium perenne)*** | < 93.75 | ≤ 93.75 | 210.10 (174.05 – 242.37) |
| **Maize (*Zea mays)*** | 93.75 | 187.5 | > 1500 [b)] |

n.d. not determined
a) $ER_{50}$ could not be calculated as no clear dose response relationship occurred.
b) $ER_{50}$ could not be determined due to a lack of inhibition ≥ 50%.

## C. MORTALITY

Mortality occurred in sugar beet, turnip, lettuce and ryegrass. The highest mortality was observed for sugar beet with 100% at the two highest test rates (23.44 and 46.88 mL product/ha) and for lettuce with 100% at the highest test item rate (93.75 mL product/ha). The resulting endpoints are presented in the following table.

**Table 9.10.3-6: Effect of SAE053H/01 on mortality**

| Plant species | NOER [mL product/ha] | LOER [mL product/ha] | $ER_{50}$ (95% CL) [mL product/ha] |
|---|---|---|---|
| **Sugar beet (*Beta vulgaris)*** | 9.38 | 23.44 | n.d. [b)] |
| **White cabbage (*Brassica oleracea)*** | ≥ 187.5 | n.d. [a)] | > 187.5 [c)] |
| **Turnip (*Brassica rapa)*** | 93.75 | 187.5 | 127.88 (108.72 – 150.93) |
| **Cucumber *(Cucumis sativus)*** | ≥ 187.5 | n.d. [a)] | > 187.5 [c)] |
| **Soy bean (*Glycine max)*** | ≥ 1500 | n.d. [a)] | > 1500 [c)] |
| **Lettuce (*Lactuca sativa)*** | 23.43 | 46.88 | 39.17 (32.56 – 47.00) |
| **Onion (*Allium cepa)*** | ≥ 1500 | n.d. [a)] | > 1500 [c)] |
| **Oat *(Avena sativa )*** | ≥ 1500 | n.d. [a)] | > 1500 [c)] |
| **Ryegrass (*Lolium perenne)*** | ≥ 1500 | n.d. [a)] | > 1500 [c)] |
| **Maize (*Zea mays)*** | ≥ 1500 | n.d. [a)] | > 1500 [c)] |

n.d. not determined
a) LOER could not be calculated due to a lack of effects.
b) $ER_{50}$ could not be determined due to a lack of clear dose-response relationship.
c) $ER_{50}$ could not be determined due to a lack of effects ≥ 50%.

## D. PHYTOTOXICITY

Phytotoxicity (i.e. stunted growth, necrosis, chlorosis, wilting and leaf deformation) was observed in all test species. Observed effects are summarized in the following table.

**Table 9.10.3-7: Phytotoxic effects of SAE053H/01 on non-target plants (21 days after application)**

| Plant species | Rates of observation [mL product/ha] | | | | | Maximum median rating [a)] |
|---|---|---|---|---|---|---|
| | Chlorosis | Necrosis | Stunted growth | Leaf deforma-tion | Wilting | |
| **Sugar beet (*Beta vulgaris)*** | 4.69 and 9.38 | 9.38 | 9.38 | - | - | 4 |
| **White cabbage (*Brassica oleracea)*** | ≥ 9.38 | ≥ 46.88 | ≥ 46.88 | - | - | 5 |
| **Turnip (*Brassica rapa)*** | ≥ 9.38 | ≥ 9.38 | ≥ 46.88 | ≥ 23.43 | - | 5 |
| **Cucumber *(Cucumis sativus)*** | ≥ 9.38 | ≥ 46.88 | ≥ 46.88 | ≥ 46.88 | - | 4 |
| **Soy bean (*Glycine max)*** | ≥ 93.75 | ≥ 93.75 | ≥ 93.75 | ≥ 93.75 | - | 4 |
| **Lettuce (*Lactuca sativa)*** | 9.38, 23.43 and 46.88 | 23.43 and 46.88 | 23.43 and 46.88 | - | 9.38, 23.43 and 46.88 | 4 |
| **Onion (*Allium cepa)*** | ≥ 187.5 | ≥ 187.5 | ≥ 187.5 | ≥ 375 | - | 4 |
| **Oat *(Avena sativa )*** | ≥ 375 | 1500 | ≥ 750 | ≥ 375 | - | 3 |
| **Ryegrass (*Lolium perenne)*** | ≥ 93.75 | ≥ 187.5 | ≥ 187.5 | 187.5 and 375 | ≥ 375 | 5 |
| **Maize (*Zea mays)*** | ≥ 375 | - | - | - | - | 2 |

- not observed

[a)] Rating according to EPPO 1/135(3) (2006) including all phytotoxic effects; 1 = normal plant appearance, 2 = slight symptoms, 3 = moderate symptoms, 4 = strong symptoms, 5 = plants totally affected

## E. VALIDITY CRITERIA

The control seedling emergence was ≥ 70% (actual: 86 - 99%). Control plants did not exhibit visible phytotoxic effects and mean plant survival was ≥ 90% (actual: 100%). Furthermore, environmental conditions and growing media for a particular species were identical. Therefore, the validity criteria of the guideline were met.

### Conclusion

Based on the results of this vegetative vigour test with SAE053H/01, the lowest $ER_{50}$ values for the parameter biomass (measured as shoot dry weight) were determined to be 8.47 (95% CL: 7.33 – 9.84) mL product/ha for lettuce. The most sensitive species for mortality was sugar beet, however, no $ER_{50}$ could be determined. The lowest $ER_{50}$ was calculated for lettuce with 39.17 (95% CL: 32.56 – 47.00) mL product/ha. All plants were affected in different severity in terms of phytotoxicity. All validity criteria in the study were fulfilled.

## A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants

No additional data submitted.

## A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

No additional data submitted.

## A 2.8 KCP 10.8 Monitoring data

No additional data submitted.