REGISTRATION REPORT

**Part B**

**Section 9**

**Ecotoxicology**

Detailed summary of the risk assessment

Product code: GLOB2112dH

Product name: Walkover Trio

Chemical active substances:

Mesotrione, 375 g/L
Thiencarbazone-methyl, 75 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Globachem NV

Submission date: September 2024

zRMS Assessment : 31/03/2025

Version after commenting: 03/07/2025

List of references update: 10/07/2025

# Version history

| When | What |
|---|---|
| September 2024 | Initial dossier submission by applicant for approval of new product. |
| March 2025 | zRMS assessment |
| July 2025 | zRMS – after commenting period |
| July 2025 | List of references update |

# Table of Contents

# 9 Ecotoxicology (KCP 10)

## 9.1 Critical GAP and overall conclusions

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

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Use-No. * | Member state(s) | Crop and/or situation (crop destination / purpose of crop) | F, Fn, Fpn G, Gn, Gpn or I ** | Pests or Group of pests controlled (additionally: 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) | L product/ha a) max. rate per appl. b) max. total rate per crop/season | g 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 arthropods | Soil organisms | Non-target plants |
| **Zonal uses (field or outdoor uses, certain types of protected crops)** | | | | | | | | | | | | | | | | | | | | |
| 1 | PL, RO, HU, SK | Maize (ZEAMX) | F | *Annual dicotyledonous weed plants* (3ANDIT) *Annual grasses* (3ANMNT) | Downwards spraying – Broadcast application | BBCH 10-18 | a) 1 b) 1 | / | a) 0.2 b) 0.2 | a) Thiencarbazone-methyl: 15 + Mesotrione: 75 b) Thiencarbazone-methyl 15 + Mesotrione: 75 | 100 – 300 | N/A | Safener: 22.4 g/ha cyprosulfamide | | | | | | | |
| 2 | PL, RO, HU, SK | Maize (ZEAMX) | F | *Annual dicotyledonous weed plants* (3ANDIT) *Annual grasses* (3ANMNT) | Downwards spraying – Broadcast application | BBCH 10-18 | a) 1 b) 1 | / | a) 0.13 b) 0.13 | a) Thiencarbazone-methyl: 9.75 + Mesotrione: 48.75 b) Thiencarbazone-methyl 9.75 + Mesotrione: 48.75 | 100 – 300 | N/A | Safener: 14.6 g/ha cyprosulfamide Optional lower rate as backup or dose range. | | | | | | | |
| 3 | PL, RO, HU, SK | Maize (ZEAMX) | F | *Annual dicotyledonous weed plants* (3ANDIT) *Annual grasses* (3ANMNT) | Downwards spraying – Banded application | BBCH 10-18 | a) 1 b) 1 | / | a) 0.2 b) 0.2 | a) Thiencarbazone-methyl: 15 + Mesotrione: 75 b) Thiencarbazone-methyl 15 | 100 – 300 | N/A | Safener: 22.4 g/ha cyprosulfamide Dose rate is concentration within the | | | | | | | |

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | + Mesotrione: 75 | | | band. | | | | | | | |
| 4 | PL, RO, HU, SK | Maize (ZEAMX) | F | *Annual dicotyledonous weed plants* (3ANDIT) *Annual grasses* (3ANMNT) | Downwards spraying – Banded application | BBCH 10-18 | a) 1<br>b) 1 | / | a) 0.13<br>b) 0.13 | a) Thiencarbazone-methyl: 9.75 + Mesotrione: 48.75<br>b) Thiencarbazone-methyl 9.75 + Mesotrione: 48.75 | 100 – 300 | N/A | Safener: 14.6 g/ha cyprosulfamide<br>Optional lower rate as backup or dose range.<br>Dose rate is concentration within the band. | | | | | | | |

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

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

## Explanation for column 15 – 21 "Conclusion"

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

**Remarks table:**

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

### 9.1.1 Overall conclusions

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

The risk to birds, mammals and other terrestrial vertebrates is acceptable when applying GLOB2112dH according to the intended uses.

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

The risk for aquatic organisms is acceptable when applying GLOB2112dH according to the intended uses when taking into account the required mitigation measures.

#### 9.1.1.3 Effects on bees (KCP 10.3.1)

The risk to bees is acceptable when applying GLOB2112dH according to the intended uses.

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

The risk to non-target arthropods other than bees is acceptable when applying GLOB2112dH according to the intended uses.

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

The risk to non-target soil meso-, macro- and micro-organisms is acceptable when applying GLOB2112dH according to the intended uses.

#### 9.1.1.6 Effects on non-target terrestrial plants (KCP 10.6)

The risk to non-target terrestrial plant is acceptable when applying GLOB2112dH according to the intended uses and taking into account the following mitigation measures: a buffer zone of 1 m in combination with 50% drift reducing techniques or a buffer zone of 5 m.

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

Not required.

### 9.1.2 Grouping of intended uses for risk assessment

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

**Table 9.1-2: Critical use pattern of GLOB2112dH grouped according to application rate**

| Grouping according to application rate | | | |
|---|---|---|---|
| **Group** | **Intended uses** | **relevant use parameters for grouping** | **relevant parameter or value for sorting** |
| 1 | 1, 3 | 0.2 L/ha | Application rate |
| 2 | 2, 4 | 0.13 L/ha | |

## 9.1.3 Consideration of metabolites

A list of metabolites found in environmental compartments is provided below. The need for conducting a metabolite-specific risk assessment in the context of the evaluation of GLOB2112dH is indicated in the table.

**Table 9.1-3 Metabolites of mesotrione**

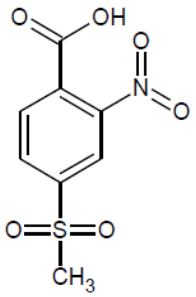
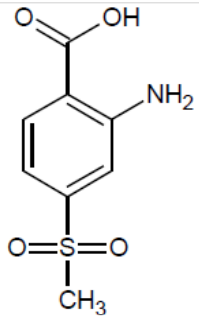
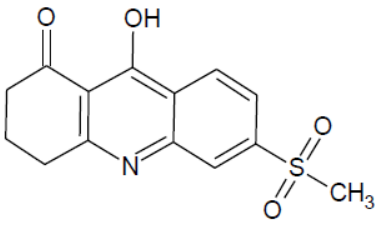
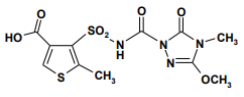
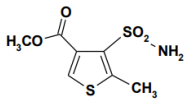
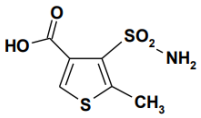
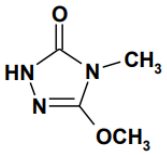
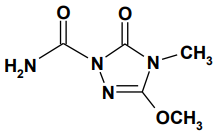
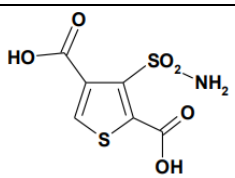
| Metabolite | Chemical structure | Molar mass | Maximum occurrence in compartments | Risk assessment required? |
|---|---|---|---|---|
| MNBA |  | 245 | Soil:<br>> 10 % of a.s<br><br>Water/Sediment:<br>Max. 7.4% | Yes, soil and aquatic organisms |
| AMBA |  | 215 | Soil: Max. 9.7%<br><br>Water/Sediment:<br>> 10 % of a.s | Yes, soil and aquatic organisms |
| SYN 546974 |  | 291 | Water/Sediment:<br>> 10 % of a.s | Yes, aquatic organisms |

**Table 9.1-4 Metabolites of thiencarbazone-methyl**

| Metabolite | Chemical structure | Molar mass | Maximum occurrence in compartments | Risk assessment required? |
|---|---|---|---|---|
| BYH 18636-carboxylic acid (AE 1394083) |  | 376.4 | Soil: 53.6% after 84 d<br>Water: 25.7% after 14 d<br>Sediment: 14.1% after 30 | Yes, soil and aquatic organisms |

| Metabolite | Chemical structure | Molar mass | Maximum occurrence in compartments | Risk assessment required? |
|---|---|---|---|---|
| | | | d | |
| BYH 18636-sulfonamide (AE 1364547) |  | 235.3 | Soil: 15.6% after 1 d<br>Water-sediment: 7.0% | Yes, soil and aquatic organisms |
| BYH 18636-sulfonamide carboxylic acid (AE 1395853) |  | 221.3 | Soil: 21.2% after 42 d<br>Water: 45.6% after 120 d<br>Sediment: 21.3% after 120 d | Yes, soil and aquatic organisms |
| BYH 18636-MMT (AE 1277106) |  | 129.1 | Soil: 20.6% after 28 d<br>Water: 24.9% after 90 d<br>Sediment: 7.8% after 120 d | Yes, soil and aquatic organisms |
| BYH 18636-triazolinonecarboxamide (AE 1430601) |  | 172.1 | Soil: > 5% of AR in more than two consecutive sampling dates<br>Water: minor non-transient | Yes, soil and aquatic organisms |
| BYH 18636-dicarboxysulfonamide (BCS-AA10007) |  | 251.2 | Water: 18.9% after 120 d<br>Sediment: 6.1% after 120 d | Yes, aquatic organisms |

## 9.2 Effects on birds (KCP 10.1.1)

### 9.2.1 Toxicity data

Avian toxicity studies have been carried out with mesotrione, thiencarbazone-methyl and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.
Effects on birds of GLOB2112dH were not evaluated as part of the EU assessment of mesotrione and thiencarbazone-methyl.
However, the provision of further data on the GLOB2112dH is not considered essential, because the risk for birds from GLOB2112dH can be adequately assessed from the risk assessment of the active substance since birds are typically exposed to dry residues on their food items following the dilution and spraying of the formulated product. During these processes, much of the formulation constituents are likely to be lost by volatilisation. Since oral exposure is the main route of exposure, toxicity data for the active substance are therefore used in preference to data from tests with the formulated material.
The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

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

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Colinus virginianus* | Mesotrione | Acute:<br>Oral (single dose)<br>1 d | **$LD_{50}$ > 2000 mg/kg bw** (corrected to 3776 mg/kg bw) | EFSA, 2016<br>[redacted]., 1995 |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| | | | NOEL = 2000 mg/kg bw | |
| *Anas platyrhynchos* | Mesotrione | Short-term<br>5 days<br>dietary | $LC_{50}$ > 5200 mg/kg diet<br>NOEC = 5200 mg/kg diet | EFSA, 2016<br>[redacted]., 1995 |
| *Colinus virginianus* | Mesotrione | Short-term<br>5 days<br>dietary | $LC_{50}$ > 5200 mg/kg diet<br>NOEC = 5200 mg/kg diet | EFSA, 2016<br>[redacted]., 1995 |
| *Colinus virginianus* | Mesotrione | Long-term:<br>20 weeks<br>Sub-chronic and reproductive | NOEC = 3000 mg/kg diet | EFSA, 2016<br>[redacted]., 1997 |
| *Anas platyrhynchos* | Mesotrione | Long-term:<br>20 weeks<br>Sub-chronic and reproductive | **NOEL** = 120 mg/kg diet =<br>**20.6 mg/kg bw/d** (reproduction) | EFSA, 2016<br>[redacted], 1997 |
| *Colinus virginianus* | Thiencarbazone-methyl | Acute | **$LD_{50}$ > 2000 mg/kg bw/d** | EFSA, 2013<br>[redacted]., 2005 |
| *Anas platyrynchos* | Thiencarbazone-methyl | Long-term | **NOEL = 24 mg/kg bw/d** | EFSA, 2013<br>[redacted]<br>2007 |

#### 9.2.1.1 Justification for new endpoints

Effects on birds of GLOB2112dH were not evaluated as part of the EU assessment of mesotrione and thiencarbazone-methyl. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

### 9.2.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).
To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group 1 also covers the risk for birds from all other intended uses in group 2 (see 9.1.2).

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

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

**Table 9.2-2: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of GLOB2112dH in maize**

| **Intended use** | | **Maize** | | | |
|---|---|---|---|---|---|
| **Active substance/product** | | Mesotrione | | | |
| **Application rate (g/ha)** | | 1 × 75 | | | |
| **Acute toxicity (mg/kg bw)** | | 2000 | | | |
| **TER criterion** | | 10 | | | |
| **Crop scenario Growth stage** | **Indicator/generic focal species** | **$SV_{90}$** | **$MAF_{90}$** | **$DDD_{90}$ (mg/kg bw/d)** | **$TER_a$** |
| Maize | Small omnivorous bird | 158.8 | 1 | 11.91 | 168 |
| **Reprod. toxicity (mg/kg bw/d)** | | 20.6 | | | |
| **TER criterion** | | 5 | | | |
| **Crop scenario Growth stage** | **Indicator/generic focal species** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$ (mg/kg bw/d)** | **$TER_{lt}$** |
| Maize | Small omnivorous bird | 64.8 | 0.53 | 2.576 | 8.0 |
| **Active substance/product** | | Thiencarbazone-methyl | | | |
| **Application rate (g/ha)** | | 1 × 15 | | | |
| **Acute toxicity (mg/kg bw)** | | 2000 | | | |
| **TER criterion** | | 10 | | | |
| **Crop scenario Growth stage** | **Indicator/generic focal species** | **$SV_{90}$** | **$MAF_{90}$** | **$DDD_{90}$ (mg/kg bw/d)** | **$TER_a$** |
| Maize | Small omnivorous bird | 158.8 | 1 | 2.382 | 840 |
| **Reprod. toxicity (mg/kg bw/d)** | | 24 | | | |
| **TER criterion** | | 5 | | | |
| **Crop scenario Growth stage** | **Indicator/generic focal species** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$ (mg/kg bw/d)** | **$TER_{lt}$** |
| Maize | Small omnivorous bird | 64.8 | 0.53 | 0.515 | 47 |

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.

**Combined toxicity**

In the absence of acute and/or chronic study endpoints on the formulated product, the possible risk to birds exposed to the formulated product is predicted on the basis of data for the individual active substances in a combined toxicity assessment.

A quantitative toxicity risk assessment according to concentration addition is not needed if one of the following points applies:

- The risk assessment for all active substances in the product passes with a high margin of safety
- One active substance clearly drives the risk assessment

These conditions are assessed following a step-wise approach.

1st step: margin of safety

Condition: all TER values are > trigger x n (n = number of active substances in the mixture)

| Scenario/indicator species | TER values | | Trigger x n | All TER > trigger x n? |
|---|---|---|---|---|
| | Mesotrione | Thiencarbazone-methyl | | |
| Acute/small omnivorous bird | 168 | 840 | 10 x 2 | yes |
| Chronic/small omnivorous bird | 8.0 | 47 | 5 x 2 | no |

For the acute exposure, the TER values are > trigger × n (n = number of active substances in the mixture), indicating no unacceptable acute risk from the use of the product.
For the chronic exposure, the TER value of mesotrione is < trigger × n (n = number of active substances in the mixture). Therefore, a check for driver of toxicity is made in the next step.

2nd step: risk per fraction

Condition: one a.s. contributes to ≥ 90% of the predicted combined toxicity of the product.

The contribution of each individual a.s. to the combined toxicity (risk per fraction, rpf) is estimated based on the following equation:

$$rpf_{a.s.1} = \frac{1}{TER_{a.s.1}} / (\frac{1}{TER_{a.s.1}} + \frac{1}{TER_{a.s.2}} ... + \frac{1}{TER_{a.s.i}})$$

The estimation is based on TER values from the same refinement level to assure comparability.

| Active substance | $TER_{a.s.}$ | $rpf_{a.s.}$ | Driver? |
|---|---|---|---|
| Mesotrione | 8.0 | 0.85 | no |
| Thiencarbazone-methyl | 47 | 0.15 | no |

No driver of toxicity is identified. Therefore, the $TER_{mix}$ is calculated in the next step.

3rd step: $TER_{mix}$ calculation

Condition: the combined toxicity is acceptable if $TER_{mix} \geq 10$ (acute) or 5 (long-term).

The combined toxicity risk ($TER_{mix}$) with concentration-addition is estimated based on the following equation.

$$TER_{mix} = 1 / (\frac{1}{TER_{a.s.1}} + \frac{1}{TER_{a.s.2}} ... + \frac{1}{TER_{a.s.i}})$$

$TER_{mix} = 1/(1/8.0 + 1/47) = 6.8$

The $TER_{mix}$ for chronic exposure is above the trigger of 5, indicating no unacceptable chronic risk from the use of the product.

### 9.2.2.2 Higher-tier risk assessment

Not required.

### 9.2.2.3 Drinking water exposure

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

**Leaf scenario**

Since GLOB2112dH is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not have to be considered.

**Puddle scenario**

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

With a K(f)oc ranging between 14 and 354 L/kg, mesotrione belongs to the group of less sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group 1 also covers the risk for birds from all other intended uses in group 2 (see 9.1.2).

| | | | | |
|---|---|---|---|---|
| Effective application rate (g/ha) | = | 75 | | |
| Acute toxicity (mg/kg bw) | = | 2000 | quotient = | 0.0375 |
| Reprod. toxicity (mg/kg bw/d) | = | 20.6 | quotient = | 3.64 |

With a K(f)oc of 82.8 L/kg, thiencarbazone-methyl belongs to the group of less sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group 1 also covers the risk for birds from all other intended uses in group 2 (see 9.1.2).

| | | | | |
|---|---|---|---|---|
| Effective application rate (g/ha) | = | 15 | | |
| Acute toxicity (mg/kg bw) | = | 2000 | quotient = | 0.0075 |
| Reprod. toxicity (mg/kg bw/d) | = | 24 | quotient = | 0.625 |

### 9.2.2.4 Effects of secondary poisoning

The log $P_{ow}$ of mesotrione amounts to 0.11 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.
The log $P_{ow}$ of thiencarbazone-methyl amounts to -1.98 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

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

Not required.

**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 for birds is acceptable when using GLOB2112dH according to the intended GAP.

**zRMS comments:**

The risk assessment to birds was performed in accordance with the recommendation of Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA (EFSA Journal 2009; 7(12):1438).

The results of the 'screening phase' acute dietary risk assessment and Tier-1 long term dietary risk assessment - Toxicity Exposure Ratios ($TER_A$ and $TER_{LT}$) were calculated taking into account the EU agreed and accepted in core assessment endpoints for most sensitive species for the active substances and using the EFSA Bird and Mammal risk assessment calculator for the higher predicted application rate than it is foreseen in GAP exceeding the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects. Revealed that there is no potential of risk for birds resulting from acute and long-term exposure to active substances following use of Walkover Trio (GLOB2112dH) in compliance with proposed GAP.

**Mixture toxicity:**

**Acute risk**

For the assessment of the acute risk to birds, the following formula is used to derive a surrogate $LD_{50}$ for the mixture of active substances with known toxicity assuming additivity:

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

With:

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

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

For GLOB2112dH, the $LD_{50}$ (mix) amounts to 2000 mg/kg bw = $((0.833/2000) + (0.1667/2000))^{-1}$

No single substance is identified as the driver of the risk assessment.

The combined toxicity assessment supplied by the applicant is not in line with Appendix B of the EFSA guidance document for birds and mammals (2009).
The mixture $LD_{50}$ should be calculated considering the fractions of active substances and the endpoints:

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

| | **Mesotrione** | **Thiencarbazone** |
|---|---|---|
| Content in the formulation | 375 | 75 |
| Fraction in the a.s. mixture | 0.8333 | 0.1666 |
| $LD_{50}$ of a.s. [mg/kg bw] | >2000 | >2000 |
| Fraction / $LD_{50}$ | 0.8333/2000 = 0.000416667 | 0.1666/2000 = 8.33333E-05 |
| Sum | 0.0005 | |
| 1/ sum = predicted $LD_{50}$ (mix) | 2000 | |

In order to analyse if one of the active substances is leading the toxicity of the mixture, a "tox per fraction" quotient can be calculated, using the following equation:

$$\text{tox per fraction (a.s.)} = \frac{LD_{50}(\text{a.s.}_i)}{X(\text{a.s.}_i)}$$

$$\text{tox per fraction (mix)} = \frac{LD_{50}(\text{mix})}{\sum_i X(\text{a.s.}_i)}$$

Tox per fraction (**Mesotrione**) = 2000/0.8333 = 2400 mg/kg bw/d (deviation 20%)
Tox per fraction (**Thiencarbazone**) = 2000/0.1666 = 12000 mg/kg bw/d (deviation 500%)

It should be noted that none of the active substances contributes to ≥ 90% to mixture toxicity, so a combitox risk assessment should be done, according to the EFSA Guidance document (2009).

**Screening and first-tier assessment of the acute risk for birds due to the use of GLOB2021dF in maize (2 x 893 g/ha)**

| **Intended use** | **maize** | | | | |
|---|---|---|---|---|---|
| **Active substance/product** | GLOB2112dH | | | | |
| **Application rate (g/ha)** | 1 x 243* | | | | |
| **Acute toxicity (mg/kg bw)** | 2000 | | | | |
| **TER criterion** | 10 | | | | |
| **Crop scenario Growth stage** | **Indicator/generic focal species** | **$SV_{90}$** | **$MAF_{90}$** | **$DDD_{90}$ (mg/kg bw/d)** | **$TER_a$** |
| Screening step | Small omnivorous bird | 158.8 | 1.0 | 38.59 | 51.8 |

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger. *Based on a density of 1.2153 g/mL.

Based on the screening step and Tier 1, the combined acute risk is acceptable for the use on maize. Therefore, no further risk assessment was performed for combination of active substances.

**Long-term risk**

A calculated NOEL for the mixture is unlikely to constitute a reliable measure of toxicity as NOEL values may represent varying risk or response levels for different compounds. It is therefore not the current practice to derive a surrogate NOEL for the mixture of active substances as has been done for the acute risk. Alternatively, to assess the long-term risk to birds, a common accepted approach by different member states is to calculate a combined TER(mix) for each exposure scenario using the following equation:

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

where:

$TER_{(a.s.i)}$= calculated TER for the active substance i

$TER_{(mix)chronic} = ((1/8) + (1/47))^{-1} = 6.8$

The combined toxicity assessment indicates that the acute and long-term risks are acceptable.

A quantitative drinking water risk assessment is not triggered for the proposed use pattern of Walkover Trio (GLOB2112dH) according to EFSA/2009/1438 criteria and therefore the risk to birds via drinking water is acceptable.

No unacceptable effects to fish-eating and earthworm-eating birds are expected following application of Walkover Trio (GLOB2112dH) according to the proposed use pattern..

No risk mitigation measures are required.

## 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, thiencarbazone-methyl and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on mammals of GLOB2112dH were not evaluated as part of the EU assessment of mesotrione, and thiencarbazone-methyl.
However, the provision of further data on the formulation GLOB2112dH is not considered essential, because the risk for mammals from GLOB2112dH can be adequately assessed from the risk assessment for the active substance, since mammals are typically exposed to dry residues on their food items following the dilution and spraying of the formulated product. During these processes, much of the formulation constituents are likely to be lost by volatilisation. Since oral exposure is the main route of exposure, toxicity data for the active substance are therefore used in preference to data from tests with the formulated material.
The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

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

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| Rat | Mesotrione | Oral<br>1 d<br>Acute<br>Single dose | **$LD_{50}$ > 5000 mg/kg bw** | EFSA, 2016<br>[redacted]., 1994 |
| Rat | MNBA | Single dose/<br>Acute oral | $LD_{50}$ > 5000 mg/kg bw | EFSA, 2016<br>[redacted]., 1996 |
| Rat | AMBA | Single dose/<br>Acute oral | $LD_{50}$ > 5000 mg/kg bw | EFSA, 2016<br>[redacted]., 1996 |
| Rat | Mesotrione | 3-generation study | **NOAEL** = 2.5 mg/kg feed =<br>**0.3 mg/kg bw/d** | EFSA, 2016<br>[redacted]., 1997 |
| Rat | Thiencarbazone-methyl | Acute | **$LD_{50}$ > 2000 mg/kg bw/d** | EFSA, 2013<br>Anonymous, 2004 |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| Rat | Thiencarbazone-methyl | Long-term | **NOEL = 946 mg/kg bw/d** | EFSA, 2013<br>Anonymous, 2006 |

### 9.3.1.1 Justification for new endpoints

/

## 9.3.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Mammals and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).
To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group 1 also covers the risk for mammals from all other intended uses in group 2 (see 9.1.2).

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

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

**Table 9.3-2: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of GLOB2112dH in maize**

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | Maize | | | | |
| **Active substance/product** | Mesotrione | | | | |
| **Application rate (g/ha)** | 1 × 75 | | | | |
| **Acute toxicity (mg/kg bw)** | 5000 | | | | |
| **TER criterion** | 10 | | | | |
| **Crop scenario<br>Growth stage** | **Indicator/generic focal species** | **$SV_{90}$** | **$MAF_{90}$** | **$DDD_{90}$<br>(mg/kg bw/d)** | **$TER_a$** |
| Maize | Small herbivorous mammal | 136.4 | 1 | 10.23 | 489 |
| **Reprod. toxicity (mg/kg bw/d)** | 0.3 | | | | |
| **TER criterion** | 5 | | | | |
| **Crop scenario<br>Growth stage** | **Indicator/generic focal species** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$<br>(mg/kg bw/d)** | **$TER_{lt}$** |
| Maize | Small herbivorous mammal | 72.3 | 0.53 | 2.874 | **0.10** |
| Maize, BBCH 10-19 | Small insectivorous mammal shrew | 4.2 | 0.53 | 0.167 | **1.80** |
| Maize, BBCH 10-29 | Small herbivorous mammal vole | 72.3 | 0.53 | 2.874 | **0.10** |
| Maize, BBCH 10-29 | Small omnivorous mammal mouse | 7.8 | 0.53 | 0.310 | **0.97** |
| **Active substance/product** | Thiencarbazone-methyl | | | | |
| **Application rate (g/ha)** | 1 × 15 | | | | |
| **Acute toxicity (mg/kg bw)** | 2000 | | | | |
| **TER criterion** | 10 | | | | |

| **Crop scenario Growth stage** | **Indicator/generic focal species** | **$SV_{90}$** | **$MAF_{90}$** | **$DDD_{90}$ (mg/kg bw/d)** | **$TER_a$** |
|---|---|---|---|---|---|
| Maize | Small herbivorous mammal | 136.4 | 1 | 2.046 | 978 |
| **Reprod. toxicity (mg/kg bw/d)** | 946 | | | | |
| **TER criterion** | 5 | | | | |
| **Crop scenario Growth stage** | **Indicator/generic focal species** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$ (mg/kg bw/d)** | **$TER_{lt}$** |
| Maize | Small herbivorous mammal | 72.3 | 0.53 | 0.575 | 1646 |
| Maize, BBCH 10-19 | Small insectivorous mammal shrew | 4.2 | 0.53 | 0.033 | 28332 |
| Maize, BBCH 10-29 | Small herbivorous mammal vole | 72.3 | 0.53 | 0.57 | 1646 |
| Maize, BBCH 10-29 | Small omnivorous mammal mouse | 7.8 | 0.53 | 0.062 | 15256 |
| Maize BBCH 10-29 | Brown hare Lepus europaeus* (100% grass) | 17.3[1)] | 0.53 | 0.138 | 6855 |

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)

**Combined toxicity**

In the absence of acute and/or chronic study endpoints on the formulated product, the possible risk to birds exposed to the formulated product is predicted on the basis of data for the individual active substances in a combined toxicity assessment.

A quantitative toxicity risk assessment according to concentration addition is not needed if one of the following points applies:

- The risk assessment for all active substances in the product passes with a high margin of safety
- One active substance clearly drives the risk assessment

These conditions are assessed following a step-wise approach.

1st step: margin of safety

Condition: all TER values are > trigger x n (n = number of active substances in the mixture)

| **Scenario/indicator species** | **TER values** | | **Trigger x n** | **All TER > trigger x n?** |
|---|---|---|---|---|
| | **Mesotrione** | **Thiencarbazone-methyl** | | |
| Acute/small herbivorous mammal | 489 | 978 | 10 x 2 | yes |
| Chronic/small herbivorous mammal | 0.10 | 1646 | 5 x 2 | no |

For the acute exposure, the TER values are > trigger × n (n = number of active substances in the mixture), indicating no unacceptable acute risk from the use of the product.
For the chronic exposure, the TER value of mesotrione is < trigger × n (n = number of active substances in the mixture). Therefore, a check for driver of toxicity is made in the next step.

2nd step: risk per fraction

Condition: one a.s. contributes to ≥ 90% of the predicted combined toxicity of the product.

The contribution of each individual a.s. to the combined toxicity (risk per fraction, rpf) is estimated based on the following equation:

$$rpf_{a.s.1} = \frac{1}{TER_{a.s.1}} / (\frac{1}{TER_{a.s.1}} + \frac{1}{TER_{a.s.2}} ... + \frac{1}{TER_{a.s.i}})$$

The estimation is based on TER values from the same refinement level to assure comparability.

| Active substance | $TER_{a.s.}$ | $rpf_{a.s.}$ | Driver? |
|---|---|---|---|
| Mesotrione | 0.10 | 0.9999 | yes |
| Thiencarbazone-methyl | 1646 | 0.0001 | no |

Mesotrione is identified as the driver of chronic toxicity. Therefore, a combined risk assessment is not needed.

### 9.3.2.2 Higher-tier risk assessment

**Table 9.3-3: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of GLOB2112dH in maize – refined parameters (*) are further described and justified in the text**

| Crop scenario | Focal species | PD | RUD (mean) | $f_{twa}$ | FIR/bw | PT | Dose rate (kg/ha) | DDD | DDD (sum) |
|---|---|---|---|---|---|---|---|---|---|
| Maize BBCH 10-29 | Wood mouse* | 0.25 ~~(non-grass herbs)~~ maize shoots | ~~28.7~~ 54.2 | 0.09* | 0.27 | 0.139 * | 0.075 | ~~0.0018~~ 0.0034 | ~~0.026~~ 0.028 |
| | | 0.5 (weed seeds) | 40.2 | 0.53 | | | | 0.0230 | |
| | | 0.25 (ground arthropods with interception) | 3.5[1)] | 0.53 | | | | 0.0013 | |

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) according to EFSA B&M guidance (2009) RUD values for arthropods with interception are relevant for scenario maize at BBCH 10-29

| | |
|---|---|
| **Intended use** | Maize |
| **Active substance** | Mesotrione |
| **Application rate (g/ha)** | 1 × 75 |
| **Reprod. toxicity (mg/kg bw/d)** | 0.3 |
| **TER criterion** | 5 |

| Crop scenario Growth stage | Focal species | $DDD_m$ (mg/kg bw/d) | $TER_{lt}$ |
|---|---|---|---|
| Maize BBCH 10-29 | Wood mouse | ~~0.026~~ 0.028 | ~~11.5~~ 10.7 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Intended use** | Maize | | | | | |
| **Active substance/product** | Mesotrione | | | | | |
| **Application rate (g a.s./ha)** | 1 × 75 | | | | | |
| **Reprod. toxicity (mg/kg bw/d)** | 0.3 | | | | | |
| **TER criterion** | 5 | | | | | |
| **Crop scenario Growth stage** | **Focal species** | **SVm** | **PT** | **MAFm × TWA** | **DDDm (mg/kg bw/d)** | **TERlt** |
| Maize BBCH 10-29 | Brown hare Lepus europaeus* (100% grass) | 17.3[1)] | 0.62 | ~~0.0398*~~ 0.063* | ~~0.032~~ 0.05 | ~~9.4~~ 6 |

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

1) SVm from EFSA B&M guidance (2009) for Brown hare (grassland scenario)

***Refinement of focal species**

A generic field study monitoring the use of maize crops by small mammals (Wolf, 2005) was described in the RAR. This study involved live trapping of small mammals (BBCH 00-14; 2340 trapnights for maize). The only small mammal species trapped in maize fields was the wood mouse. This species was found at the highest number in the surrounding area, as were several other small mammal species (yellow-necked mouse, common vole, field vole, common pine vole, water vole and common shrew). The results indicate that the omnivorous **wood mouse can be an appropriate focal species for early maize (BBCH 00-14**; in this study the results on wood mouse were not reported separately for the pre- and post-emergence periods).

In a second field monitoring study described in the RAR (Grimm et al., 2013; maize at BBCH 00-12 in Germany; 27 April – 17 May 2013; 3040 trapnights) bank vole and yellow-necked mouse were also captured in the surrounding area of maize fields, however, only the wood mouse was trapped in-field. There were a total of 1560 trapnights between BBCH 00-09 and 720 trapnights between BBCH 10-12 in maize fields. The only in-field trapped wood mouse individual gives a 0.14 capture/100 trapnights trapping efficiency for the post-emergence period until BBCH 12. Whilst this result only relates to one individual, it is in line with the above studies and suggests that **wood mouse can be an appropriate focal species in freshly emerged maize (BBCH 10-12)**.

In addition to wood mouse, European brown hare (*Lepus europaeus*) was detected within the germinated maize fields (BBCH 10-14) in Austria with abundance of 0.12 individuals/hectare (5 transect tracks comprising 65.4 ha, 10 transect counts during the whole study; Wolf, 2005). Only European rabbit was observed in nocturnal scan sampling in Germany (0.05 individuals/ha in BBCH 10-16; 186 scan sessions comprising 0.43 ha; Grimm et al., 2013).

While the rabbit has a smaller bodyweight, the RMS considers the European **brown hare to be the appropriate focal species for lagomorphs in early maize**, given the high PT values indicated for this species in arable crops.

***Refinement of PT wood mouse**

A generic field study monitoring the use of maize crops by small mammals (Grimm et al., 2013) was described in the RAR. 17 tracking sessions of 14 individuals of wood mouse were conducted in freshly germinated maize fields (on days 2-18 after emergence; BBCH 10-16). 13 sessions contained wood mice with maize in their home range. The 9 tracking sessions of the "consumer only" group comprised 7 individuals (8 of their sessions were conducted on the same plot No. 2). The 10 tracking sessions of 7 consumer individuals resulted in 9 PT values – one did not enter maize – ranged between 0.4 and 13.9%. The PT data are briefly summarised in the table below.

| Wood mouse individual No. | PT by session (%) | PT by individual (%) |
|---|---|---|
| 3 | 3.4 | 3.4 |
| 7 | 4.5 | 2.6 |
| 7 | 0.8 | |
| 8 | 6.8 | 6.8 |
| 9 | 13.9 | **13.9** |
| 10 | 2.8 | 3.0 |
| 10 | 3.1 | |
| 13 | 1.3 | 0.7 |
| 13 | 0.0 | |
| 14 | 0.4 | 0.4 |

Considering the relatively small number of consumer individuals (n = 7) and the lack of data on the later stages of maize (BBCH 17-18), the worst-case PT of 13.9% was used for the wood mouse.

***Refinement of PT brown hare**

A generic field study monitoring the use of maize crops by the brown hare was performed (Grimm and Katzschner, 2019). The PT value to be used for the risk assessment was 0.62.

***Refinement of DT50 (for use on grasses and cereal shoots)**

Normally a $DT_{50}$ of 10 days is assumed in the birds and mammals risk assessment as a default value. For mesotrione however, a lower $DT_{50}$ could be expected based on five plant residue trials that were conducted in Europe (see dRR Part B7 for a description of these studies). The studies were conducted with Mesotrione 100 g/L SC which composition can be found in the Part C. The results on this latter formulation can be extrapolated to the GLOB2112dH because of the same formulation type and a similar dose rate per hectare. A kinetic analysis of the dissipation of mesotrione in maize was conducted by Enviresearch and a report of this study was submitted to support this refinement of the $DT_{50}$ of mesotrione on monocots (Hazlerigg, 2016).
FOCUS (2006, 2014) degradation kinetics guidance was applied to calculate $DT_{50}$ endpoints for mesotrione modelling from residues measured in five plant residue trials in Europe. The data were described reasonably well by either SFO kinetics or bi-phasic FOMC kinetics and acceptable endpoints were derived for all five studies.
The calculated $DT_{50}$ values and statistics for the decline of mesotrione in maize are shown in the table below. The $DT_{50}$ values ranged from 10.1 to 21.9 hours. The final $DT_{50}$ recommended for modelling is the geomean of 14 hours. This leads to a new TWA of 0.0398.

**Summary of fitted parameters for the decline of mesotrione.**

| Study | Kinetic model | t-test | $\chi$2-error | Visual fit | DT50 (hours) |
|---|---|---|---|---|---|
| B5116 AN1 | FOMC | n/a | 7.93 | Good | 13.3 * |
| B5116 MA1 | SFO | Pass | 6.92 | Good | 21.9 |
| B5116 BM1 | SFO | Pass | 14.3 | Medium | 10.1 |
| B5116 ND1 | FOMC | n/a | 6.74 | Good | 15.2 * |
| B5116 EF1 | FOMC | n/a | 10.9 | Good | 12.1 * |
| **Geomean of all trials** | | | | | **14.0** |

* Pseudo first-order $DT_{50}$ calculated as FOMC $DT_{90}$/3.32 (FOCUS 2006, 2014)

In general, the kinetic evaluation is considered acceptable.
As results from 5 trials performed only in one country (France) is available (4 are Northern France not belonging to the Central Zone, although conditions in Northern France are similar to the Central Europe and 1 is Southern France), it is proposed by the zRMS to use the worst case DT50 of 21.9 hours for purposes of the risk refinement. This leads to a new TWA of 0.063

***Refinement of DT50 (for use on dicots)**

Normally a $DT_{50}$ of 10 days is assumed in the birds and mammals risk assessment as a default value. For mesotrione, a lower $DT_{50}$ could be expected based on six plant residue trials that were conducted in Europe (see dRR Part B7 for a description of these studies). The studies were conducted with Mesotrione 100 g/L SC which composition can be found in the Part C. The results on this latter formulation can be extrapolated to GLOB2112dH because of the same formlation type the and a similar dose rate per hectare. A kinetic analysis of the dissipation of mesotrione in oilseed rape was conducted by Anadiag and a report was submitted to support this refinement of the $DT_{50}$ of mesotrione on dicots (Ertus C., 2020).
FOCUS (2006, 2014) degradation kinetics guidance was applied to calculate $DT_{50}$ endpoints for mesotrione modelling from residues measured in six plant residue trials in Europe. The data were described well by SFO kinetics and acceptable endpoints were derived for all six studies.
The calculated $DT_{50}$ values and statistics for the decline of mesotrione in maize are shown in the table below. The $DT_{50}$ values ranged from 3.7 to 31.5 hours. As some of the trials were performed in the southern zone, the final $DT_{50}$ used for the risk assessment is the highest value of 31.5 hours. This leads to a new TWA of 0.09.

**Summary of fitted parameters for the decline of mesotrione.**

| Study | Kinetic model | k | χ2-error | Residual sum of squares | DT50 (hours) |
|---|---|---|---|---|---|
| B7314 HU1 | SFO | 0.025 | 9.9 | 507.8 | 27.7 |
| B7314 MA1 | SFO | 0.037 | 15.4 | 1087.0 | 18.7 |
| B7314 ND1 | SFO | 0.022 | 30.6 | 7326.0 | 31.5 |
| B7314 PL1 | SFO | 0.187 | 28.8 | 1664.9 | 3.7 |
| B7314 EF1 | SFO | 0.032 | 21.0 | 1612.1 | 21.7 |
| B7314 ES1 | SFO | 0.051 | 13.3 | 556.0 | 13.6 |
| **Geomean of all trials** | | | | | **16.2** |

Higher tier risk assessment mammals: comparability of mesotrione residues from GLOB205H vs. GLOB2112dH

In the dossier of Walkover Trio (GLOB2112dH), a higher tier risk assessment for mammals was needed for mesotrione. Residue decline trials performed with Mesotrione 100 SC (GLOB205H) on monocots (Schneider E., 2016) and dicots (Ertus C., 2020) were used to refine the $DT_{50}$.

Although GLOB205H is not identical to GLOB2112dH, mesotrione residue levels and their residue decline behaviour can be expected to be similar.

Indeed, in the OECD Guidance Document on crop field trials (OECD No. 509) is explained that experience shows that emulsifiable concentrates (EC), wettable powders (WP), dispersible granules (WG), and suspension concentrates (SC) formulations usually produce comparable residues (especially if the last application is more than seven days prior to harvest). Please find below an excerpt from this guidance.

***Formulations diluted in water***

24 The most common formulation types which are diluted in water prior to application include EC, WP, water dispersible granules (WG), suspension concentrates (SC, also called flowable concentrates), and soluble concentrates (SL). Residue data may be translated among these formulation types for applications that are made to seeds, prior to crop emergence (i.e., pre-plant, at-plant, and pre-emergence applications) or just after crop emergence. Data may also be translated among these formulation types for applications directed to the soil, such as row middle or post-directed applications as opposed to foliar treatments.

25 In a publication by Maclachlan and Hamilton (2010) it was shown by evaluation of side by side trials with the same application rate and similar spray volumes that WP, EC, CS (capsule suspension) and SC formulations do not show a significant difference in day-zero residues after foliar treatment (JMPR data from 2000 to 2004). The evaluation includes trials with PHIs of less than seven days. If the PHI is exceeding 7 days for mid-season and late-season foliar applications, formulations diluted in water are considered equivalent to each other within two groups: formulations not containing oils or organic solvents (e.g., WG, SC) and those containing oils or organic solvents (e.g., EC, OD).

Since GLOB205H and GLOB2112dH are both SC formulations applied early post-emergence at the same dose rate per hectare, no significant difference in mesotrione residues is expected.

In addition, in the majority of the trials in Schneider E. (2016) and Ertus C. (2020), the residue data have been fitted using first-order kinetics, which means that the obtained $DT_{50}$ is completely independent of the initial concentration. The first-order representation is convenient because the rate is summarized with a single parameter (the rate constant, k), and the rate of transformation is independent of the initial concentration. The half-life, t1/2 =ln(2)/k, indicates the time required to reduce the concentration by 50% from any concentration point in time.

The obtained $DT_{50}$ values have also been confirmed in several other mesotrione residue decline trials. A comparison of all available trials was made by the German authorities. The $DT_{50}$ on monocots in the applicant's trials range from 0.42 d to 0.91 d. These values are in line with the $DT_{50}$ 0f 0.92 d derived by Henkes (2016)[1]. The lowest available half-life for residues on maize on monocotyl plants for the central zone was a $DT_{50}$ of 0.8 d (North, 2016)[2], the highest $DT_{50}$ of 1 d was observed by Loriau (2016)[3]. Allen (2019)[4] investigated the residue decline of mesotrione in dicotyl plants. the maximal value for mesotrione decline in Germany is 2.76 d. The minimal value is a $DT_{50}$ of 1.18 d (Hohnheiser, 2018[5]). This is also in line with the $DT_{50}$ on dicots obtained in the applicant's trials which are ranging from 0.15 d to 1.3 d.

It can be concluded, based on the OECD No. 509 guidance and other available mesotrione residue decline trials, that the $DT_{50}$ values obtained with GLOB205H are reliable and representative, and can be used to refine the mammals higher tier risk assessment of GLOB2112dH.

[1] Henkes (2016): Residues of Mesotrione on maize plants after spray application of Mesotrione 100 SC in early growth stages of maize in Germany – magnitude of residues and time course of residue decline. Rifcon reports. Submitted to ZV8651, Daneva
[2] North (2016): Mesotrione – Foliage Decline Study with A12739A on Maize in Northern France and the United Kingdom in 2015. Eurofins report. Submitted to ZV 4660, Callisto
[3] Loriau (2016): Residues of mesotrione (and its metabolites) in maize in open field conditions after one foliar application of AG-M3-100 SC1 in 4 DCS tials in Nort EU (Belgium, Germany, The Netherlands and Poland) in 2016. Redebel report. Submitted to ZV 8117, Starship
[4] Allen (2019): Mesotrione - Foliar Residue Decline Study on Clover in Hungary, Poland, Germany, United Kingdom, Northern France and Belgium in 2018. Cemas report. Submitted to ZV 4660, Callisto
[5] Hohnheiser (2018): Determination of Residues of Mesotrione after One Spray Application of Mesotrione in Maize at 4 Field Sites in the United Kingdom and 1 Field Site in Northern France 2018. Eurofins report. Submitted to ZV 8517, Mesotstar

### 9.3.2.3 Drinking water exposure

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

**Puddle scenario**

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

With a K(f)oc ranging between 14 and 354 L/kg, mesotrione belongs to the group of less sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group 1 also covers the risk for mammals from all other intended uses in group 2 (see 9.1.2).

| | | | | |
|---|---|---|---|---|
| Effective application rate (g/ha) | = | 75 | | |
| Acute toxicity (mg/kg bw) | = | 5000 | quotient = | 0.015 |
| Reprod. toxicity (mg/kg bw/d) | = | 0.3 | quotient = | 250 |

With a K(f)oc ranging between 14 and 354 L/kg, mesotrione belongs to the group of less sorptive substances. Since the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) exceeds the critical value of 50 for at least one use scenario, a quantitative risk assessment (calculation of TER values) is necessary.
To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group 1 also covers the risk for mammals from all other intended uses in group 2 (see 9.1.2).

**Table 9.3-4: Assessment of the risk for mammals due to exposure to mesotrione via contaminated drinking water in puddles**

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | Maize | | | | |
| **Active substance** | Mestrione | | | | |
| **Application rate (g/ha)** | 1 × 75 | | | | |
| **Reprod. toxicity (mg/kg bw/d)** | 0.3 | | | | |
| **TER criterion** | 5 | | | | |
| **Soil-relevant applic. rate (g/ha)** | **Koc (L/kg)** | **$PEC_{puddle}$ (mg/L)** | **DW uptake (L/kg bw/d)** | **Daily dose (mg/kg bw/d)** | **$TER_{lt}$** |
| 75 | 14 (worst-case) | 0.18 | 0.24 | 0.044 | 6.8 |

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

With a K(f)oc of 82.8 L/kg, thiencarbazone-methyl belongs to the group of less sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group 1 also covers the risk for birds from all other intended uses in group 2 (see 9.1.2).

| | | | | |
|---|---|---|---|---|
| Effective application rate (g/ha) | = | 15 | | |
| Acute toxicity (mg/kg bw) | = | 2000 | quotient = | 0.0075 |
| Reprod. toxicity (mg/kg bw/d) | = | 946 | quotient = | 0.0159 |

### 9.3.2.4 Effects of secondary poisoning

The log $P_{ow}$ of mesotrione amounts to 0.11 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.
The log $P_{ow}$ of thiencarbazone-methyl amounts to -1.98 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

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

Not required.

**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 for mammals is acceptable when using GLOB2112dH according to the intended GAP.

**zRMS comments:**

The risk assessment to mammals was performed in accordance with the recommendation of Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA (EFSA Journal 2009; 7(12):1438).

The results of the 'screening phase' acute dietary risk assessment and Tier-1 long term dietary risk assessment - Toxicity Exposure Ratios ($TER_A$ and $TER_{LT}$) were calculated taking into account the EU agreed and accepted in core assessment endpoints for most sensitive species for the active substances and using the EFSA Bird and Mammal risk assessment calculator for the higher predicted application rate than it is foreseen in GAP exceeding the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects. Revealed that there is no potential of risk for ~~birds~~ mammals resulting from acute and long-term exposure to active substances following use of Walkover Trio (GLOB2112dH) in compliance with proposed GAP.

**Mixture toxicity:**

**Acute risk**

The combined toxicity assessment supplied by the applicant is not in line with Appendix B of the EFSA guidance document for birds and mammals (2009).
The mixture $LD_{50}$ should be calculated considering the fractions of active substances and the endpoints:

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

| | Mesotrione | Thiencarbazone |
|---|---|---|
| Content in the formulation | 375 | 75 |
| Fraction in the a.s. mixture | 0.8333 | 0.1666 |
| $LD_{50}$ of a.s. [mg/kg bw] | >5000 | >2000 |
| Fraction / $LD_{50}$ | 0.8333/5000 = 0.0001667 | 0.1666/2000 = 8.33333E-05 |
| Sum | 0.00025 | |
| 1/ sum = predicted $LD_{50}$ (mix) | 4000 | |

In order to analyse if one of the active substances is leading the toxicity of the mixture, a "tox per fraction" quotient can be calculated, using the following equation:

$$\text{tox per fraction (a.s.)} = \frac{LD_{50}(\text{a.s.}_i)}{X(\text{a.s.}_i)}$$

$$\text{tox per fraction (mix)} = \frac{LD_{50}(\text{mix})}{\sum_i X(\text{a.s.}_i)}$$

Tox per fraction (**Mesotrione**) = 5000/0.8333 = 6000 mg/kg bw/d (deviation 50%)
Tox per fraction (**Thiencarbazone**) = 2000/0.1666 = 12000 mg/kg bw/d (deviation 200%)

It should be noted that none of the active substances contributes to ≥ 90% to mixture toxicity, so a combitox risk assessment should be done, according to the EFSA Guidance document (2009).

**Screening and first-tier assessment of the acute risk for mammals due to the use of GLOB2112dH in Maize (1 x 243 g/ha)**

| **Intended use** | **Maize** | | | | |
|---|---|---|---|---|---|
| **Active substance/product** | GLOB2112dH | | | | |
| **Application rate (g/ha)** | 1 x 243* | | | | |
| **Acute toxicity (mg/kg bw)** | 4000 | | | | |
| **TER criterion** | 10 | | | | |
| **Crop scenario Growth stage** | **Indicator/generic focal species** | **$SV_{90}$** | **$MAF_{90}$** | **$DDD_{90}$ (mg/kg bw/d)** | **$TER_a$** |
| Screening step | Small herbivorous mammal | 136.4 | 1.0 | 33.15 | 12.1 |

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. *Based on a density of 1.2153 g/mL.

Based on the screening step and Tier 1, the combined acute risk is acceptable for the use on maize. Therefore, no further risk assessment was performed for combination of active substances.

**Long-term risk**

A calculated NOEL for the mixture is unlikely to constitute a reliable measure of toxicity as NOEL values may represent varying risk or response levels for different compounds. It is therefore not the current practice to derive a surrogate NOEL for the mixture of active substances as has been done for the acute risk. Alternatively, to assess the long-term risk to ~~birds~~ mammals, a common accepted approach by different

member states is to calculate a combined TER(mix) using the following equation:

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

where:

$TER_{(a.s.i)}$= calculated TER for the active substance i

$TER_{(mix)chronic\ brown\ hare} = ((1/6) + (1/6855))^{-1} = 6$
$TER_{(mix)chronic\ mouse} = ((1/10.7) + (1/15256))^{-1} = 10.7$

A refined risk assessment for the reproductive risk to mammals for mesotrione was performed by applicant based on the relevant focal species in early maize (BBCH 12-18) and PT values, Food Intake Rate, RUD and fTWA values. Generally evaluator agrees with the proposed refinement.

Additionally the RUD value of 54.2 for maize currently included in the B&M EFSA guidance document 2009; 7(12):1438 is likely a conservative value since the trials it is based upon do not include maize, the lower RUD value of 29.7 for maize from the database developed by Lahr et al. (2018) has been proposed in the draft of the new Birds and Mammal guidance document (2021).
Taking into consideration the conservativeness of the approach, the risk to brown hare is considered acceptable by the zRMS.

The combined toxicity assessment indicates that the acute and long-term risks are acceptable.

A quantitative drinking water risk assessment is not triggered for the proposed use pattern of Walkover Trio (GLOB2112dH) according to EFSA/2009/1438 criteria and therefore the risk to ~~birds~~ mammals via drinking water is acceptable.

No unacceptable effects to fish-eating and earthworm-eating ~~birds~~ mammals are expected following application of Walkover Trio (GLOB2112dH) according to the proposed use pattern..

No risk mitigation measures are required.

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

Birds and mammals are regarded as adequate surrogates for terrestrial stages of amphibians and reptiles. For the aquatic stages of amphibians, please refer to the risk assessment for fish presented in KCP 10.2.

## 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, thiencarbazone-methyl and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on aquatic organisms of GLOB2112dH were not evaluated as part of the EU assessment of meso-

trione and thiencarbazone-methyl. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| **Fish** | | | | |
| *Oncorhynchus mykiss* | Mesotrione | 96 h, s | **$LC_{50}$ > 120 mg/L $_{nom}$** | EFSA, 2016 [redacted], 1994 |
| *Lepomis macrochirus* | Mesotrione | 96 h, s | $LC_{50}$ > 120 mg/L $_{nom}$ | EFSA, 2016 [redacted], 1994 |
| *Pimephales promelas* | Mesotrione | 36 d, f | **NOEC = 12.5 mg/L $_{nom}$** | EFSA, 2016 [redacted], 1997 |
| *Oncorhynchus mykiss* | MNBA | 96 h, s | **$LC_{50}$ > 120 mg/L $_{nom}$** | EFSA, 2016 [redacted] 1997 |
| *Oncorhynchus mykiss* | AMBA | 96 h, s | **$LC_{50}$ = 150 mg/L $_{nom}$** | EFSA, 2016 [redacted], 1998 |
| *Oncorhynchus mykiss* | Thiencarbazone-methyl | 96 h, s | **$LC_{50}$ > 104 mg/L$_{mm}$** | EFSA, 2013 [redacted] 2005 |
| *Pimephales promelas* | Thiencarbazone-methyl | 35 d, f | **NOEC = 4.8 mg/L$_{mm}$** | EFSA, 2013 [redacted], 2006 |
| *Oncorhynchus mykiss* | BYH 18636-sulfonamide (M15) | 96 h, s | **$LC_{50}$ = 98.3 mg/L$_{mm}$** | EFSA, 2013 [redacted], 2005 |
| **Aquatic invertebrates** | | | | |
| *Daphnia magna* | Mesotrione | 48 h, s | **$EC_{50}$ > 622 mg/L $_{mm}$** | EFSA, 2016 Gentle W.E., Hamer M.J., 1995 |
| *Daphnia magna* | Mesotrione | 21 d, ss | **NOEC = 180 mg/L $_{nom}$** | EFSA, 2016 Morris D.S. *et al.*, 1996 |
| *Daphnia magna* | MNBA | 48 h, s | **$EC_{50}$ = 130 mg/L $_{nom}$** | EFSA, 2016 Kent S.J., Shillabeer N., 1997 |
| *Daphnia magna* | AMBA | 48 h, s | **$EC_{50}$ = 160 mg/L $_{nom}$** | EFSA, 2016 Magor S.E., Gore N.R., 1998 |
| *Daphnia magna* | Thiencarbazone-methyl | 48 h, s | **$EC_{50}$ > 98.6 mg/L$_{mm}$** | EFSA, 2013 Banman C.S.; Lam |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| | | | | C.V., 2005 |
| *Daphnia magna* | Thiencarbazone-methyl | 21 d, sr | **NOEC = 3.54 mg/$L_{mm}$** | EFSA, 2013<br>Kern M.E., Lam C.V., 2006 |
| *Daphnia magna* | BYH 18636-sulfonamide (M15) | 48 h, s | **$EC_{50}$ > 100 mg/$L_{nom}$** | EFSA, 2013<br>Bruns E., 2007 |
| **Sediment-dwelling organisms** | | | | |
| *Chironimus riparius* | Thiencarbazone-methyl | 48 h, s | **$EC_{50}$ > 100 mg/$L_{nom}$** | EFSA, 2013<br>Bruns E., 2006 |
| *Chironimus riparius* | BYH 18636-carboxylic acid (M01) | 48 h, s | **$EC_{50}$ > 100 mg/$L_{nom}$** | EFSA, 2013<br>Bruns E., 2006 |
| *Chironimus riparius* | BYH 18636-sulfonamide-carboxylic acid (M03) | 28 d, s | **$EC_{50}$ > 100 mg/$L_{nom}$** | EFSA, 2013<br>Bruns E., 2006 |
| **Algae** | | | | |
| *Pseudokirchneriella subcapitata* | Mesotrione | 72 h, s<br>120 h, s | **$E_rC_{50}$ = 13 mg/L $_{nom}$**<br>$E_bC_{50}$ = 3.5 mg/L $_{nom}$ | EFSA, 2016<br>Shillabeer N., Kent S.J., Smyth D.V., 1997 |
| *Pseudokirchneriella subcapitata* | MNBA | 72 h, s | **$E_rC_{50}$ = 42 mg/L $_{nom}$**<br>$E_bC_{50}$ = 38 mg/L $_{nom}$ | EFSA, 2016<br>Smyth D.V. e*t al.*, 1997 |
| *Pseudokirchneriella subcapitata* | AMBA | 72 h, s | **$E_rC_{50}$ = 14 mg/L $_{nom}$**<br>$E_bC_{50}$ = 9.4 mg/L $_{nom}$ | EFSA, 2016<br>Smyth D.V., Magor S.E., Shillabeer N., 1998 |
| *Pseudokirchneriella subcapitata* | Thiencarbazone-methyl | 96 h, s | $E_bC_{50}$ = 0.17 mg/$L_{mm}$<br>**$E_rC_{50}$ = 1.02 mg/$L_{mm}$** | EFSA, 2013<br>Kern M.E., Banman C.S., Lam C.V., 2005 |
| *Pseudokirchneriella subcapitata* | BYH 18636-sulfonamide (M15) | 72 h, s | $E_bC_{50}$ = 0.50 mg/$L_{mm}$<br>**$E_rC_{50}$ = 1.61 mg/$L_{mm}$** | EFSA, 2013<br>Banman C.S., Lam C.V., 2005 |
| *Navicula pelliculosa* | Thiencarbazone-methyl | 96 h, s | $E_bC_{50}$ = 64.0 mg/$L_{mm}$<br>$E_rC_{50}$ = 64.0 mg/$L_{mm}$ | EFSA, 2013<br>Kern ME., Roberts J.A., Lam C.K., 2005 |
| *Anabaena flos-aquae* | Thiencarbazone-methyl | 96 h, s | $E_bC_{50}$ = 4.25 mg/$L_{mm}$<br>$E_rC_{50}$ = 8.92 mg/$L_{mm}$ | EFSA, 2013<br>Kern M.E., Lam C.V., 2006 |
| **Higher plants** | | | | |
| *Lemna gibba* | Mesotrione | 14 d, ss | **$E_bC_{50}$ = 0.0077 mg/L $_{nom}$**<br>$E_rC_{50}$ frond no.= 0.022 mg a.s./$L_{nom}$ | EFSA, 2016<br>Smyth D.V. e*t al.*, 1997 |
| *Lemna gibba* | Mesotrione | 7 d, ss | $E_rC_{50}$ frond no or | Hengsberger A., |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| | | | biomass = 0.028 mg /L $_{nom}$<br>$E_bC_{50}$ yield = 0.006 mg/L $_{nom}$<br>$E_rC_{10}$ biomass = 0.0011 mg/L $_{nom}$<br><br>Corrected for purity:<br>**$E_rC_{50}$ frond no or biomass = 0.0241 mg/L $_{nom}$**<br>$E_bC_{50}$ yield = 0.0045 mg/L $_{nom}$<br>$E_rC_{10}$ biomass = 0.0009 mg/L $_{nom}$ | Wydra V. 2015 (report amendment); Kosak & Wydra, 2016 |
| *Myriophyllum spicatum* | Mesotrione | 14d, ss | $E_rC_{50}$ total shoot length = 0.0339 mg/L $_{nom}$<br>$E_yC_{50}$ yield = 0.0301 mg/L $_{nom}$<br><br>$E_rC_{10}$ (total shoot length) = 0.000149 mg/L $_{nom}$<br>$E_rC_{20}$ (total shoot length) = 0.00096 mg/L $_{nom}$<br><br>Corrected for purity:<br>$E_rC_{50}$ total shoot length = 0.0287 mg /L $_{nom}$<br>$E_yC_{50}$ yield = 0.00255 mg/L $_{nom}$<br><br>$E_rC_{10}$ (total shoot length) = 0.000126 mg/L $_{nom}$<br>$E_rC_{20}$ (total shoot length) = 0.00081 mg/L $_{nom}$ | Gonsior, 2017 |
| Aquatic macrophytes | Mesotrione | Geometric mean | **$E_rC_{50}$ = 0.0263 mg/L**<br>$E_yC_{50}$ = 0.00339 mg/L | See chapter 9.5.1.1 |
| *Lemna gibba* | MNBA | 7 d, ss | **$E_rC_{50}$ > 97 mg/L $_{nom}$**<br>$E_yC_{50}$ > 97 mg/L $_{nom}$ | EFSA, 2016<br>Liedtke A., 2013 |
| *Lemna gibba* | AMBA | 7 d, ss | **$E_rC_{50}$ > 90 mg/L $_{nom}$**<br>$E_yC_{50}$ > 90 mg/L $_{nom}$ | EFSA, 2016<br>Liedtke A., 2013 |
| *Lemna gibba* | SYN 546974 | 7 d, ss | **$E_rC_{50}$ > 95 mg/L $_{nom}$**<br>$E_yC_{50}$ = 93 mg/L $_{nom}$ | EFSA, 2016<br>Liedtke A., 2013 |
| *Lemna gibba* | Thiencarbazone-methyl | 7 d, sr | $E_bC_{50}$ = 0.8 µg/$L_{mm}$<br>$E_yC_{50}$ = 1.52 µg/$L_{mm}$<br>$E_rC_{50}$ = 1.31 µg/$L_{mm}$ | EFSA, 2013<br>Kern M.E., Lam C.V., 2006 |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| | | | NOEC = 0.2 µg/$L_{mm}$ | |
| *Myriophyllum spicatum* | Thiencarbazone-methyl | 14 d, s | $E_yC_{50}$ = 0.58 µg/L<br>**$E_rC_{50}$ = 0.94 µg/L** | EFSA, 2013<br>Christ M.T., Lam C.V., 2007 |
| *Potamogeton pectinatus* | Thiencarbazone-methyl | 14 d, s | $E_bC_{50}$ = 8.3 µg/L<br>$E_rC_{50}$ = 5.3 µg/L | EFSA, 2013<br>Hoberg J.R., 2007 |
| Aquatic macrophytes | Thiencarbazone-methyl | - | Geomean $E_bC_{50}$ = 1.35 µg/L<br>**Geomean $E_rC_{50}$ = 1.87 µg/L** | EFSA, 2013 |
| *Lemna gibba* | Thiencarbazone-methyl | Pulsed exposure – recovery study | 1 peak:<br>Frond number:<br>$E_yC_{50}$ = 3.94 µg/L<br>**$E_rC_{50}$ = 27.5 µg/L**<br>Dry weight:<br>$E_yC_{50}$ = 4.91 µg/L<br>$E_rC_{50}$ = 47.0 µg/L<br><br>2 peaks:<br>Frond number:<br>$E_yC_{50}$ = 3.30 µg/L<br>**$E_rC_{50}$ = 12.2 µg/L**<br>Dry weight:<br>$E_yC_{50}$ = 5.33 µg/L<br>$E_rC_{50}$ = 52.2 µg/L | Minati R., 2024 |
| *Myriophyllum spicatum* | Thiencarbazone-methyl | Pulsed exposure – recovery study | 1 peak:<br>Total shoot length:<br>$E_yC_{50}$ = 7.01 µg/L<br>**$E_rC_{50}$ = 15.4 µg/L**<br>Fresh weight:<br>$E_yC_{50}$ = 6.92 µg/L<br>$E_rC_{50}$ = 18.8 µg/L<br>Dry weight:<br>$E_yC_{50}$ > 27.3 µg/L<br>$E_rC_{50}$ > 27.3 µg/L<br><br>2 peaks:<br>Total shoot length:<br>$E_yC_{50}$ = 4.98 µg/L<br>$E_rC_{50}$ = 7.04 µg/L<br>Fresh weight:<br>$E_yC_{50}$ = 3.60 µg/L<br>**$E_rC_{50}$ = 6.30 µg/L**<br>Dry weight:<br>$E_yC_{50}$ > 27.3 µg/L<br>$E_rC_{50}$ > 27.3 µg/L | Bebon R., 2024 |
| *Lemna gibba* | BYH 18636-carboxylic acid (M01) | 7 d, sr | $E_bC_{50}$ = 2.08 mg/$L_{mm}$<br>**$E_rC_{50}$ = 3.54 mg/$L_{mm}$** | EFSA, 2013<br>Banman C.S., Lam C.V., 2005 |
| *Lemna gibba* | BYH 18636-sulfonamide-carboxylic acid | 7 d, sr | $E_bC_{50}$ > 100 mg/$L_{nom}$<br>**$E_rC_{50}$ > 100 mg/$L_{nom}$** | EFSA, 2013<br>Dorgerloh M., 2006 |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| | (M03) | | | |
| *Lemna gibba* | BYH 18636-sulfonamide (M15) | 7 d, sr | $E_bC_{50}$ = 61.6 mg/$L_{mm}$<br>**$E_rC_{50}$ = 90.5 mg/$L_{mm}$** | EFSA, 2013<br>Christ M.T., Lam C.V., 2006 |
| *Lemna gibba* | BYH 18636-MMT (M21) | 7 d, sr | $E_bC_{50}$ > 95.7 mg/$L_{mm}$<br>**$E_rC_{50}$ > 95.7 mg/$L_{mm}$** | EFSA, 2013<br>Christ M.T., Lam C.V., 2007 |
| *Lemna gibba* | BYH 18636-dicarboxy-sulfonamide (M25) | 7 d, sr | $E_bC_{50}$ > 104 mg/$L_{mm}$<br>**$E_rC_{50}$ > 104 mg/$L_{mm}$** | EFSA, 2013<br>Christ M.T., Hoffmann J.M., Lam C.V., 2007 |
| **Other aquatic organisms*** | | | | |
| *Cyprinodon variegatus* | Thiencarbazone-methyl | 96 h, s | $LC_{50}$ > 106 mg/L | EFSA, 2013<br>Banman C.S., Lam C.V., 2005 |
| *Americamysis bahia* | Thiencarbazone-methyl | 96 h, f | $EC_{50}$ > 94 mg/$L_{mm}$ | EFSA, 2013<br>Putt A.E., 2006 |
| *Americamysis bahia* | Thiencarbazone-methyl | 28 d, f | NOEC = 5.9 mg/$L_{mm}$ | EFSA, 2013<br>Putt A.E., 2006 |
| *Crassostrea virginica* | Thiencarbazone-methyl | 96 h, f | $EC_{50}$ > 100 mg/$L_{mm}$ | EFSA, 2013<br>Cafarella M.A., 2006 |
| *Skeletonema costatum* | Thiencarbazone-methyl | 96 h, s | $E_cC_{50}$ > 114 mg/$L_{mm}$<br>$E_rC_{50}$ > 114 mg/$L_{mm}$ | EFSA, 2013<br>Christ M.T., Lam C.V., 2006 |
| **Higher-tier studies (micro- or mesocosm studies)** | | | | |
| - | | | | |

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
*The acute (and chronic) toxicity of the active substances to saltwater species (i.e. marine fish, marine invertebrates and marine algae) has been investigated to fulfill the specific registration requirements in the USA. There, the above mentioned studies on marine organisms are in support of the development of the compounds for its use in field crops in the US, particularly for those selected areas, where application is in direct vicinity of brackish or estuarine water bodies. The marine species showed sensitivity comparable to the freshwater fish and invertebrate species and are therefore not used in the risk assessment.

**Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – GLOB2112dH**

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Pseudokirchneriella subcapitata* | GLOB2112dH | 72 h, s | **$E_rC_{50}$ = 87.1 mg/$L_{nom}$**<br>$E_yC_{50}$ = 13.3 mg/$L_{nom}$<br>$E_rC_{50}$ = 14.5 mg/$L_{nom}$ | Bauer J., 2024a |
| *Lemna gibba* | GLOB2112dH | 7 d, s | $E_yC_{50}$ frond number = 8.28 µg/$L_{nom}$<br>**$E_rC_{50}$ frond number = 18.3 µg/$L_{nom}$** | Bauer J., 2024b |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| | | | $E_yC_{50}$ dry weight = 9.94 µg/L $_{nom}$<br>$E_rC_{50}$ dry weight = 83.6 µg/L $_{nom}$ | |
| Myriophyllum | GLOB2112dH | 14 d, s | $E_yC_{50}$ shoot length = 18.0 µg/L $_{mm}$<br>$E_rC_{50}$ shoot lenght = 27.0 µg/L $_{mm}$<br>$E_yC_{50}$ fresh weight = 7.81 µg/L $_{mm}$<br>**$E_rC_{50}$ fresh weight = 14.2 µg/L $_{mm}$**<br>$E_yC_{50}$ dry weight = 8.50 µg/L $_{mm}$<br>$E_rC_{50}$ dry weight = 22.0 µg/L $_{mm}$ | Bauer J., 2024c |

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

#### 9.5.1.1 Justification for new endpoints

When an endpoint based on growth rate is available, this is used instead of the endpoint based on biomass or yield since $E_rC_{50}$ values are considered more relevant for the risk assessment according to the guidance document for aquatic organisms (EFSA, 2013).

The laboratory study with mesotrione for *Lemna* was repeated due to issues in the original study submitted in which concentrations were not maintained within 20% of nominal throughout the exposure period, and endpoints were not reported in terms of growth rate. The new 7d study (Kosak & Wydra, 2016) fulfils all the current acceptability criteria, and concentrations were maintained within 20% of nominal throughout the study. The biomass endpoints of the Kosak & Wydra study ($E_bC_{50\ dry\ weight}$ = 0.0045 mg a.s./L) and the previous study of Smyth et al. (1997, $E_bC_{50}$ = 0.0077 mg a.s./L) are very similar, and the new endpoints will be used in the risk assessment, in preference, as they are considered more reliable.
In the EU review of mesotrione a data gap was identified for a dicot aquatic macrophyte, and therefore a new test has been carried out with *Myriophyllum spicatum*. The results for *Lemna* and *Myriophyllum* are remarkable similar as shown in the table above, indicating that there is no indication of selectivity to dicot or monocot aquatic macrophytes.
In accordance with the Aquatic Guidance document ('Tier 2a'), the geometric mean of endpoints can be used when there are data from more than 1 species, and when these differ by less than a factor of 10. Since data are available from studies with *Myriophyllum* ($E_rC_{50}$ 0.0287 mg a.s./L) and *Lemna* ($E_rC_{50}$ 0.0241 mg a.s./L), the geometric mean $E_rC_{50}$ of 0.0263 mg a.s./L can be used.

**Derivation of RAC values used in the Tier 1 risk assessment – Mesotrione**

| Species | Substance | Exposure System | Results (µg/L) | Assessment Safety factor | RAC (µg/L) |
|---|---|---|---|---|---|
| *Lemna gibba* | Mesotrione | 7 d, ss | $E_rC_{50}$ = 24.1 | 10 | 2.41 |
| Macrophytes | Mesotrione | Geometric mean | $E_rC_{50}$ = 26.3 | 10 | 2.63 |

**zRMS comments:**

According to the EFSA aquatic guidance (2013): "*According to the data requirements, additional testing*

*may be required by the Member State competent authorities on other macrophyte species depending on the mode of action of the substance, or if clear indications of higher toxicity are apparent to dicotyledonous (for example auxin inhibitor, broad leaf herbicides) or other monocotyledonous (e.g. grass herbicides) plant species from efficacy or testing with terrestrial non-target plants. Additional aquatic macrophyte species tests may be undertaken on a dicotyledonous species, such as Myriophyllum spicatum, Myriophyllum aquaticum or a monocotyledonous species, such as aquatic grass Glyceria maxima, as appropriate*." And "*Growth rate is the preferred endpoint to be used since it is more robust considering varying test conditions*."

In EFSA Journal 2016;14(3):4419 for Mesotrione, for aquatic macrophyte data only for *Lemna gibba* was available but the EU agreed endpoint was not based on growth rates and a data gap regarding testing of additional aquatic macrophyte species was identified.

The applicant provided Letter of Access to results of two additional studies on toxicity of mesotrione to *Lemna gibba* (Hengsberger & Wydra, 2015 report amendment 2; Kosak & Wydra, 2016) and *Myriophyllum spicatum* (Gonsior, 2017). These studies were assessed an accepted in Core dossier for Callisto 100 SC. The use of geometric mean value on their basis in the risk assessment was also accepted in Core dossier for Callisto 100 SC.

From the active substance endpoints provided in the table above, it can be concluded that Lemna are clearly more sensitive compared to the other groups of aquatic organisms. Therefore, a study on fish with the formulated product was not provided. There is a ratio of approximately 80000 between the thiencarbazone-methyl endpoint for fish and the endpoint for Lemna, and a ratio of approximately 5000 between the mesotrione endpoint for fish and the endpoint for Lemna. It is clear that a much lower toxicity to fish can be expected for the formulated product based on the data on the active ingredients compared to Lemna. Therefore, and in the interest of animal welfare, a study with fish is not considered required.

For thiencarbazone-methyl metabolites where no test data is available, the approach for metabolite risk assessment refers to part 10.2.4 decision scheme of the guidance document for aquatic organisms (EFSA, 2013):

- Step 1: none of the studies with the active substance is adequate for assessing the potential effect of the metabolites. Go to step 3.
- Step 3: No information is available to demonstrate that the toxophore is lost. Go to step 4.
- Step 4: Identify the species or taxonomic group determining the lowest tier 1 RACsw,ac for the active substance. Is the acute metabolite L(E)$C_{50}$> 10 times the a.s. L(E)$C_{50}$ (on a molar basis)? The active substance is not acutely toxic on fish and daphnia. Consequently it is proposed to use the macrophyte endpoint to compare the level of effects of the parent and the metabolites even though it is not considered as an acute endpoint. This approach shows that metabolites are more than 10x less toxic to Lemna than the parent. Therefore parent endpoints are used to demonstrate safe uses also for the metabolites, when test data are not available.

For the thiencarbazone-methyl metabolite triazolinone carboxamide, the endpoints of the parent divided by 10 are used.

**zRMS comments:**

The zRMS agrees that all metabolites can be assessed by using the parent endpoints where no studies are available.

Following the recommendations of the EFSA "Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology" (EFSA Supporting publication 2019: EN-1673), it is necessary to consider whether the formulation is more or less toxic than the parent compound. When the endpoint of the formulation expressed in terms of active substance is at least three times lower than the equivalent endpoint for the active substance, it should be considered more toxic.
The measured toxicity data available for the given endpoint is shown in the table below for the formulation and the active substance thiencarbazone-methyl.

| Test species | Measured toxicity of formulation | Measured toxicity of a.s. | Formulation end-point recalculated for a.s. | Ratio a.s. end-point/recalculated formulation endpoint |
|---|---|---|---|---|
| Algae | 87.1 mg/L | 1.02 mg/L | 5.55 mg/L | 0.18 |
| Lemna | 18.3 µg/L | 1.31 µg/L | 1.17 µg/L | 1.12 |
| Myriophyllum | 14.2 µg/L | 0.94 µg/L | 0.90 µg/L | 1.04 |

*Based on a thiencarbazone-methyl content of 6.37% w/w in the formulation studies.

The measured toxicity data available for the given endpoint is shown in the table below for the formulation and the active substance mesotrione.

| Test species | Measured toxicity of formulation | Measured toxicity of a.s. | Formulation end-point recalculated for a.s. | Ratio a.s. end-point/recalculated formulation endpoint |
|---|---|---|---|---|
| Algae | 87.1 mg/L | 13 mg/L | 28.09 mg/L | 0.46 |
| Lemna | 18.3 µg/L | 24.1 µg/L | 5.90 µg/L | 4.08 |
| Myriophyllum | 14.2 µg/L | 28.7 µg/L | 4.58 µg/L | 6.3 |

*Based on a mesotrione content of 32.25% w/w in the formulation studies.

The endpoint of the formulation expressed in terms of thiencarbazone-methyl is not more than three times lower for algae and Lemna.
The endpoint of the formulation expressed in terms of mesotrione is not more than three times lower for algae. For Lemna and Myriophyllum, the factor is 4.08 and 6.3 respectively. This finding is considered not very informative as the product is a mixture of two active substances and a safener. A comparison of back-calculated formulation endpoints with endpoints from active substance studies is therefore generally not meaningful and will inevitably lead to unrealistic and over-conservative results. Th actual toxicity of the product is properly assessed in this dossier with the combined toxicity assessment presented further below.
Consequently, the endpoints of the formulations expressed in terms of thiencarbazone-methyl or mesotrione are not used in the active substance risk assessment.

### 9.5.2 Risk assessment

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

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

**Table 9.5-3: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mesotrione for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB2112dH in maize – pH 5.1**

| **Group** | | **Fish acute** | **Fish prolonged** | **Inverteb. acute** | **Inverteb. prolonged** | **Algae** | **Plants** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Oncorhynchus mykiss*** | ***Daphnia magna*** | ***Daphnia magna*** | ***Pseudokirchn. subcapitata*** | ***Lemna*** | ***Lemna*** | **Macrophytes** |
| **Endpoint** | | $LC_{50}$ | NOEC | $EC_{50}$ | NOEC | $E_rC_{50}$ | $E_bC_{50}$ | $E_rC_{50}$ | Geomean $E_rC_{50}$ |
| **(µg/L)** | | 120000 | 12500 | 622000 | 180000 | 13000 | 7.7 | 24.1 | 26.3 |
| **AF** | | 100 | 10 | 100 | 10 | 10 | 10 | 10 | 10 |
| **RAC (µg/L)** | | 1200 | 1250 | 6220 | 18000 | 1300 | 0.77 | 2.41 | 2.63 |
| **FOCUS Scenario** | **PEC $_{gl-max}$ (µg/L)** | | | | | | | | |
| **Step 1** | | | | | | | | | |
| | 21.37 | 0.018 | 0.017 | 0.003 | 0.001 | 0.016 | **27.75** | **8.867** | **8.125** |
| **Step 2** | | | | | | | | | |
| N-Europe | 3.17 | 0.003 | 0.003 | 0.001 | 0.0002 | 0.002 | **4.117** | **1.315** | **1.205** |
| S-Europe | 5.98 | 0.005 | 0.005 | 0.001 | 0.0003 | 0.005 | **7.766** | **2.481** | **2.274** |
| **Step 3** | | | | | | | | | |
| *75 g/ha* | | | | | | | | | |
| D3/ditch | 0.3935 | - | - | - | - | - | 0.511 | 0.163 | 0.150 |
| D4/pond | 0.04175 | - | - | - | - | - | 0.054 | 0.017 | 0.016 |
| D4/stream | 0.3385 | - | - | - | - | - | 0.440 | 0.140 | 0.129 |
| D5/pond | 0.02331 | - | - | - | - | - | 0.030 | 0.010 | 0.009 |
| D5/stream | 0.3437 | - | - | - | - | - | 0.446 | 0.143 | 0.131 |

| Group | | Fish acute | Fish prolonged | Inverteb. acute | Inverteb. prolonged | Algae | Plants | | |
|---|---|---|---|---|---|---|---|---|---|
| D6/ditch | 0.3954 | - | - | - | - | - | 0.514 | 0.164 | 0.150 |
| R1/pond | 0.05680 | - | - | - | - | - | 0.074 | 0.024 | 0.022 |
| R1/stream | 1.201 | - | - | - | - | - | **1.560** | 0.498 | 0.457 |
| R2/stream | 0.8774 | - | - | - | - | - | **1.139** | 0.364 | 0.334 |
| R3/stream | 2.325 | - | - | - | - | - | **3.019** | 0.965 | 0.884 |
| R4/stream | 2.672 | - | - | - | - | - | **3.470** | **1.109** | **1.016** |
| *48.75 g/ha* | | | | | | | | | |
| D3/ditch | 0.2558 | - | - | - | - | - | 0.332 | 0.106 | 0.097 |
| D4/pond | 0.02708 | - | - | - | - | - | 0.035 | 0.011 | 0.010 |
| D4/stream | 0.2200 | - | - | - | - | - | 0.286 | 0.091 | 0.084 |
| D5/pond | 0.01504 | - | - | - | - | - | 0.020 | 0.006 | 0.006 |
| D5/stream | 0.2232 | - | - | - | - | - | 0.290 | 0.093 | 0.085 |
| D6/ditch | 0.2569 | - | - | - | - | - | 0.334 | 0.107 | 0.098 |
| R1/pond | 0.03697 | - | - | - | - | - | 0.048 | 0.015 | 0.014 |
| R1/stream | 0.7822 | - | - | - | - | - | **1.016** | 0.325 | 0.297 |
| R2/stream | 0.5598 | - | - | - | - | - | 0.727 | 0.232 | 0.213 |
| R3/stream | 1.494 | - | - | - | - | - | **1.940** | 0.620 | 0.568 |
| R4/stream | 1.725 | - | - | - | - | - | **2.240** | 0.716 | 0.656 |
| *75 g/ha – banded application* | | | | | | | | | |
| D3/ditch | 0.3935 | - | - | - | - | - | 0.511 | 0.163 | 0.150 |
| D4/pond | 0.02081 | - | - | - | - | - | 0.027 | 0.009 | 0.008 |
| D4/stream | 0.3377 | - | - | - | - | - | 0.439 | 0.140 | 0.128 |
| D5/pond | 0.01686 | - | - | - | - | - | 0.022 | 0.007 | 0.006 |
| D5/stream | 0.3395 | - | - | - | - | - | 0.441 | 0.141 | 0.129 |

| Group | | Fish acute | Fish prolonged | Inverteb. acute | Inverteb. prolonged | Algae | Plants | | |
|---|---|---|---|---|---|---|---|---|---|
| D6/ditch | 0.3943 | - | - | - | - | - | 0.512 | 0.164 | 0.150 |
| R1/pond | 0.03216 | - | - | - | - | - | 0.042 | 0.013 | 0.012 |
| R1/stream | 0.6013 | - | - | - | - | - | 0.781 | 0.250 | 0.229 |
| R2/stream | 0.4248 | - | - | - | - | - | 0.552 | 0.176 | 0.162 |
| R3/stream | 1.139 | - | - | - | - | - | **1.479** | 0.473 | 0.433 |
| R4/stream | 1.318 | - | - | - | - | - | **1.712** | 0.547 | 0.501 |
| *48.75 g/ha – banded application* | | | | | | | | | |
| D3/ditch | 0.2558 | - | - | - | - | - | 0.332 | 0.106 | 0.097 |
| D4/pond | 0.01351 | - | - | - | - | - | 0.018 | 0.006 | 0.005 |
| D4/stream | 0.2195 | - | - | - | - | - | 0.285 | 0.091 | 0.083 |
| D5/pond | 0.01093 | - | - | - | - | - | 0.014 | 0.005 | 0.004 |
| D5/stream | 0.2205 | - | - | - | - | - | 0.286 | 0.091 | 0.084 |
| D6/ditch | 0.2563 | - | - | - | - | - | 0.333 | 0.106 | 0.097 |
| R1/pond | 0.02091 | - | - | - | - | - | 0.027 | 0.009 | 0.008 |
| R1/stream | 0.3912 | - | - | - | - | - | 0.508 | 0.162 | 0.149 |
| R2/stream | 0.2708 | - | - | - | - | - | 0.352 | 0.112 | 0.103 |
| R3/stream | 0.7322 | - | - | - | - | - | 0.951 | 0.304 | 0.278 |
| R4/stream | 0.8512 | - | - | - | - | - | **1.105** | 0.353 | 0.324 |

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

**Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mesotrione for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB2112dH in maize – pH 6.5**

| Group | | Fish acute | Fish prolonged | Inverteb. acute | Inverteb. prolonged | Algae | Plants | | |
|---|---|---|---|---|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Oncorhynchus mykiss*** | ***Daphnia magna*** | ***Daphnia magna*** | ***Pseudokirchn. subcapitata*** | ***Lemna*** | ***Lemna*** | **Macrophytes** |
| **Endpoint** | | $LC_{50}$ | NOEC | $EC_{50}$ | NOEC | $E_rC_{50}$ | $E_bC_{50}$ | $E_rC_{50}$ | Geomean $E_rC_{50}$ |

| **Group** | | **Fish acute** | **Fish prolonged** | **Inverteb. acute** | **Inverteb. prolonged** | **Algae** | **Plants** | | |
|---|---|---|---|---|---|---|---|---|---|
| **(µg/L)** | | 120000 | 12500 | 622000 | 180000 | 13000 | 7.7 | 24.1 | 26.3 |
| **AF** | | 100 | 10 | 100 | 10 | 10 | 10 | 10 | 10 |
| **RAC (µg/L)** | | 1200 | 1250 | 6220 | 18000 | 1300 | 0.77 | 2.41 | 2.63 |
| **FOCUS Scenario** | **PEC $_{gl-max}$ (µg/L)** | | | | | | | | |
| **Step 1** | | | | | | | | | |
| | 24.06 | 0.020 | 0.019 | 0.004 | 0.001 | 0.019 | **31.25** | **9.983** | **9.148** |
| **Step 2** | | | | | | | | | |
| N-Europe | 3.28 | 0.003 | 0.003 | 0.0005 | 0.0002 | 0.003 | **4.26** | **1.361** | **1.247** |
| S-Europe | 6.17 | 0.005 | 0.005 | 0.001 | 0.0003 | 0.005 | **8.01** | **2.560** | **2.346** |
| **Step 3** | | | | | | | | | |
| *75 g/ha* | | | | | | | | | |
| D3/ditch | 0.3936 | - | - | - | - | - | 0.511 | 0.163 | 0.150 |
| D4/pond | 0.01618 | - | - | - | - | - | 0.021 | 0.007 | 0.006 |
| D4/stream | 0.3379 | - | - | - | - | - | 0.439 | 0.140 | 0.128 |
| D5/pond | 0.01692 | - | - | - | - | - | 0.022 | 0.007 | 0.006 |
| D5/stream | 0.3393 | - | - | - | - | - | 0.441 | 0.141 | 0.129 |
| D6/ditch | 0.3947 | - | - | - | - | - | 0.513 | 0.164 | 0.150 |
| R1/pond | 0.03674 | - | - | - | - | - | 0.048 | 0.015 | 0.014 |
| R1/stream | 0.8203 | - | - | - | - | - | **1.065** | 0.340 | 0.312 |
| R2/stream | 1.605 | - | - | - | - | - | **2.084** | 0.666 | 0.610 |
| R3/stream | 2.952 | - | - | - | - | - | **3.834** | **1.225** | **1.122** |
| R4/stream | 3.116 | - | - | - | - | - | **4.047** | **1.293** | **1.185** |

| Group | | Fish acute | Fish prolonged | Inverteb. acute | Inverteb. prolonged | Algae | Plants | | |
|---|---|---|---|---|---|---|---|---|---|
| *48.75 g/ha* | | | | | | | | | |
| D3/ditch | 0.2559 | - | - | - | - | - | 0.332 | 0.106 | 0.097 |
| D4/pond | 0.01051 | - | - | - | - | - | 0.014 | 0.004 | 0.004 |
| D4/stream | 0.2196 | - | - | - | - | - | 0.285 | 0.091 | 0.083 |
| D5/pond | 0.01099 | - | - | - | - | - | 0.014 | 0.005 | 0.004 |
| D5/stream | 0.2205 | - | - | - | - | - | 0.286 | 0.091 | 0.084 |
| D6/ditch | 0.2566 | - | - | - | - | - | 0.333 | 0.106 | 0.098 |
| R1/pond | 0.02423 | - | - | - | - | - | 0.031 | 0.010 | 0.009 |
| R1/stream | 0.5318 | - | - | - | - | - | 0.691 | 0.221 | 0.202 |
| R2/stream | 1.032 | - | - | - | - | - | **1.340** | 0.428 | 0.392 |
| R3/stream | 1.915 | - | - | - | - | - | **2.487** | 0.795 | 0.728 |
| R4/stream | 2.021 | - | - | - | - | - | **2.625** | 0.839 | 0.768 |
| *75 g/ha – banded application* | | | | | | | | | |
| D3/ditch | 0.3936 | - | - | - | - | - | 0.511 | 0.163 | 0.150 |
| D4/pond | 0.01603 | - | - | - | - | - | 0.021 | 0.007 | 0.006 |
| D4/stream | 0.3374 | - | - | - | - | - | 0.438 | 0.140 | 0.128 |
| D5/pond | 0.01640 | - | - | - | - | - | 0.021 | 0.007 | 0.006 |
| D5/stream | 0.3375 | - | - | - | - | - | 0.438 | 0.140 | 0.128 |
| D6/ditch | 0.3941 | - | - | - | - | - | 0.512 | 0.164 | 0.150 |
| R1/pond | 0.02259 | - | - | - | - | - | 0.029 | 0.009 | 0.009 |
| R1/stream | 0.4078 | - | - | - | - | - | 0.530 | 0.169 | 0.155 |
| R2/stream | 0.7863 | - | - | - | - | - | **1.021** | 0.326 | 0.299 |
| R3/stream | 1.468 | - | - | - | - | - | **1.906** | 0.609 | 0.558 |
| R4/stream | 1.551 | - | - | - | - | - | **2.014** | 0.644 | 0.590 |

| Group | | Fish acute | Fish prolonged | Inverteb. acute | Inverteb. prolonged | Algae | Plants | | |
|---|---|---|---|---|---|---|---|---|---|
| *48.75 g/ha – banded application* | | | | | | | | | |
| D3/ditch | 0.2558 | - | - | - | - | - | 0.332 | 0.106 | 0.097 |
| D4/pond | 0.01042 | - | - | - | - | - | 0.014 | 0.004 | 0.004 |
| D4/stream | 0.2193 | - | - | - | - | - | 0.285 | 0.091 | 0.083 |
| D5/pond | 0.01066 | - | - | - | - | - | 0.014 | 0.004 | 0.004 |
| D5/stream | 0.2194 | - | - | - | - | - | 0.285 | 0.091 | 0.083 |
| D6/ditch | 0.2562 | - | - | - | - | - | 0.333 | 0.106 | 0.097 |
| R1/pond | 0.01487 | - | - | - | - | - | 0.019 | 0.006 | 0.006 |
| R1/stream | 0.2644 | - | - | - | - | - | 0.343 | 0.110 | 0.101 |
| R2/stream | 0.5052 | - | - | - | - | - | 0.656 | 0.210 | 0.192 |
| R3/stream | 0.9528 | - | - | - | - | - | **1.237** | 0.395 | 0.362 |
| R4/stream | 1.006 | - | - | - | - | - | **1.306** | 0.417 | 0.383 |

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 9.5-5: 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 GLOB2112dH in maize – pH 7.9**

| Group | | Fish acute | Fish prolonged | Inverteb. acute | Inverteb. prolonged | Algae | Plants | | |
|---|---|---|---|---|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Oncorhynchus mykiss*** | ***Daphnia magna*** | ***Daphnia magna*** | ***Pseudokirchn. subcapitata*** | ***Lemna*** | ***Lemna*** | **Macrophytes** |
| **Endpoint** | | $LC_{50}$ | NOEC | $EC_{50}$ | NOEC | $E_rC_{50}$ | $EC_{50}$ | $E_rC_{50}$ | Geomean $E_rC_{50}$ |
| **(µg/L)** | | 120000 | 12500 | 622000 | 180000 | 13000 | 7.7 | 24.1 | 26.3 |
| **AF** | | 100 | 10 | 100 | 10 | 10 | 10 | 10 | 10 |
| **RAC (µg/L)** | | 1200 | 1250 | 6220 | 18000 | 1300 | 0.77 | 2.41 | 2.63 |
| **FOCUS Scenario** | **PEC $_{gl-max}$ (µg/L)** | | | | | | | | |

| Group | | Fish acute | Fish prolonged | Inverteb. acute | Inverteb. prolonged | Algae | Plants | | |
|---|---|---|---|---|---|---|---|---|---|
| **Step 1** | | | | | | | | | |
| | 25.12 | 0.021 | 0.020 | 0.004 | 0.0014 | 0.019 | **32.62** | **10.42** | **9.551** |
| **Step 2** | | | | | | | | | |
| N-Europe | 0.69 | 0.001 | 0.001 | 0.0001 | < 0.0001 | 0.001 | 0.896 | 0.286 | 0.262 |
| S-Europe | 0.69 | 0.001 | 0.001 | 0.0001 | < 0.0001 | 0.001 | 0.896 | 0.286 | 0.262 |
| **Step 3** | | | | | | | | | |
| *75 g/ha* | | | | | | | | | |
| D3/ditch | 0.3935 | - | - | - | - | - | 0.511 | 0.163 | 0.150 |
| D4/pond | 0.01589 | - | - | - | - | - | 0.021 | 0.007 | 0.006 |
| D4/stream | 0.3370 | - | - | - | - | - | 0.438 | 0.140 | 0.128 |
| D5/pond | 0.01589 | - | - | - | - | - | 0.021 | 0.007 | 0.006 |
| D5/stream | 0.3358 | - | - | - | - | - | 0.436 | 0.139 | 0.128 |
| D6/ditch | 0.3935 | - | - | - | - | - | 0.511 | 0.163 | 0.150 |
| R1/pond | 0.01588 | - | - | - | - | - | 0.021 | 0.007 | 0.006 |
| R1/stream | 0.2700 | - | - | - | - | - | 0.351 | 0.112 | 0.103 |
| R2/stream | 0.3648 | - | - | - | - | - | 0.474 | 0.151 | 0.139 |
| R3/stream | 0.3844 | - | - | - | - | - | 0.499 | 0.160 | 0.146 |
| R4/stream | 0.2719 | - | - | - | - | - | 0.353 | 0.113 | 0.103 |
| *48.75 g/ha* | | | | | | | | | |
| D3/ditch | 0.2558 | - | - | - | - | - | 0.332 | 0.106 | 0.097 |
| D4/pond | 0.01033 | - | - | - | - | - | 0.013 | 0.004 | 0.004 |
| D4/stream | 0.2190 | - | - | - | - | - | 0.284 | 0.091 | 0.083 |
| D5/pond | 0.01033 | - | - | - | - | - | 0.013 | 0.004 | 0.004 |
| D5/stream | 0.2183 | - | - | - | - | - | 0.284 | 0.091 | 0.083 |

| Group | | Fish acute | Fish prolonged | Inverteb. acute | Inverteb. prolonged | Algae | Plants | | |
|---|---|---|---|---|---|---|---|---|---|
| D6/ditch | 0.2558 | - | - | - | - | - | 0.332 | 0.106 | 0.097 |
| R1/pond | 0.01032 | - | - | - | - | - | 0.013 | 0.004 | 0.004 |
| R1/stream | 0.1755 | - | - | - | - | - | 0.228 | 0.073 | 0.067 |
| R2/stream | 0.2371 | - | - | - | - | - | 0.308 | 0.098 | 0.090 |
| R3/stream | 0.2488 | - | - | - | - | - | 0.323 | 0.103 | 0.095 |
| R4/stream | 0.1767 | - | - | - | - | - | 0.229 | 0.073 | 0.067 |

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

**Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for MNBA for each organism group based on FOCUS Steps 1, 2 calculations for the use of GLOB2112dH in maize (worst-case PECsw from all simulations)**

| Group | | Fish acute | Inverteb. acute | Algae | Plants |
|---|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Daphnia magna*** | ***Pseudokirchn. subcapi-tata*** | ***Lemna*** |
| **Endpoint (µg/L)** | | $LC_{50}$ > 120000 | NOEC 130000 | $E_rC_{50}$ 42000 | $EC_{50}$ > 97000 |
| **AF** | | 100 | 100 | 10 | 10 |
| **RAC (µg/L)** | | > 1200 | 1300 | 4200 | > 9700 |
| **FOCUS Scenario** | **PEC $_{gl-max}$ (µg/L)** | | | | |
| **Step 1** | | | | | |
| | 11.75 | < 0.010 | 0.009 | 0.003 | < 0.001 |
| **Step 2** | | | | | |
| N-Europe | 0.92 | < 0.001 | 0.001 | 0.0002 | < 0.0001 |
| S-Europe | 1.79 | < 0.001 | 0.001 | 0.000 | < 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 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AMBA for each organism group based on FOCUS Steps 1, 2 calculations for the use of GLOB2112dH in maize (worst-case PECsw from all simulations)**

| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** | **Plants** |
|---|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Daphnia magna*** | ***Pseudokirchn. subcapitata*** | ***Lemna*** |
| **Endpoint (µg/L)** | | $LC_{50}$ 150000 | $EC_{50}$ 160000 | $E_rC_{50}$ 14000 | $EC_{50}$ > 90000 |
| **AF** | | 100 | 100 | 10 | 10 |
| **RAC (µg/L)** | | 1500 | 1600 | 1400 | > 9000 |
| **FOCUS Scenario** | **PEC $_{gl-max}$ (µg/L)** | | | | |
| **Step 1** | | | | | |
| | 5.42 | 0.004 | 0.003 | 0.004 | < 0.001 |
| **Step 2** | | | | | |
| N-Europe | 0.73 | 0.0005 | 0.0005 | 0.0005 | < 0.0001 |
| S-Europe | 1.37 | 0.001 | 0.001 | 0.001 | < 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 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for SYN 546974 for each organism group based on FOCUS Steps 1, 2 calculations for the use of GLOB2112dH in maize (worst-case PECsw from all simulations)**

| **Group** | | **Plants** |
|---|---|---|
| **Test species** | | ***Lemna*** |
| **Endpoint (µg/L)** | | $EC_{50}$ > 95000 |
| **AF** | | 10 |

| **Group** | | **Plants** |
|---|---|---|
| **RAC (µg/L)** | | > 9500 |
| **FOCUS Scenario** | **PEC $_{gl-max}$ (µg/L)** | |
| **Step 1** | | |
| | 0.80 | < 0.0001 |
| **Step 2** | | |
| N-Europe | 0.20 | < 0.0001 |
| S-Europe | 0.20 | < 0.0001 |

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

**Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for thiencarbazone-methyl for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB2112dH in maize**

| **Group** | | **Fish acute** | **Fish pro-longed** | **Inverteb. acute** | **Inverteb. prolonged** | **Algae** | **Sed. dwell. prolonged** | **Crustacean acute** | **Crustacean prolonged** | **Aquatic plants** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Oncorhynchus mykiss*** | ***Daphnia magna*** | ***Daphnia magna*** | ***Pseudokirchn. subcapitata*** | ***Chironomus riparius*** | ***Americamysis bahia*** | ***Americamysis bahia*** | ~~***Lemna gibba***~~ ***Myriophyllum*** | ***Lemna gibba*** | **Macrophytes** |
| **Endpoint** | | $LC_{50}$ | NOEC | $EC_{50}$ | NOEC | $E_rC_{50}$ | $EC_{50}$ | $EC_{50}$ | NOEC | $E_rC_{50}$ | $E_rC_{50}$ | Geomean $E_rC_{50}$ / $EC_{50}$* |
| **(µg/L)** | | > 104000 | 4800 | > 98600 | 3540 | 1020 | > 100000 | > 94000 | 5900 | 0.94 | 1.31* | 1.87 / 1.35 |
| **AF** | | 100 | 10 | 100 | 10 | 10 | 100 | 100 | 10 | 10 | 10 | 10 |
| **RAC (µg/L)** | | > 1040 | 480 | > 986 | 354 | 102.0 | > 1000 | > 940 | 590 | 0.094 | 0.131 | 0.187 / 0.135 |
| **FOCUS Scenario** | **PEC $_{gl-max}$ (µg/L)** | | | | | | | | | | | |
| **Step 1** | | | | | | | | | | | | |

| Group | | Fish acute | Fish pro-longed | Inverteb. acute | Inverteb. prolonged | Algae | Sed. dwell. prolonged | Crustacean acute | Crustacean prolonged | Aquatic plants | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 4.64 | < 0.004 | 0.010 | < 0.005 | 0.013 | 0.045 | < 0.005 | < 0.005 | 0.008 | **49.36** | **35.42** | **24.81 / 34.37** |
| **Step 2** | | | | | | | | | | | | |
| N-Europe | 0.65 | < 0.0006 | 0.001 | < 0.0007 | 0.002 | 0.006 | < 0.0007 | < 0,0007 | 0.001 | **6.915** | **4.96** | **3.476 / 4.81** |
| S-Europe | 1.18 | < 0.001 | 0.002 | < 0.001 | 0.003 | 0.012 | < 0.001 | < 0.001 | 0.002 | **12.55** | **9.01** | **6.310 / 8.74** |
| **Step 3** | | | | | | | | | | | | |
| *15 g/ha* | | | | | | | | | | | | |
| D3/ditch | 0.07870 | - | - | - | - | - | - | | | 0.837 | 0.60 | 0.421 / 0.583 |
| D4/pond | 0.003202 | - | - | - | - | - | - | | | 0.034 | 0.024 | 0.017 / 0.024 |
| D4/stream | 0.06741 | - | - | - | - | - | - | | | 0.717 | 0.515 | 0.360 / 0.499 |
| D5/pond | 0.003417 | - | - | - | - | - | - | | | 0.036 | 0.026 | 0.018 / 0.025 |
| D5/stream | 0.06730 | - | - | - | - | - | - | | | 0.716 | 0.514 | 0.360 / 0.499 |
| D6/ditch | 0.07881 | - | - | - | - | - | - | | | 0.838 | 0.602 | 0.421 / 0.584 |
| R1/pond | 0.01057 | - | - | - | - | - | - | | | 0.112 | 0.081 | 0.057 / 0.078 |
| R1/stream | 0.1930 | - | - | - | - | - | - | | | **2.053** | **1.473** | **1.032 / 1.430** |
| R2/stream | 0.2037 | - | - | - | - | - | - | | | **2.167** | **1.555** | **1.089 / 1.509** |
| R3/stream | 0.4951 | - | - | - | - | - | - | | | **5.267** | **3.779** | **2.648 / 3.667** |
| R4/stream | 0.5472 | - | - | - | - | - | - | | | **5.821** | **4.177** | **2.926 / 4.053** |
| *9.75 g/ha* | | | | | | | | | | | | |
| D3/ditch | 0.05115 | - | - | - | - | - | - | | | 0.544 | 0.390 | 0.274 / 0.379 |
| D4/pond | 0.002080 | - | - | - | - | - | - | | | 0.022 | 0.016 | 0.011 / 0.015 |
| D4/stream | 0.04382 | - | - | - | - | - | - | | | 0.466 | 0.335 | 0.234 / 0.326 |
| D5/pond | 0.002210 | - | - | - | - | - | - | | | 0.024 | 0.015 | 0.012 / 0.016 |
| D5/stream | 0.04374 | - | - | - | - | - | - | | | 0.465 | 0.334 | 0.234 / 0.324 |

| Group | | Fish acute | Fish pro-longed | Inverteb. acute | Inverteb. prolonged | Algae | Sed. dwell. prolonged | Crustacean acute | Crustacean prolonged | Aquatic plants | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D6/ditch | 0.05122 | - | - | - | - | - | - | | | 0.545 | 0.391 | 0.274 / 0.379 |
| R1/pond | 0.006911 | - | - | - | - | - | - | | | 0.074 | 0.053 | 0.037 / 0.051 |
| R1/stream | 0.1265 | - | - | - | - | - | - | | | **1.346** | 0.966 | 0.676 / 0.973 |
| R2/stream | 0.1281 | - | - | - | - | - | - | | | **1.363** | 0.978 | 0.685 / 0.937 |
| R3/stream | 0.3160 | - | - | - | - | - | - | | | **3.362** | **2.412** | **1.690 / 2.341** |
| R4/stream | 0.3507 | - | - | - | - | - | - | | | **3.731** | **2.677** | **1.875 / 2.598** |
| *15 g/ha – banded application* | | | | | | | | | | | | |
| D3/ditch | 0.07870 | - | - | - | - | - | - | | | 0.837 | 0.601 | 0.421 / 0.583 |
| D4/pond | 0.003188 | - | - | - | - | - | - | | | 0.034 | 0.024 | 0.017 / 0.024 |
| D4/stream | 0.06740 | - | - | - | - | - | - | | | 0.717 | 0,515 | 0.360 / 0.499 |
| D5/pond | 0.003284 | - | - | - | - | - | - | | | 0.035 | 0.025 | 0.018 / 0.499 |
| D5/stream | 0.06722 | - | - | - | - | - | - | | | 0.715 | 0.513 | 0.359 / 0.498 |
| D6/ditch | 0.07875 | - | - | - | - | - | - | | | 0.838 | 0.601 | 0.421 / 0.583 |
| R1/pond | 0.006583 | - | - | - | - | - | - | | | 0.070 | 0.050 | 0.035 / 0.049 |
| R1/stream | 0.09855 | - | - | - | - | - | - | | | **1.048** | 0.752 | 0.527 / 0.730 |
| R2/stream | 0.09740 | - | - | - | - | - | - | | | **1.036** | 0.744 | 0.521 / 0.721 |
| R3/stream | 0.2423 | - | - | - | - | - | - | | | **2.578** | **1.850** | **1.296 / 1.795** |
| R4/stream | 0.2698 | - | - | - | - | - | - | | | **2.870** | **2.060** | **1.443 / 1.999** |
| *9.75 g/ha – banded application* | | | | | | | | | | | | |
| D3/ditch | 0.05115 | - | - | - | - | - | - | | | 0.544 | 0.390 | 0.274 / 0.379 |
| D4/pond | 0.002071 | - | - | - | - | - | - | | | 0.022 | 0.016 | 0.011 / 0.015 |
| D4/stream | 0.04381 | - | - | - | - | - | - | | | 0.466 | 0.334 | 0.234 / 0.325 |
| D5/pond | 0.002130 | - | - | - | - | - | - | | | 0.023 | 0.016 | 0.011 / 0.016 |

| Group | | Fish acute | Fish pro-longed | Inverteb. acute | Inverteb. prolonged | Algae | Sed. dwell. prolonged | Crustacean acute | Crustacean prolonged | Aquatic plants | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D5/stream | 0.04369 | - | - | - | - | - | - | | | 0.465 | 0.334 | 0.234 / 0.324 |
| D6/ditch | 0.05118 | - | - | - | - | - | - | | | 0.544 | 0.391 | 0.274 / 0.379 |
| R1/pond | 0.004319 | - | - | - | - | - | - | | | 0.046 | 0.033 | 0.023 / 0.032 |
| R1/stream | 0.06511 | - | - | - | - | - | - | | | 0.693 | 0.497 | 0.348 / 0.482 |
| R2/stream | 0.06186 | - | - | - | - | - | - | | | 0.658 | 0.472 | 0.331 / 0.458 |
| R3/stream | 0.1561 | - | - | - | - | - | - | | | **1.661** | **1.192** | 0.835 / **1.156** |
| R4/stream | 0.1747 | - | - | - | - | - | - | | | **1.859** | **1.334** | 0.934 / **1.294** |

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

**Table 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for BYH 18363-carboxylic acid for each organism group based on FOCUS Steps 1, 2 calculations for the use of GLOB2112dH in maize (worst-case PECsw from all simulations)**

| Group | | Fish acute | Fish prolonged | Inverteb. acute | Inverteb. pro-longed | Algae | Sed. dwell. prolonged | Crustacean acute | *Crustacean prolonged* | Aquatic plants |
|---|---|---|---|---|---|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Oncorhynchus mykiss*** | ***Daphnia magna*** | ***Daphnia magna*** | ***Pseudokirchn. subcapitata*** | ***Chironomus riparius*** | ***Americamysis bahia*** | ***Americamysis bahia*** | ***Lemna gibba*** |
| **Endpoint (µg/L)** | | $LC_{50}$ > 104000 | NOEC 4800 | $EC_{50}$ > 98600 | NOEC 3540 | $E_rC_{50}$ 1020 | $EC_{50}$ > 100000 | $EC_{50}$ > 94000 | NOEC 5900 | $E_rC_{50}$ 3540 |
| **AF** | | 100 | 10 | 100 | 10 | 10 | 100 | 100 | 10 | 10 |
| **RAC (µg/L)** | | > 1040 | 480 | > 986 | 354 | 102.0 | > 1000 | > 940 | 590 | 354 |
| **FOCUS Scenario** | **PEC $_{gl-max}$ (µg/L)** | | | | | | | | | |
| **Step 1** | | | | | | | | | | |
| | 4.35 | < 0.004 | 0.009 | < 0.004 | 0.012 | 0.043 | < 0.004 | < 0.005 | 0.007 | 0.012 |

| Group | | Fish acute | Fish prolonged | Inverteb. acute | Inverteb. pro-longed | Algae | Sed. dwell. prolonged | Crustacean acute | *Crustacean prolonged* | Aquatic plants |
|---|---|---|---|---|---|---|---|---|---|---|
| **Step 2** | | | | | | | | | | |
| N-Europe | 0.62 | < 0.0006 | 0.001 | < 0.0006 | 0.002 | 0.006 | < 0.0006 | < 0,0007 | 0.001 | 0.002 |
| S-Europe | 1.19 | < 0.001 | 0.002 | < 0.001 | 0.003 | 0.012 | < 0.001 | < 0.001 | 0.002 | 0.003 |

**Table 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for BYH 18363-sulfonamide for each organism group based on FOCUS Steps 1, 2 calculations for the use of GLOB2112dH in maize (worst-case PECsw from all simulations)**

| Group | | Fish acute | Fish prolonged | Inverteb. acute | Inverteb. pro-longed | Algae | Sed. dwell. prolonged | Crustacean acute | *Crustacean prolonged* | Aquatic plants |
|---|---|---|---|---|---|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Oncorhynchus mykiss*** | ***Daphnia magna*** | ***Daphnia magna*** | ***Pseudokirchn. subcapitata*** | ***Chironomus riparius*** | ***Americamysis bahia*** | ***Americamysis bahia*** | ***Lemna gibba*** |
| **Endpoint (µg/L)** | | $LC_{50}$ 98300 | NOEC 4800 | $EC_{50}$ > 100000 | NOEC 3540 | $E_rC_{50}$ 1610 | $EC_{50}$ > 100000 | $EC_{50}$ > 94000 | NOEC 5900 | $E_rC_{50}$ 90500 |
| **AF** | | 100 | 10 | 100 | 10 | 10 | 100 | 100 | 100 | 10 |
| **RAC (µg/L)** | | 983 | 480 | > 1000 | 354 | 161 | > 1000 | > 940 | 590 | 9050 |
| **FOCUS Scenario** | **PEC $_{gl\text{-}max}$ (µg/L)** | | | | | | | | | |
| **Step 1** | | | | | | | | | | |
| | 0.61 | 0.001 | 0.001 | < 0.001 | 0.002 | 0.004 | < 0.001 | > 0.0006 | 0.001 | 0.0001 |
| **Step 2** | | | | | | | | | | |
| N-Europe | 0.07 | 0.0001 | 0.0001 | < 0.0001 | 0.0002 | 0.0004 | < 0.0001 | < 0.00007 | 0.0001 | < 0.0001 |
| S-Europe | 0.15 | 0.0002 | 0.0003 | < 0.0002 | 0.0004 | 0.001 | < 0.0002 | < 0.0002 | 0.0003 | < 0.0001 |

**Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for BYH 18363-sulfonamide carboxylic acid for each organism group based on FOCUS Steps 1, 2 calculations for the use of GLOB2112dH in maize (worst-case PECsw from all simulations)**

| **Group** | | **Fish acute** | **Fish prolonged** | **Inverteb. acute** | **Inverteb. pro-longed** | **Algae** | **Sed. dwell. prolonged** | **Crustacean acute** | **Crustacean prolonged** | **Aquatic plants** |
|---|---|---|---|---|---|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Oncorhynchus mykiss*** | ***Daphnia magna*** | ***Daphnia magna*** | ***Pseudokirchn. subcapitata*** | ***Chironomus riparius*** | ***Americamysis bahia*** | ***Americamysis bahia*** | ***Lemna gibba*** |
| **Endpoint** | | $LC_{50}$ | NOEC | $EC_{50}$ | NOEC | $E_rC_{50}$ | $EC_{50}$ | $EC_{50}$ | NOEC | $E_rC_{50}$ |
| **(µg/L)** | | > 104000 | 4800 | > 98600 | 3540 | 1020 | > 100000 | > 94000 | 5900 | > 100000 |
| **AF** | | 100 | 10 | 100 | 10 | 10 | 100 | 100 | 10 | 10 |
| **RAC (µg/L)** | | > 1040 | 480 | > 986 | 354 | 102.0 | > 1000 | > 940 | 590 | > 10000 |
| **FOCUS Scenario** | **PEC gl-max (µg/L)** | | | | | | | | | |
| **Step 1** | | | | | | | | | | |
| | 2.53 | < 0.002 | 0.005 | < 0.003 | 0.007 | 0.025 | < 0.003 | < 0.003 | 0.004 | < 0.0003 |
| **Step 2** | | | | | | | | | | |
| N-Europe | 0.34 | < 0.0003 | 0.0007 | < 0.0003 | 0.0010 | 0.003 | < 0.0003 | < 0.0004 | 0.0006 | < 0.0001 |
| S-Europe | 0.62 | < 0.0006 | 0.001 | < 0.0006 | 0.002 | 0.006 | < 0.0006 | < | 0.0011 | < 0.0001 |

**Table 9.5-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for BYH 18363-MMT for each organism group based on FOCUS Steps 1, 2 calculations for the use of GLOB2112dH in maize (worst-case PECsw from all simulations)**

| **Group** | | **Fish acute** | **Fish prolonged** | **Inverteb. acute** | **Inverteb. pro-longed** | **Algae** | **Sed. dwell. prolonged** | **Crustacean acute** | **Crustacean prolonged** | **Aquatic plants** |
|---|---|---|---|---|---|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Oncorhynchus mykiss*** | ***Daphnia magna*** | ***Daphnia magna*** | ***Pseudokirchn. subcapitata*** | ***Chironomus riparius*** | ***Americamysis bahia*** | ***Americamysis bahia*** | ***Lemna gibba*** |
| **Endpoint** | | $LC_{50}$ | NOEC | $EC_{50}$ | NOEC | $E_rC_{50}$ | $EC_{50}$ | $EC_{50}$ | NOEC | $E_rC_{50}$ |
| **(µg/L)** | | > 104000 | 4800 | > 98600 | 3540 | 1020 | > 100000 | > 94000 | 5900 | > 95700 |
| **AF** | | 100 | 10 | 100 | 10 | 10 | 100 | | | 10 |

| Group | | Fish acute | Fish prolonged | Inverteb. acute | Inverteb. pro-longed | Algae | Sed. dwell. prolonged | Crustacean acute | Crustacean prolonged | Aquatic plants |
|---|---|---|---|---|---|---|---|---|---|---|
| **RAC (µg/L)** | | > 1040 | 480 | > 986 | 354 | 102.0 | > 1000 | >940 | 590 | > 9570 |
| **FOCUS Scenario** | **PEC gl-max (µg/L)** | | | | | | | | | |
| **Step 1** | | | | | | | | | | |
| | 0.85 | < 0.001 | 0.002 | < 0.001 | 0.002 | 0.008 | < 0.001 | < 0.0009 | 0.0002 | < 0.0001 |
| **Step 2** | | | | | | | | | | |
| N-Europe | 0.12 | < 0.0001 | 0.0003 | < 0.0001 | 0.0003 | 0.001 | < 0.0001 | < 0.0001 | 0.0002 | < 0.0001 |
| S-Europe | 0.23 | < 0.0002 | 0.0005 | < 0.0002 | 0.001 | 0.002 | < 0.0002 | < 0.0002 | 0.004 | < 0.0001 |

**Table 9.5-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for BYH 18363-dicarboxysulfonamide for each organism group based on FOCUS Steps 1, 2 calculations for the use of GLOB2112dH in maize (worst-case PECsw from all simulations)**

| Group | | Fish acute | Fish prolonged | Inverteb. acute | Inverteb. pro-longed | Algae | Sed. dwell. prolonged | Crustacean acute | Crustacean prolonged | Aquatic plants |
|---|---|---|---|---|---|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Oncorhynchus mykiss*** | ***Daphnia magna*** | ***Daphnia magna*** | ***Pseudokirchn. subcapitata*** | ***Chironomus riparius*** | ***Americamysis bahia*** | ***Americamysis bahia*** | ***Lemna gibba*** |
| **Endpoint (µg/L)** | | $LC_{50}$ > 104000 | NOEC 4800 | $EC_{50}$ > 98600 | NOEC 3540 | $E_rC_{50}$ 1020 | $EC_{50}$ > 100000 | $EC_{50}$ > 94000 | NOEC 5900 | $E_rC_{50}$ > 104000 |
| **AF** | | 100 | 10 | 100 | 10 | 10 | 100 | 100 | 10 | 10 |
| **RAC (µg/L)** | | > 1040 | 480 | > 986 | 354 | 102.0 | > 1000 | > 940 | 590 | > 10400 |
| **FOCUS Scenario** | **PEC gl-max (µg/L)** | | | | | | | | | |
| **Step 1** | | | | | | | | | | |
| | 0.79 | < 0.001 | 0.002 | < 0.001 | 0.002 | 0.008 | < 0.001 | < 0.001 | 0.001 | < 0.0001 |

| Group | | Fish acute | Fish prolonged | Inverteb. acute | Inverteb. pro-longed | Algae | Sed. dwell. prolonged | Crustacean acute | Crustacean prolonged | Aquatic plants |
|---|---|---|---|---|---|---|---|---|---|---|
| **Step 2** | | | | | | | | | | |
| N-Europe | 0.11 | < 0.0001 | 0.0002 | < 0.0001 | 0.0003 | 0.001 | < 0.0001 | < 0.0001 | 0.0002 | < 0.0001 |
| S-Europe | 0.20 | < 0.0002 | 0.0004 | < 0.0002 | 0.001 | 0.002 | < 0.0002 | < 0.0002 | 0.0003 | < 0.0001 |

**Table 9.5-12: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for BYH 18363-triazolinone carboxamide for each organism group based on FOCUS Steps 1, 2 calculations for the use of GLOB2112dH in maize (worst-case PECsw from all simulations)**

| Group | | Fish acute | Fish prolonged | Inverteb. acute | Inverteb. pro-longed | Algae | Sed. dwell. prolonged | Crustacean acute | Crustacean prolonged | Aquatic plants |
|---|---|---|---|---|---|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Oncorhynchus mykiss*** | ***Daphnia magna*** | ***Daphnia magna*** | ***Pseudokirchn. subcapitata*** | ***Chironomus riparius*** | ***Americamysis bahia*** | ***Americamysis bahia*** | ***Lemna gibba*** |
| **Endpoint (µg/L)** | | $LC_{50}$ > 10400 | NOEC 480 | $EC_{50}$ > 9860 | NOEC 354 | $E_rC_{50}$ 102 | $EC_{50}$ > 10000 | $EC_{50}$ > 94000 | NOEC 5900 | $E_rC_{50}$ > 10400 |
| **AF** | | 100 | 10 | 100 | 10 | 10 | 100 | 100 | 10 | 10 |
| **RAC (µg/L)** | | > 104 | 48 | > 98.6 | 35.4 | 10.2 | > 100 | > 940 | 590 | > 1040 |
| **FOCUS Scenario** | **PEC gl-max (µg/L)** | | | | | | | | | |
| **Step 1** | | | | | | | | | | |
| | 0.17 | < 0.002 | 0.004 | < 0.002 | 0.005 | 0.017 | < 0.002 | < 0.0002 | 0.0003 | 0.0002 |
| **Step 2** | | | | | | | | | | |
| N-Europe | 0.02 | < 0.0002 | 0.0004 | < 0.0002 | 0.001 | 0.002 | < 0.0002 | 0.00002 | 0.00003 | < 0.0001 |
| S-Europe | 0.04 | < 0.0004 | 0.001 | < 0.0004 | 0.001 | 0.004 | < 0.0004 | < 0.00004 | 0.00007 | < 0.0001 |

For the intended uses in maize, calculated PEC/RAC ratios of mesotrione did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for aquatic plants as characterised by an $EC_{50}$ for *Lemna gibba* of 2.41 or 2.63 µg/L in connection with an assessment factor of 10) in several FOCUS Steps 1-3 scenarios. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 $PEC_{SW}$ considering reduced exposure of surface water bodies.

**Table 9.5-17: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for mesotrione based on FOCUS Step 4 calculations and toxicity data for aquatic plants with mitigation of spray drift and run-off for the use of GLOB2112dH in maize – 75 g/ha**

| **Intended use**<br>**Active substance**<br>**Application rate (g/ha)** | | Maize<br>Mesotrione<br>1 × 75 | | |
|---|---|---|---|---|
| **Nozzle reduction** | **No-spray buffer (m)** | 10 | 10 vfsmod | 20 |
| | **Vegetated filter strip (m)** | 10 | 10 | 20 |
| *pH 5.1* | | | | |
| None | R1 stream | 0.5437 | 0.05973 | 0.2843 |
| None | R2 stream | 0.3872 | - | - |
| None | R3 stream | 1.049 | 0.08547 | 0.5488 |
| None | R4 stream | 1.214 | | |
| **RAC (µg/L)**<br>**0.77** | | **PEC/RAC ratio** | | |
| None | R1 stream | 0.706 | | |
| None | R2 stream | 0.497 | | |
| None | R3 stream | 1.362 | 0.111 | 0.713 |
| None | R4 stream | 1.577 | 0.079 | 0.826 |
| **RAC (µg/L)**<br>2.41 | | **PEC/RAC ratio** | | |
| None | R4 stream | 0.504 | | |
| **RAC (µg/L)**<br>2.63 | | **PEC/RAC ratio** | | |
| None | R4 stream | 0.462 | | |
| *pH 6.5* | | | | |
| None | R1 stream | 0.3366 | | |
| None | R2 stream | 0.7083 | | |
| None | R3 stream | 1.333 | 0.08548 | 0.6973 |
| None | R4 stream | 1.416 | 0.06071 | 0.7422 |
| **RAC (µg/L)**<br>0.77 | | **PEC/RAC ratio** | | |
| None | R1 stream | 0.437 | | |
| None | R2 stream | 0.920 | | |

| | | | | |
|---|---|---|---|---|
| None | R3 stream | 1.731 | 0.111 | 0.906 |
| None | R4 stream | 1.839 | 0.079 | 0.964 |
| **RAC (µg/L)**<br>2.41 | | **PEC/RAC ratio** | | |
| None | R3 stream | 0.553 | | |
| None | R4 stream | 0.588 | | |
| **RAC (µg/L)**<br>2.63 | | **PEC/RAC ratio** | | |
| None | R3 stream | 0.507 | | |
| None | R4 stream | 0.538 | | |

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

For the intended uses in maize, calculated PEC/RAC ratios of thiencarbazone-methyl did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for aquatic plants as characterised by a geomean $EC_{50}$ of 1.87 µg/L in connection with an assessment factor of 10) in several FOCUS Steps 1-3 scenarios. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 $PEC_{SW}$ considering reduced exposure of surface water bodies.

**Table 9.5-13: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for thiencarbazone-methyl based on FOCUS Step 4 calculations and toxicity data for aquatic plants with mitigation of spray drift and run-off for the use of GLOB2112dH in maize – 15 g/ha**

| **Intended use**<br>**Active substance**<br>**Application rate (g/ha)** | | Maize<br>Thiencarbazone-methyl<br>1 × 15 | | |
|---|---|---|---|---|
| **Nozzle reduction** | **No-spray buffer (m)** | 10 | 10 vfsmod | 20 |
| | **Vegetated filter strip (m)** | 10 | 10 vfsmod | 20 |
| None | R1 stream | 0.08737 | - | - |
| None | R2 stream | 0.08990 | - | - |
| None | R3 stream | 0.2237 | 0.01709 | 0.1170 |
| None | R4 stream | 0.2487 | 0.01214 | 0.1304 |
| **RAC (µg/L)**<br>0.187 / 0.135* | | **PEC/RAC ratio** | | |
| None | R1 stream | 0.467 / 0.647 | - | - |
| None | R2 stream | 0.481 / 0.666 | - | - |
| None | R3 stream | **1.196 / 1.657** | 0.091 / 0.127 | 0.626 / 0.867 |
| None | R4 stream | **1.330 / 1.842** | 0.065 / 0.090 | 0.697 / 0.966 |

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

**Table 9.5-14: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for thiencarbazone-methyl based on FOCUS Step 4 calculations and toxicity data for aquatic plants with mitigation of spray drift and run-off for the use of GLOB2112dH in maize – 9.75 g/ha**

| **Intended use**<br>**Active substance**<br>**Application rate (g/ha)** | | Maize<br>Thiencarbazone-methyl<br>1 × 9.75 | | |
|---|---|---|---|---|
| **Nozzle reduction** | **No-spray buffer (m)** | 10 | 10 vfsmod | 20 |
| | **Vegetated filter strip (m)** | 10 | 10 vfsmod | 20 |
| None | R3 stream | 0.1428 | 0.01105 | 0.07466 |
| None | R4 stream | 0.1595 | 0.07849 | 0.08354 |
| **RAC (µg/L)**<br>0.187 / 0.135* | | **PEC/RAC ratio** | | |
| None | R3 stream | 0.764 /1,058 | 0.082 | 0.553 |
| None | R4 stream | 0.853 / 1.181 | 0.581 | 0.619 |

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

**Table 9.5-15: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for thiencarbazone-methyl based on FOCUS Step 4 calculations and toxicity data for aquatic plants with mitigation of spray drift and run-off for the use of GLOB2112dH in maize – 15 g/ha – band application**

| **Intended use**<br>**Active substance**<br>**Application rate (g/ha)** | | Maize<br>Thiencarbazone-methyl<br>1 × 15 |
|---|---|---|
| **Nozzle reduction** | **No-spray buffer (m)** | 10 |
| | **Vegetated filter strip (m)** | 10 |
| None | R3 stream | 0.1095 |
| None | R4 stream | 0.1227 |
| **RAC (µg/L)**<br>0.187 / 0.135* | | **PEC/RAC ratio** |
| None | R3 stream | 0.586 / 0.811 |
| None | R4 stream | 0.656 / 0.909 |

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

**Table 9.5-16: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for thiencarbazone-methyl based on FOCUS Step 4 calculations and toxicity data for aquatic plants with mitigation of spray drift and run-off for the use of GLOB2112dH in maize – 9.75 g/ha – band application**

| | | |
|---|---|---|
| **Intended use** | | Maize |
| **Active substance** | | Thiencarbazone-methyl |
| **Application rate (g/ha)** | | 1 × 9.75 |
| **Nozzle reduction** | **No-spray buffer (m)** | 10 |
| | **Vegetated filter strip (m)** | 10 |
| None | R3 stream | 0.07053 |
| None | R4 stream | 0.07941 |
| **RAC (µg/L)**<br>0.187 / 0.135* | | **PEC/RAC ratio** |
| None | R3 stream | 0.377 / 0.522 |
| None | R4 stream | 0.425 / 0.582 |

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

**Tier 2C: higher tier assessment based on refined exposure testing combined with exposure pattern analysis for thiencarbazone-methyl**

The Tier 2C refined exposure approach is based on the concentration-time profiles of those FOCUS Step 3 PECsw simulations used for assessment at Tier 1 before, and the results of refined exposure type laboratory tests studying the effects of a pulsed exposure on the most sensitive group of organisms for thiencarbazone-methyl which is macrophytes.

For the assessment, an exposure pattern is characterized by four properties which are:

- the PECmax
- the number of peaks above the Tier 1 RAC
- the duration of these peak events
- the interval between these peak events

A peak event is identified as such when a concentration in the exposure profile exceeds the relevant Tier 1 RAC value which in the case of thiencarbazone-methyl is 0.187 µg a.s./L (geomean $E_rC_{50}$ = 1.87 µg a.s./L for macrophytes, divided by standard assessment factor 10).

The exposure profiles for the entire FOCUS year of simulation are plotted graphically from the model output files and are amended with a numeric characterisation for event identification according to the above descriptors, extracted by the EPAT Exposure Profile Analysis Tool.
These characterised exposure patterns are then assessed versus the findings from refined exposure type tests, which studied the effects of one single or two sequential pulse exposure events. Such tests were performed with Lemna and Myriophyllum.

Tier 2C assessment for Lemna

The results of the pulse exposure studies performed with thiencarbazone-methyl and Lemna are suitable to address two different peak exposure situations as predicted by FOCUS:

- A single peak exceeding the Tier 1 geomean RAC: can be addressed with study Minati R., 2024, which delivered a peak $E_rC_{50}$ of 27.5 µg/L, resulting in a peak RAC of 2.75 µg/L.
- Two peak events with an interval of ≥ 7 days: can be addressed with study Minati R., 2024, which delivered a peak $E_rC_{50}$ of 12.2 µg/L, resulting in a peak RAC of 1.22 µg/L.

**Table 9.5-18: Peak event summary for FOCUS scenarios exceeding the Tier 1 RAC at Step 3**

| Application | Scenario | PECmax (µg/L) | Number of events above Tier 1 RAC | Duration of event above Tier 1 RAC (d) | Interval between events above Tier 1 RAC (d) | Relevant peak RAC (µg/L) | RQ = PECmax/peak RAC |
|---|---|---|---|---|---|---|---|
| STEP 3 | | | | | | | |
| 15 g/ha | R1 stream | 0.1930 | 1 | 0.375 | - | 2.75 | 0.070 |
| | R2 stream | 0.2037 | 1 | 0.542 | - | 2.75 | 0.074 |
| | R3 stream | 0.4951 | 1 | 0.625 | - | 2.75 | 0.180 |
| | R4 stream | 0.5472 | 1 | 0.833 | - | 2.75 | 0.199 |
| 9.75 g/ha | R3 stream | 0.3160 | 1 | 0.542 | - | 2.75 | 0.115 |
| | R4 stream | 0.3507 | 1 | 0.791 | - | 2.75 | 0.128 |
| 15 g/ha, banded | R3 stream | 0.2423 | 1 | 0.459 | - | 2.75 | 0.088 |
| | R4 stream | 0.2698 | 1 | 0.750 | - | 2.75 | 0.098 |

Tier 2C assessment for Myriophyllum

The results of the pulse exposure studies performed with thiencarbazone-methyl and Myriophyllum are suitable to address two different peak exposure situations as predicted by FOCUS:

- A single peak exceeding the Tier 1 geomean RAC: can be addressed with study Bebon R., 2024, which delivered a peak $E_rC_{50}$ of 15.4 µg/L, resulting in a peak RAC of 1.54 µg/L.
- Two peak events with an interval of ≥ 7 days: can be addressed with study Bebon R., 2024, which delivered a peak $E_rC_{50}$ of 6.3 µg/L, resulting in a peak RAC of 0.63 µg/L.

**Table 9.5-19: Peak event summary for FOCUS scenarios exceeding the Tier 1 RAC at Step 3**

| Application | Scenario | PECmax (µg/L) | Number of events above Tier 1 RAC | Duration of event above Tier 1 RAC (d) | Interval between events above Tier 1 RAC (d) | Relevant peak RAC (µg/L) | RQ = PECmax/peak RAC |
|---|---|---|---|---|---|---|---|
| STEP 3 | | | | | | | |
| 15 g/ha | R1 stream | 0.1930 | 1 | 0.375 | - | 1.54 | 0.125 |
| | R2 stream | 0.2037 | 1 | 0.542 | - | 1.54 | 0.132 |
| | R3 stream | 0.4951 | 1 | 0.625 | - | 1.54 | 0.321 |
| | R4 stream | 0.5472 | 1 | 0.833 | - | 1.54 | 0.355 |
| 9.75 g/ha | R3 stream | 0.3160 | 1 | 0.542 | - | 1.54 | 0.205 |
| | R4 stream | 0.3507 | 1 | 0.791 | - | 1.54 | 0.228 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 15 g/ha, banded | R3 stream | 0.2423 | 1 | 0.459 | - | 1.54 | 0.157 |
| | R4 stream | 0.2698 | 1 | 0.750 | - | 1.54 | 0.175 |

**Combined risk assessment for the formulation GLOB2112dH**

The evaluation of the mixture toxicity risks for aquatic (and sediment-dwelling) organisms was performed in accordance with the "Guidance 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) and in EFSA Journal 2013;11(7):3290 (Aquatic Guidance Document, abbreviated as EFSA, 2013). The whole mixture assessment is based on the model of concentration addition (CA) and particularly the decision scheme as provided by EFSA (2013, section 10.3.11) was applied. The calculations were performed with the tool "AGD_AquaMix v1.22"

A discussion of all details of every decision is not intended in this section of this registration report. However, at least data for some important steps are documented in this section, beginning with the check for (and potentially assessment of) synergistic or antagonistic effects (Step 2 and Steps 7, 9 and 10) as well as the $ETR_{mix}$ or $RQ_{mix}$ calculation results for the assessed FOCUS Scenarios (Steps 4 and 8a/b).

**Screening Assessment**

Fish

High dose rate

All ETRi ≤ ETR trigger/n; acceptable risk can be concluded on screening level for the highest dose rate and for all mesotrione pH scenarios. Therefore, the combined risk to fish is not considered further for any of the other uses.

| Fish | ETR | | | | Any a.s. above | ETRtrigger/n (if needed) | All ETRi ≤ ETRtrigger/n? |
|---|---|---|---|---|---|---|---|
| | TCM | MST pH 5.1 | | | | | |
| Step 1 | 0.000 | 0.000 | | | no | 0.005 | yes |
| Step 2 | | | | | | | |
| N-Europe | 0.000 | 0.000 | | | no | 0.005 | yes |
| S-Europe | 0.000 | 0.000 | | | no | 0.005 | yes |
| Step 3 | | | | | | | |
| D3 Ditch | 0.000 | 0.000 | | | no | 0.005 | yes |
| D4 Pond | 0.000 | 0.000 | | | no | 0.005 | yes |
| D4 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |
| D5 Pond | 0.000 | 0.000 | | | no | 0.005 | yes |
| D5 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |
| D6 Ditch | 0.000 | 0.000 | | | no | 0.005 | yes |
| R1 Pond | 0.000 | 0.000 | | | no | 0.005 | yes |
| R1 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |
| R2 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |
| R3 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |
| R4 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |

| Fish | ETR | | | | Any a.s. above | ETRtrigger/n (if needed) | All ETRi ≤ ETRtrigger/n? |
|---|---|---|---|---|---|---|---|
| | TCM | MST pH 6.5 | | | | | |
| Step 1 | 0.000 | 0.000 | | | no | 0.005 | yes |
| Step 2 | | | | | | | |
| N-Europe | 0.000 | 0.000 | | | no | 0.005 | yes |
| S-Europe | 0.000 | 0.000 | | | no | 0.005 | yes |
| Step 3 | | | | | | | |
| D3 Ditch | 0.000 | 0.000 | | | no | 0.005 | yes |
| D4 Pond | 0.000 | 0.000 | | | no | 0.005 | yes |
| D4 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |
| D5 Pond | 0.000 | 0.000 | | | no | 0.005 | yes |
| D5 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |
| D6 Ditch | 0.000 | 0.000 | | | no | 0.005 | yes |
| R1 Pond | 0.000 | 0.000 | | | no | 0.005 | yes |
| R1 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |
| R2 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |
| R3 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |
| R4 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |

| Fish | ETR | | | | Any a.s. above | ETRtrigger/n (if needed) | All ETRi ≤ ETRtrigger/n? |
|---|---|---|---|---|---|---|---|
| | TCM | MST pH 7.9 | | | | | |
| Step 1 | 0.000 | 0.000 | | | no | 0.005 | yes |
| Step 2 | | | | | | | |
| N-Europe | 0.000 | 0.000 | | | no | 0.005 | yes |
| S-Europe | 0.000 | 0.000 | | | no | 0.005 | yes |
| Step 3 | | | | | | | |
| D3 Ditch | 0.000 | 0.000 | | | no | 0.005 | yes |
| D4 Pond | 0.000 | 0.000 | | | no | 0.005 | yes |
| D4 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |
| D5 Pond | 0.000 | 0.000 | | | no | 0.005 | yes |
| D5 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |
| D6 Ditch | 0.000 | 0.000 | | | no | 0.005 | yes |
| R1 Pond | 0.000 | 0.000 | | | no | 0.005 | yes |
| R1 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |
| R2 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |
| R3 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |
| R4 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |

Daphnia

High dose rate

All ETRi ≤ ETR trigger/n; acceptable risk can be concluded on screening level for the highest dose rate and for all mesotrione pH scenarios. Therefore, the combined risk to Daphnia is not considered further for any of the other uses.

| Invertebrates | ETR | | | | Any a.s. above | ETRtrigger/n (if needed) | All ETRi ≤ ETRtrigger/n |
|---|---|---|---|---|---|---|---|
| | TCM | MST pH 5.1 | | | | | |
| Step 1 | 0.000 | 0.000 | | | no | 0.005 | yes |
| Step 2 | | | | | | | |
| N-Europe | 0.000 | 0.000 | | | no | 0.005 | yes |
| S-Europe | 0.000 | 0.000 | | | no | 0.005 | yes |
| Step 3 | | | | | | | |
| D3 Ditch | 0.000 | 0.000 | | | no | 0.005 | yes |
| D4 Pond | 0.000 | 0.000 | | | no | 0.005 | yes |
| D4 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |
| D5 Pond | 0.000 | 0.000 | | | no | 0.005 | yes |
| D5 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |
| D6 Ditch | 0.000 | 0.000 | | | no | 0.005 | yes |
| R1 Pond | 0.000 | 0.000 | | | no | 0.005 | yes |
| R1 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |
| R2 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |
| R3 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |
| R4 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |

| Invertebrates | ETR | | | | Any a.s. above | ETRtrigger/n (if needed) | All ETRi ≤ ETRtrigger/n |
|---|---|---|---|---|---|---|---|
| | TCM | MST pH 6.5 | | | | | |
| Step 1 | 0.000 | 0.000 | | | no | 0.005 | yes |
| Step 2 | | | | | | | |
| N-Europe | 0.000 | 0.000 | | | no | 0.005 | yes |
| S-Europe | 0.000 | 0.000 | | | no | 0.005 | yes |
| Step 3 | | | | | | | |
| D3 Ditch | 0.000 | 0.000 | | | no | 0.005 | yes |
| D4 Pond | 0.000 | 0.000 | | | no | 0.005 | yes |
| D4 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |
| D5 Pond | 0.000 | 0.000 | | | no | 0.005 | yes |
| D5 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |
| D6 Ditch | 0.000 | 0.000 | | | no | 0.005 | yes |
| R1 Pond | 0.000 | 0.000 | | | no | 0.005 | yes |
| R1 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |
| R2 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |
| R3 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |
| R4 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |

| Invertebrates | ETR | | | | Any a.s. above | ETRtrigger/n (if needed) | All ETRi ≤ ETRtrigger/n |
|---|---|---|---|---|---|---|---|
| | TCM | MST pH 7.9 | | | | | |
| Step 1 | 0.000 | 0.000 | | | no | 0.005 | yes |
| Step 2 | | | | | | | |
| N-Europe | 0.000 | 0.000 | | | no | 0.005 | yes |
| S-Europe | 0.000 | 0.000 | | | no | 0.005 | yes |
| Step 3 | | | | | | | |
| D3 Ditch | 0.000 | 0.000 | | | no | 0.005 | yes |
| D4 Pond | 0.000 | 0.000 | | | no | 0.005 | yes |
| D4 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |
| D5 Pond | 0.000 | 0.000 | | | no | 0.005 | yes |
| D5 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |
| D6 Ditch | 0.000 | 0.000 | | | no | 0.005 | yes |
| R1 Pond | 0.000 | 0.000 | | | no | 0.005 | yes |
| R1 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |
| R2 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |
| R3 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |
| R4 Stream | 0.000 | 0.000 | | | no | 0.005 | yes |

Algae

High dose rate

All ETRi ≤ ETR trigger/n; acceptable risk can be concluded on screening level for the highest dose rate and for all mesotrione pH scenarios. Therefore, the combined risk to algae is not considered further for any of the other uses.

| Algae | ETR | | | | Any a.s. above | ETRtrigger/n (if needed) | All ETRi ≤ ETRtrigger/n? |
|---|---|---|---|---|---|---|---|
| | TCM | MST pH 5.1 | | | | | |
| Step 1 | 0.005 | 0.002 | | | no | 0.05 | yes |
| Step 2 | | | | | | | |
| N-Europe | 0.001 | 0.000 | | | no | 0.05 | yes |
| S-Europe | 0.001 | 0.000 | | | no | 0.05 | yes |
| Step 3 | | | | | | | |
| D3 Ditch | 0.000 | 0.000 | | | no | 0.05 | yes |
| D4 Pond | 0.000 | 0.000 | | | no | 0.05 | yes |
| D4 Stream | 0.000 | 0.000 | | | no | 0.05 | yes |
| D5 Pond | 0.000 | 0.000 | | | no | 0.05 | yes |
| D5 Stream | 0.000 | 0.000 | | | no | 0.05 | yes |
| D6 Ditch | 0.000 | 0.000 | | | no | 0.05 | yes |
| R1 Pond | 0.000 | 0.000 | | | no | 0.05 | yes |
| R1 Stream | 0.000 | 0.000 | | | no | 0.05 | yes |
| R2 Stream | 0.000 | 0.000 | | | no | 0.05 | yes |
| R3 Stream | 0.000 | 0.000 | | | no | 0.05 | yes |
| R4 Stream | 0.001 | 0.000 | | | no | 0.05 | yes |

| Algae | ETR | | | | Any a.s. above | ETRtrigger/n (if needed) | All ETRi ≤ ETRtrigger/n? |
|---|---|---|---|---|---|---|---|
| | TCM | MST pH 6.5 | | | | | |
| Step 1 | 0.005 | 0.002 | | | no | 0.05 | yes |
| Step 2 | | | | | | | |
| N-Europe | 0.001 | 0.000 | | | no | 0.05 | yes |
| S-Europe | 0.001 | 0.000 | | | no | 0.05 | yes |
| Step 3 | | | | | | | |
| D3 Ditch | 0.000 | 0.000 | | | no | 0.05 | yes |
| D4 Pond | 0.000 | 0.000 | | | no | 0.05 | yes |
| D4 Stream | 0.000 | 0.000 | | | no | 0.05 | yes |
| D5 Pond | 0.000 | 0.000 | | | no | 0.05 | yes |
| D5 Stream | 0.000 | 0.000 | | | no | 0.05 | yes |
| D6 Ditch | 0.000 | 0.000 | | | no | 0.05 | yes |
| R1 Pond | 0.000 | 0.000 | | | no | 0.05 | yes |
| R1 Stream | 0.000 | 0.000 | | | no | 0.05 | yes |
| R2 Stream | 0.000 | 0.000 | | | no | 0.05 | yes |
| R3 Stream | 0.000 | 0.000 | | | no | 0.05 | yes |
| R4 Stream | 0.001 | 0.000 | | | no | 0.05 | yes |

| Algae | ETR | | | | Any a.s. above | ETRtrigger/n (if needed) | All ETRi ≤ ETRtrigger/n? |
|---|---|---|---|---|---|---|---|
| | TCM | MST pH 7.9 | | | | | |
| Step 1 | 0.005 | 0.002 | | | no | 0.05 | yes |
| Step 2 | | | | | | | |
| N-Europe | 0.001 | 0.000 | | | no | 0.05 | yes |
| S-Europe | 0.001 | 0.000 | | | no | 0.05 | yes |
| Step 3 | | | | | | | |
| D3 Ditch | 0.000 | 0.000 | | | no | 0.05 | yes |
| D4 Pond | 0.000 | 0.000 | | | no | 0.05 | yes |
| D4 Stream | 0.000 | 0.000 | | | no | 0.05 | yes |
| D5 Pond | 0.000 | 0.000 | | | no | 0.05 | yes |
| D5 Stream | 0.000 | 0.000 | | | no | 0.05 | yes |
| D6 Ditch | 0.000 | 0.000 | | | no | 0.05 | yes |
| R1 Pond | 0.000 | 0.000 | | | no | 0.05 | yes |
| R1 Stream | 0.000 | 0.000 | | | no | 0.05 | yes |
| R2 Stream | 0.000 | 0.000 | | | no | 0.05 | yes |
| R3 Stream | 0.000 | 0.000 | | | no | 0.05 | yes |
| R4 Stream | 0.001 | 0.000 | | | no | 0.05 | yes |

Macrophytes

High dose rate

All ETRi ≤ ETR trigger/n at Step 3 for the D scenarios and the R1 pond scenario at the high dose rate and for all mesotrione pH scenarios; acceptable risk can be concluded on screening level. Therefore, the combined risk to Lemna is not considered further for these scenarios for any of the other uses.
The R stream scenarios will be further addressed below.

| Macrophytes | ETR | | | | Any a.s. above ETRtrigger? | ETRtrigger/n (if needed) | All ETRi ≤ ETRtrigger/n? |
|---|---|---|---|---|---|---|---|
| | TCM | MST pH 5.1 | | | | | |
| Step 1 | 2.481 | 0.813 | | | yes | | |
| Step 2 | | | | | | | |
| N-Europe | 0.348 | 0.121 | | | yes | | |
| S-Europe | 0.631 | 0.227 | | | yes | | |
| Step 3 | | | | | | | |
| D3 Ditch | 0.042 | 0.015 | | | no | 0.05 | yes |
| D4 Pond | 0.002 | 0.002 | | | no | 0.05 | yes |
| D4 Stream | 0.036 | 0.013 | | | no | 0.05 | yes |
| D5 Pond | 0.002 | 0.001 | | | no | 0.05 | yes |
| D5 Stream | 0.036 | 0.013 | | | no | 0.05 | yes |
| D6 Ditch | 0.042 | 0.015 | | | no | 0.05 | yes |
| R1 Pond | 0.006 | 0.002 | | | no | 0.05 | yes |
| R1 Stream | 0.103 | 0.046 | | | yes | | |
| R2 Stream | 0.109 | 0.033 | | | yes | | |
| R3 Stream | 0.265 | 0.088 | | | yes | | |
| R4 Stream | 0.293 | 0.102 | | | yes | | |

| Macrophytes | ETR | | | | Any a.s. above | ETRtrigger/n (if needed) | All ETRi ≤ ETRtrigger/n |
|---|---|---|---|---|---|---|---|
| | TCM | MST pH 6.5 | | | | | |
| Step 1 | 2.481 | 0.915 | | | yes | | |
| Step 2 | | | | | | | |
| N-Europe | 0.348 | 0.125 | | | yes | | |
| S-Europe | 0.631 | 0.235 | | | yes | | |
| Step 3 | | | | | | | |
| D3 Ditch | 0.042 | 0.015 | | | no | 0.05 | yes |
| D4 Pond | 0.002 | 0.001 | | | no | 0.05 | yes |
| D4 Stream | 0.036 | 0.013 | | | no | 0.05 | yes |
| D5 Pond | 0.002 | 0.001 | | | no | 0.05 | yes |
| D5 Stream | 0.036 | 0.013 | | | no | 0.05 | yes |
| D6 Ditch | 0.042 | 0.015 | | | no | 0.05 | yes |
| R1 Pond | 0.006 | 0.001 | | | no | 0.05 | yes |
| R1 Stream | 0.103 | 0.031 | | | yes | | |
| R2 Stream | 0.109 | 0.061 | | | yes | | |
| R3 Stream | 0.265 | 0.112 | | | yes | | |
| R4 Stream | 0.293 | 0.118 | | | yes | | |

| Macrophytes | ETR | | | | Any a.s. above | ETRtrigger/n (if needed) | All ETRi ≤ ETRtrigger/n |
|---|---|---|---|---|---|---|---|
| | TCM | MST pH 7.9 | | | | | |
| Step 1 | 2.481 | 0.955 | | | yes | | |
| Step 2 | | | | | | | |
| N-Europe | 0.348 | 0.026 | | | yes | | |
| S-Europe | 0.631 | 0.026 | | | yes | | |
| Step 3 | | | | | | | |
| D3 Ditch | 0.042 | 0.015 | | | no | 0.05 | yes |
| D4 Pond | 0.002 | 0.001 | | | no | 0.05 | yes |
| D4 Stream | 0.036 | 0.013 | | | no | 0.05 | yes |
| D5 Pond | 0.002 | 0.001 | | | no | 0.05 | yes |
| D5 Stream | 0.036 | 0.013 | | | no | 0.05 | yes |
| D6 Ditch | 0.042 | 0.015 | | | no | 0.05 | yes |
| R1 Pond | 0.006 | 0.001 | | | no | 0.05 | yes |
| R1 Stream | 0.103 | 0.010 | | | yes | | |
| R2 Stream | 0.109 | 0.014 | | | yes | | |
| R3 Stream | 0.265 | 0.015 | | | yes | | |
| R4 Stream | 0.293 | 0.010 | | | yes | | |

Low dose rate

| Macrophytes | ETR | | | | Any a.s. above | ETRtrigger/n (if needed) | All ETRi ≤ ETRtrigger/n |
|---|---|---|---|---|---|---|---|
| | TCM | MST pH 5.1 | | | | | |
| R1 Stream | 0.068 | 0.030 | | | no | 0.05 | no |
| R2 Stream | 0.069 | 0.021 | | | no | 0.05 | no |
| R3 Stream | 0.169 | 0.057 | | | yes | | |
| R4 Stream | 0.188 | 0.066 | | | yes | | |

| Macrophytes | ETR | | | | Any a.s. above | ETRtrigger/n (if needed) | All ETRi ≤ ETRtrigger/n |
|---|---|---|---|---|---|---|---|
| | TCM | MST pH 6.5 | | | | | |
| R1 Stream | 0.068 | 0.020 | | | no | 0.05 | no |
| R2 Stream | 0.069 | 0.039 | | | no | 0.05 | no |
| R3 Stream | 0.169 | 0.073 | | | yes | | |
| R4 Stream | 0.188 | 0.077 | | | yes | | |

| Macrophytes | ETR | | | | Any a.s. above | ETRtrigger/n (if needed) | All ETRi ≤ ETRtrigger/n |
|---|---|---|---|---|---|---|---|
| | TCM | MST pH 7.9 | | | | | |
| R1 Stream | 0.068 | 0.007 | | | no | 0.05 | no |
| R2 Stream | 0.069 | 0.009 | | | no | 0.05 | no |
| R3 Stream | 0.169 | 0.009 | | | yes | | |
| R4 Stream | 0.188 | 0.007 | | | yes | | |

No driver is detected for the R1 and R2 stream scenario at pH 5.1 and 6.5. Therefore, all R scenarios will be further addressed below.
Thiencarbazone-methyl is the driver for the R1 stream scenario at pH 7.9. Therefore, reference is made to the risk assessment with the individual active substance. The other R scenarios will be further addressed below.

<u>High dose rate – banded application</u>

| Macrophytes | ETR | | | | Any a.s. above | ETRtrigger/n (if needed) | All ETRi ≤ ETRtrigger/n |
|---|---|---|---|---|---|---|---|
| | TCM | MST pH 5.1 | | | | | |
| R1 Stream | 0.053 | 0.023 | | | no | 0.05 | no |
| R2 Stream | 0.052 | 0.016 | | | no | 0.05 | no |
| R3 Stream | 0.130 | 0.043 | | | yes | | |
| R4 Stream | 0.144 | 0.050 | | | yes | | |

| Macrophytes | ETR | | | | Any a.s. above | ETRtrigger/n (if needed) | All ETRi ≤ ETRtrigger/n |
|---|---|---|---|---|---|---|---|
| | TCM | MST pH 6.5 | | | | | |
| R1 Stream | 0.053 | 0.031 | | | no | 0.05 | no |
| R2 Stream | 0.052 | 0.061 | | | no | 0.05 | no |
| R3 Stream | 0.130 | 0.112 | | | yes | | |
| R4 Stream | 0.144 | 0.118 | | | yes | | |

| Macrophytes | ETR | | | | Any a.s. above | ETRtrigger/n (if needed) | All ETRi ≤ ETRtrigger/n |
|---|---|---|---|---|---|---|---|
| | TCM | MST pH 7.9 | | | | | |
| R1 Stream | 0.053 | 0.010 | | | no | 0.05 | no |
| R2 Stream | 0.052 | 0.014 | | | no | 0.05 | no |
| R3 Stream | 0.130 | 0.015 | | | yes | | |
| R4 Stream | 0.144 | 0.010 | | | yes | | |

No driver is detected for the R1 and R2 stream scenario. Therefore, all R stream scenarios will be further addressed below.

Low dose rate – banded application

| Macrophytes | ETR | | | | Any a.s. above | ETRtrigger/n (if needed) | All ETRi ≤ ETRtrigger/n |
|---|---|---|---|---|---|---|---|
| | TCM | MST pH 5.1 | | | | | |
| R1 Stream | 0.035 | 0.015 | | | no | 0.05 | yes |
| R2 Stream | 0.033 | 0.010 | | | no | 0.05 | yes |
| R3 Stream | 0.083 | 0.028 | | | no | 0.05 | no |
| R4 Stream | 0.093 | 0.032 | | | no | 0.05 | no |

| Macrophytes | ETR | | | | Any a.s. above | ETRtrigger/n (if needed) | All ETRi ≤ ETRtrigger/n |
|---|---|---|---|---|---|---|---|
| | TCM | MST pH 6.5 | | | | | |
| R1 Stream | 0.035 | 0.010 | | | no | 0.05 | yes |
| R2 Stream | 0.033 | 0.019 | | | no | 0.05 | yes |
| R3 Stream | 0.083 | 0.036 | | | no | 0.05 | no |
| R4 Stream | 0.093 | 0.038 | | | no | 0.05 | no |

| Macrophytes | ETR | | | | Any a.s. above | ETRtrigger/n (if needed) | All ETRi ≤ ETRtrigger/n |
|---|---|---|---|---|---|---|---|
| | TCM | MST pH 7.9 | | | | | |
| R1 Stream | 0.035 | 0.007 | | | no | 0.05 | yes |
| R2 Stream | 0.033 | 0.009 | | | no | 0.05 | yes |
| R3 Stream | 0.083 | 0.009 | | | no | 0.05 | no |
| R4 Stream | 0.093 | 0.007 | | | no | 0.05 | no |

All ETRi ≤ ETR trigger/n at Step 3 for the R1 and R2 stream scenario; acceptable risk can be concluded on screening level.
No driver is detected for the R3 and R4 stream scenario at pH 5.1 and 6.5. Therefore, the R3 and R4 stream scenarios will be further addressed below.
Thiencarbazone-methyl is the driver for the R4 stream scenario at pH 7.9. Therefore, reference is made to the risk assessment with the individual active substance. The R3 stream scenario will be further addressed below.

**Step 2:** MDR calculation to check for synergistic or antagonistic effects

**Table 9.5-20**: **Calculation of the acute mixture toxicity of the formulation**

| Aquatic organisms | Fraction of Mesotrione in mixture | Fraction of Thiencarbazone-methyl in mix-ture | Mesotrione $EC_{50}$ (mg a.s./L) | Thiencarbazone-methyl $EC_{50}$ (mg a.s./L) | $ECx_{mix\text{-}CA}$. Predicted $EC_{50}$ of GLOB2112dH based on the a.s. toxicity (mg as/L) | $ECx_{ppp.}$ $EC_{50}$ of GLOB2112dH from the studies (mg prod./L) | $ECx_{ppp.}$ $EC_{50}$ of GLOB2112dH from the studies (mg sum of a.s./L) | MDR (model deviation ratio) | Comparison toxicity of the formulation and the predicted one |
|---|---|---|---|---|---|---|---|---|---|
| Macrophytes | 0.83 | 0.17 | 0.0263 | 0.00187 | 0.0083 | 0.0142 | 0.0053 | 1.57 | MDR= 0.2-5 |

The MDR for macrophytes is between 0.2 and 5 indicating that the formulation is not more toxic than what can be expected based on the data of the active substances.

**Step 3** Comparison of mixture composition of PPP and $PEC_{mix}$

According to Step 3 it has to be checked whether the mixture composition in the formulation is similar to the mixture composition at $PEC_{mix}$ (which is the sum of $PEC_i$ of individual active substances). Therefore, the "$ECx_{mix\text{-}CA}$ (a.s. in PPP) / $ECx_{mix\text{-}CA}$ (a.s. in $PEC_{mix}$)" ratios were calculated.
In case the ratio is in the range of 0.8–1.2 (mixture similar), the risk assessment can be based on the $ECx_{PPP}$ (Step 4).
In case the ratio is outside the range of 0.8–1.2 (i.e. mixture not similar), the endpoint $ECx_{mix\text{-}CA}$ normalized to the mixture composition at $PEC_{mix}$ (reported as "$ECx_{mix\text{-}CA}$ (a.s. in $PEC_{mix}$)" in EFSA, 2013, and the following parts of this report) has to be used to calculate the risk (Step 5 or Step 8, respectively).

<u>pH 5.1 – high dose rate</u>

| Macrophytes | |
|---|---|
| ECxmix-CA (a.s. in PPP)/ ECxmix-CA (a.s. in PECmix) | |
| R1 Stream | 0.88 |
| R2 Stream | 1.09 |
| R3 Stream | 1.04 |
| R4 Stream | 1.01 |

| Macrophytes | | | | | |
|---|---|---|---|---|---|
| FOCUS Step 4 | Scenario | ECxmix-CA (a.s. in PPP) / ECxmix-CA (a.s. in PECmix) | | | |
| | Vegetative strip [m] | None | 10 | 10 vfsmod | 20 |
| Nozzle reduction | No spray buffer [m] | FOCUS default | 10 | 10 | 20 |
| None | R1 stream | 0.88 | 0.88 | 1.00 | 0.88 |
| None | R2 stream | 1.09 | 1.09 | | |
| None | R3 stream | 1.04 | 1.04 | 1.01 | 1.04 |
| None | R4 stream | 1.01 | 1.01 | 1.00 | 1.01 |

At Step 3 and 4, the mixture composition in the product is similar to the mixture composition at PECmix in all R scenarios that still need to be considered.

pH 6.5 – high dose rate

| Macrophytes | |
|---|---|
| ECxmix-CA (a.s. in PPP)/ ECxmix-CA (a.s. in PECmix) | |
| R1 Stream | 1.10 |
| R2 Stream | 0.78 |
| R3 Stream | 0.91 |
| R4 Stream | 0.93 |

| Macrophytes | | | | | |
|---|---|---|---|---|---|
| FOCUS Step 4 | Scenario | ECxmix-CA (a.s. in PPP) / ECxmix-CA (a.s. in PECmix) | | | |
| | Vegetative strip [m] | None | 10 | 10 vfsmod | 20 |
| Nozzle reduction | No spray buffer [m] | FOCUS default | 10 | 10 | 20 |
| None | R1 stream | 1.10 | 1.16 | | |
| None | R2 stream | 0.78 | 0.78 | | |
| None | R3 stream | 0.91 | 0.91 | 1.00 | 0.91 |
| None | R4 stream | 0.93 | 0.93 | 1.00 | 0.93 |

At Step 3 and 4, the mixture composition in the product is similar to the mixture composition at PECmix in all R scenarios that still need to be considered, except for the R2 stream scenario.

pH 7.9 – high dose rate

| Macrophytes | |
|---|---|
| ECxmix-CA (a.s. in PPP)/ ECxmix-CA (a.s. in PECmix) | |
| R1 Stream | 2.03 |
| R2 Stream | 1.79 |
| R3 Stream | 2.63 |
| R4 Stream | 3.06 |

| Macrophytes | | | |
|---|---|---|---|
| FOCUS Step 4 | Scenario | ECxmix-CA (a.s. in PPP) / ECxm | |
| | Vegetative strip [m] | None | 10 |
| Nozzle reduction | No spray buffer [m] | FOCUS default | 10 |
| None | R2 stream | 1.79 | 2.47 |

At Step 3 and 4, the mixture composition in the product is never similar to the mixture composition at PECmix in all R scenarios that still need to be considered.

pH 5.1 – low dose rate

| Macrophytes | |
|---|---|
| ECxmix-CA (a.s. in PPP)/ ECxmix-CA (a.s. in PECmix) | |
| R1 Stream | 0.89 |
| R2 Stream | 1.08 |
| R3 Stream | 1.03 |
| R4 Stream | 1.01 |

| Macrophytes | | | | | |
|---|---|---|---|---|---|
| FOCUS Step 4 | Scenario | ECxmix-CA (a.s. in PPP) / ECxmix-CA (a.s. in PECmix) | | | |
| | Vegetative strip [m] | None | 10 | 10 vfsmod | 20 |
| Nozzle reduction | No spray buffer [m] | FOCUS default | 10 | 10 | 20 |
| None | R1 stream | 0.89 | 0.89 | | |
| None | R2 stream | 1.08 | 1.08 | | |
| None | R3 stream | 1.03 | 1.03 | 1.00 | 1.03 |
| None | R4 stream | 1.01 | 1.01 | 1.00 | 1.01 |

At Step 3 and 4, the mixture composition in the product is similar to the mixture composition at PECmix in all R scenarios that still need to be considered.

pH 6.5 – low dose rate

| Macrophytes | |
|---|---|
| ECxmix-CA (a.s. in PPP)/ ECxmix-CA (a.s. in PECmix) | |
| R1 Stream | 1.10 |
| R2 Stream | 0.77 |
| R3 Stream | 0.90 |
| R4 Stream | 0.92 |

| Macrophytes | | | | | |
|---|---|---|---|---|---|
| FOCUS Step 4 | Scenario | ECxmix-CA (a.s. in PPP) / ECxmix-CA (a.s. in PECmix) | | | |
| | Vegetative strip [m] | None | 10 | 10 vfsmod | 20 |
| Nozzle reduction | No spray buffer [m] | FOCUS default | 10 | 10 | 20 |
| None | R1 stream | 1.10 | 1.17 | | |
| None | R2 stream | 0.77 | 0.77 | | |
| None | R3 stream | 0.90 | 0.90 | 1.00 | 0.90 |
| None | R4 stream | 0.92 | 0.92 | 1.00 | 0.92 |

At Step 3 and 4, the mixture composition in the product is similar to the mixture composition at PECmix in all R scenarios that still need to be considered, except for the R2 stream scenario.

pH 7.9 – low dose rate

| Macrophytes | |
|---|---|
| ECxmix-CA (a.s. in PPP)/ ECxmix-CA (a.s. in PECmix) | |
| R1 Stream | 2.04 |
| R2 Stream | 1.76 |
| R3 Stream | 2.62 |
| R4 Stream | 3.05 |

At Step 3, the mixture composition in the product is never similar to the mixture composition at PECmix in all R scenarios that still need to be considered.

pH 5.1 – high dose rate – banded application

| Macrophytes | |
|---|---|
| ECxmix-CA (a.s. in PPP)/ ECxmix-CA (a.s. in PECmix) | |
| R1 Stream | 0.89 |
| R2 Stream | 1.08 |
| R3 Stream | 1.04 |
| R4 Stream | 1.01 |

| Macrophytes | | | | | |
|---|---|---|---|---|---|
| FOCUS Step 4 | Scenario | ECxmix-CA (a.s. in PPP) / ECxmix-CA (a.s. in PECmix) | | | |
| | Vegetative strip [m] | None | 10 | 10 vfsmod | 20 |
| Nozzle reduction | No spray buffer [m] | FOCUS default | 10 | 10 | 20 |
| None | R1 stream | 0.89 | 0.89 | | |
| None | R2 stream | | | | |
| None | R3 stream | 1.04 | 1.04 | 1.00 | 1.04 |
| None | R4 stream | 1.01 | 1.01 | 1.00 | 1.01 |

At Step 3 and 4, the mixture composition in the product is similar to the mixture composition at PECmix in all R scenarios that still need to be considered.

pH 6.5 – high dose rate – banded application

| Macrophytes | |
|---|---|
| ECxmix-CA (a.s. in PPP)/ ECxmix-CA (a.s. in PECmix) | |
| R1 Stream | 0.76 |
| R2 Stream | 0.55 |
| R3 Stream | 0.63 |
| R4 Stream | 0.64 |

| Macrophytes | | | | | |
|---|---|---|---|---|---|
| FOCUS Step 4 | Scenario | ECxmix-CA (a.s. in PPP) / ECxmix-CA (a.s. in PECmix) | | | |
| | Vegetative strip [m] | None | 10 | 10 vfsmod | 20 |
| Nozzle reduction | No spray buffer [m] | FOCUS default | 10 | 10 | 20 |
| None | R1 stream | | | | |
| None | R2 stream | 0.55 | 0.55 | | |
| None | R3 stream | 0.63 | 0.63 | 0.69 | 0.63 |
| None | R4 stream | 0.64 | 0.64 | 0.69 | 0.64 |

At Step 3 and 4, the mixture composition in the product is never similar to the mixture composition at PECmix in all R scenarios that still need to be considered.

pH 7.9 – high dose rate – banded application

| Macrophytes | |
|---|---|
| ECxmix-CA (a.s. in PPP)/ ECxmix-CA (a.s. in PECmix) | |
| R1 Stream | 1.42 |
| R2 Stream | 1.18 |
| R3 Stream | 1.91 |
| R4 Stream | 2.36 |

| Macrophytes | | | |
|---|---|---|---|
| FOCUS Step 4 | Scenario | ECxmix-CA (a.s. in PPP) / ECxmi | |
| | Vegetative strip [m] | None | 10 |
| Nozzle reduction | No spray buffer [m] | FOCUS default | 10 |
| None | R1 stream | | |
| None | R2 stream | | |
| None | R3 stream | 1.91 | 2.62 |
| None | R4 stream | | |

At Step 3 and 4, the mixture composition in the product is never similar to the mixture composition at PECmix in all R scenarios that still need to be considered, except for the R2 stream scenario.

pH 5.1 – low dose rate – banded application

| Macrophytes | |
|---|---|
| ECxmix-CA (a.s. in PPP)/ ECxmix-CA (a.s. in PECmix) | |
| R1 Stream | 0.90 |
| R2 Stream | 1.08 |
| R3 Stream | 1.04 |
| R4 Stream | 1.01 |

| Macrophytes | | | |
|---|---|---|---|
| FOCUS Step 4 | Scenario | ECxmix-CA (a.s. in PPP) / ECxm | |
| | Vegetative strip [m] | None | 10 |
| Nozzle reduction | No spray buffer [m] | FOCUS default | 10 |
| None | R3 stream | 1.04 | 1.04 |
| None | R4 stream | 1.01 | 1.01 |

At Step 3 and 4, the mixture composition in the product is similar to the mixture composition at PECmix in all R scenarios that still need to be considered.

pH 6.5 – low dose rate – banded application

| Macrophytes | |
|---|---|
| ECxmix-CA (a.s. in PPP)/ ECxmix-CA (a.s. in PECmix) | |
| R1 Stream | 1.13 |
| R2 Stream | 0.76 |
| R3 Stream | 0.89 |
| R4 Stream | 0.92 |

| Macrophytes | | | | | |
|---|---|---|---|---|---|
| FOCUS Step 4 | Scenario | ECxmix-CA (a.s. in PPP) / ECxmix-CA (a.s. in PECmix) | | | |
| | Vegetative strip [m] | None | 10 | 10 vfsmod | 20 |
| Nozzle reduction | No spray buffer [m] | FOCUS default | 10 | 10 | 20 |
| None | R3 stream | 0.89 | 0.89 | | |
| None | R4 stream | 0.92 | 0.92 | 1.00 | 0.92 |

At Step 3 and 4, the mixture composition in the product is similar to the mixture composition at PECmix in all R scenarios that still need to be considered.

pH 7.9 – low dose rate – banded application

| Macrophytes | |
|---|---|
| ECxmix-CA (a.s. in PPP)/ ECxmix-CA (a.s. in PECmix) | |
| R1 Stream | 1.43 |
| R2 Stream | 1.17 |
| R3 Stream | 1.90 |
| R4 Stream | 2.36 |

At Step 3 and 4, the mixture composition in the product is not similar to the mixture composition at PECmix in all R scenarios that still need to be considered.

**Step 5:** Driver detection

pH 6.5 – high dose rate

| Next Step for: | Macrophytes | | | | | | Product data assessed? |
|---|---|---|---|---|---|---|---|
| FOCUS | TCM | MST pH 6.5 | | | Conclusion | | |
| R1 Stream | | | | | | Go to Step 8 | yes in 4 |
| R2 Stream | | | | | | Go to Step 8 | |
| R3 Stream | | | | | | Go to Step 8 | yes in 4 |
| R4 Stream | | | | | | Go to Step 8 | yes in 4 |

There is no driver for macrophytes in the R2 stream scenario.

pH 7.9 – high dose rate

| Next Step for: | Macrophytes | | | | | |
|---|---|---|---|---|---|---|
| FOCUS | TCM | MST pH 7.9 | | | Conclusion | |
| R1 Stream | Driver | | | | Go to Step 6 | |
| R2 Stream | | | | | | Go to Step 8 |
| R3 Stream | Driver | | | | Go to Step 6 | |
| R4 Stream | Driver | | | | Go to Step 6 | |

Thiencarbazone-methyl is the driver for macrophytes in all R scenario that still need to be considered, except the R2 stream scenario. For these scenarios, reference is made to the risk assessment with the individual active substance.

pH 6.5 – low dose rate

| Next Step for: | Macrophytes | | | | | | Product data assessed? |
|---|---|---|---|---|---|---|---|
| FOCUS | TCM | MST pH 6.5 | | | Conclusion | | |
| R1 Stream | | | | | | Go to Step 8 | yes in 4 |
| R2 Stream | | | | | | Go to Step 8 | |
| R3 Stream | | | | | | Go to Step 8 | yes in 4 |
| R4 Stream | | | | | | Go to Step 8 | yes in 4 |

There is no driver for macrophytes in the R2 stream scenario.

pH 7.9 – low dose rate

| Next Step for: | Macrophytes | | | | | |
|---|---|---|---|---|---|---|
| FOCUS | TCM | MST pH 7.9 | | | Conclusion | |
| R1 Stream | Driver | | | | Go to Step 6 | |
| R2 Stream | | | | | | Go to Step 8 |
| R3 Stream | Driver | | | | Go to Step 6 | |
| R4 Stream | Driver | | | | Go to Step 6 | |

Thiencarbazone-methyl is the driver for macrophytes in all R scenario that still need to be considered, except the R2 stream scenario. For these scenarios, reference is made to the risk assessment with the individual active substance.

pH 6.5 – high dose rate – banded application

| Next Step for: | Macrophytes | | | | | |
|---|---|---|---|---|---|---|
| FOCUS | TCM | MST pH 6.5 | | | Conclusion | |
| R1 Stream | | | | | | Go to Step 8 |
| R2 Stream | | | | | | Go to Step 8 |
| R3 Stream | | | | | | Go to Step 8 |
| R4 Stream | | | | | | Go to Step 8 |

There is no driver for macrophytes.

pH 7.9 – high dose rate – banded application

| Next Step for: | Macrophytes | | | | | | Product data |
|---|---|---|---|---|---|---|---|
| R1 Stream | | | | | | Go to Step 8 | |
| R2 Stream | | | | | | Go to Step 8 | yes in 4 |
| R3 Stream | | | | | | Go to Step 8 | |
| R4 Stream | Driver | | | | Go to Step 6 | | |

Thiencarbazone-methyl is the driver for macrophytes in the R4 stream scenario. For this scenario, reference is made to the risk assessment with the individual active substance.

pH 7.9 – low dose rate – banded application

| Next Step for: | Macrophytes | | | | | | Product data assessed? |
|---|---|---|---|---|---|---|---|
| FOCUS | TCM | MST pH 7.9 | | | Conclusion | | |
| R1 Stream | | | | | | Go to Step 8 | |
| R2 Stream | | | | | | Go to Step 8 | yes in 4 |
| R3 Stream | | | | | | Go to Step 8 | |
| R4 Stream | Driver | | | | Go to Step 6 | | |

Thiencarbazone-methyl is the driver for macrophytes in the R4 stream scenario. For this scenario, reference is made to the risk assessment with the individual active substance.

**Step 4 and Step 8a/b:** Mixture risk assessment based on measured and/or calculated mixture toxicity

pH 5.1 – high dose rate – Step 4

| Macrophytes | |
|---|---|
| ETRmix-PPP | |
| R1 Stream | 0.27 |
| R2 Stream | 0.21 |
| R3 Stream | 0.54 |
| R4 Stream | 0.61 |

A FOCUS Step 4 risk assessment is needed.

| Macrophytes | | | | | |
|---|---|---|---|---|---|
| FOCUS Step 4 | Scenario | ETRmix-PPP | | | |
| | Vegetative strip [m] | None | 10 | 10 vfsmod | 20 |
| Nozzle reduction | No spray buffer [m] | FOCUS default | 10 | 10 | 20 |
| None | R1 stream | 0.27 | 0.12 | 0.01 | 0.06 |
| None | R2 stream | 0.21 | 0.09 | | |
| None | R3 stream | 0.54 | 0.24 | 0.02 | 0.13 |
| None | R4 stream | 0.61 | 0.28 | 0.01 | 0.15 |

The risk for macrophytes is acceptable in the R1 stream scenario when using a no spray buffer zone of 20 m including a 20 m vegetated filter strip unless VFS-mod is accepted. In the latter case, a no spray buffer zone of 10 m including a 10 m vegetated filter strip is sufficient to obtain an acceptable risk for macrophytes.

The risk for macrophytes is acceptable in the R2 stream scenario when using a no spray buffer zone of 10 m including a 10 m vegetated filter strip.
The ETRmix-PPP in the R3 and R4 stream scenario is slightly above the trigger, unless VFSmod is accepted. In the latter case, a no spray buffer zone of 10 m including a 10 m vegetated filter strip is sufficient to obtain an acceptable risk for macrophytes. Alternatively, a restriction of use on sloped areas could be implemented.

pH 6.5 – high dose rate – Step 4

| Macrophytes | |
|---|---|
| ETRmix-PPP | |
| R1 Stream | 0.19 |
| R2 Stream | Go to Step 5/8 |
| R3 Stream | 0.66 |
| R4 Stream | 0.70 |

A FOCUS Step 4 risk assessment is needed.

| Macrophytes | | | | | |
|---|---|---|---|---|---|
| FOCUS Step 4 | Scenario | ETRmix-PPP | | | |
| | Vegetative strip [m] | None | 10 | 10 vfsmod | 20 |
| Nozzle reduction | No spray buffer [m] | FOCUS default | 10 | 10 | 20 |
| None | R1 stream | 0.19 | 0.08 | | |
| None | R2 stream | Go to Step 5/8 | Go to Step 5/8 | | |
| None | R3 stream | 0.66 | 0.30 | 0.02 | 0.15 |
| None | R4 stream | 0.70 | 0.32 | 0.01 | 0.17 |

The risk for macrophytes is acceptable in the R1 stream scenario when using a no spray buffer zone of 10 m including a 10 m vegetated filter strip.
The ETRmix-PPP in the R3 and R4 stream scenario is slightly above the trigger, unless VFSmod is accepted. In the latter case, a no spray buffer zone of 10 m including a 10 m vegetated filter strip is sufficient to obtain an acceptable risk for macrophytes. Alternatively, a restriction of use on sloped areas could be implemented.

pH 6.5 – high dose rate – Step 8a

| Macrophytes | |
|---|---|
| R1 Stream | Go to Step 4 |
| R2 Stream | 0.17 |
| R3 Stream | Go to Step 4 |
| R4 Stream | Go to Step 4 |

A FOCUS Step 4 risk assessment is needed for the R2 stream scenario.

| Macrophytes | | | | | |
|---|---|---|---|---|---|
| FOCUS Step 4 | Scenario | ETRmix-CA | | | |
| | Vegetative strip [m] | None | 10 | 10 vfsmod | 20 |
| Nozzle reduction | No spray buffer [m] | FOCUS default | 10 | 10 | 20 |
| None | R1 stream | Go to Step 4 | Go to Step 4 | | |
| None | R2 stream | 0.17 | 0.08 | | |
| None | R3 stream | Go to Step 4 | Go to Step 4 | Go to Step 4 | Go to Step 4 |
| None | R4 stream | Go to Step 4 | Go to Step 4 | Go to Step 4 | Go to Step 4 |

The risk for macrophytes is acceptable in the R2 stream scenario when using a no spray buffer zone of 10 m including a 10 m vegetated filter strip.

pH 7.9 – high dose rate – Step 8a

| Macrophytes | |
|---|---|
| ETRmix-CA | |
| R1 Stream | 0.11 |
| R2 Stream | 0.12 |
| R3 Stream | 0.28 |
| R4 Stream | 0.30 |

A FOCUS Step 4 risk assessment is needed for the R2 stream scenario.

| Macrophytes | | | |
|---|---|---|---|
| FOCUS Step 4 | Scenario | ETRmix-CA | |
| | Vegetative strip [m] | None | 10 |
| Nozzle reduction | No spray buffer [m] | FOCUS default | 10 |
| None | R2 stream | 0.12 | 0.05 |

The risk for macrophytes is acceptable in the R2 stream scenario when using a no spray buffer zone of 10 m including a 10 m vegetated filter strip.

pH 5.1 – low dose rate – Step 4

| Macrophytes | |
|---|---|
| ETRmix-PPP | |
| R1 Stream | 0.17 |
| R2 Stream | 0.13 |
| R3 Stream | 0.34 |
| R4 Stream | 0.39 |

A FOCUS Step 4 risk assessment is needed.

| Macrophytes | | | | | |
|---|---|---|---|---|---|
| FOCUS Step 4 | Scenario | ETRmix-PPP | | | |
| | Vegetative strip [m] | None | 10 | 10 vfsmod | 20 |
| Nozzle reduction | No spray buffer [m] | FOCUS default | 10 | 10 | 20 |
| None | R1 stream | 0.17 | 0.08 | | |
| None | R2 stream | 0.13 | 0.06 | | |
| None | R3 stream | 0.34 | 0.16 | 0.01 | 0.08 |
| None | R4 stream | 0.39 | 0.18 | 0.01 | 0.09 |

The risk for macrophytes is acceptable in the R1 and R2 stream scenario when using a no spray buffer zone of 10 m including a 10 m vegetated filter strip.
The risk for macrophytes is acceptable in the R3 and R4 stream scenario when using a no spray buffer zone of 20 m including a 20 m vegetated filter strip, unless VFSmod is accepted. In the latter case, a no spray buffer zone of 10 m including a 10 m vegetated filter strip is sufficient to obtain an acceptable risk for macrophytes.

pH 6.5 – low dose rate – Step 4

| Macrophytes | |
|---|---|
| ETRmix-PPP | |
| R1 Stream | 0.13 |
| R2 Stream | Go to Step 5/8 |
| R3 Stream | 0.42 |
| R4 Stream | 0.45 |

A FOCUS Step 4 risk assessment is needed.

| Macrophytes | | | | | |
|---|---|---|---|---|---|
| FOCUS Step 4 | Scenario | ETRmix-PPP | | | |
| | Vegetative strip [m] | None | 10 | 10 vfsmod | 20 |
| Nozzle reduction | No spray buffer [m] | FOCUS default | 10 | 10 | 20 |
| None | R1 stream | 0.13 | 0.05 | | |
| None | R2 stream | Go to Step 5/8 | Go to Step 5/8 | | |
| None | R3 stream | 0.42 | 0.19 | 0.01 | 0.10 |
| None | R4 stream | 0.45 | 0.21 | 0.01 | 0.11 |

The risk for macrophytes is acceptable in the R1 stream scenario when using a no spray buffer zone of 10 m including a 10 m vegetated filter strip.
The ETRmix-PPP in the R3 and R4 stream scenario is slightly above the trigger, unless VFSmod is accepted. In the latter case, a no spray buffer zone of 10 m including a 10 m vegetated filter strip is sufficient to obtain an acceptable risk for macrophytes. Alternatively, a restriction of use on sloped areas can be implemented.

pH 6.5 – low dose rate – Step 8a

| Macrophytes | |
|---|---|
| ETRmix-CA | |
| R1 Stream | Go to Step 4 |
| R2 Stream | 0.11 |
| R3 Stream | Go to Step 4 |
| R4 Stream | Go to Step 4 |

A FOCUS Step 4 risk assessment is needed for the R2 stream scenario.

| Macrophytes | | | | | |
|---|---|---|---|---|---|
| FOCUS Step 4 | Scenario | ETRmix-CA | | | |
| | Vegetative strip [m] | None | 10 | 10 vfsmod | 20 |
| Nozzle reduction | No spray buffer [m] | FOCUS default | 10 | 10 | 20 |
| None | R1 stream | Go to Step 4 | | | |
| None | R2 stream | 0.11 | 0.05 | | |
| None | R3 stream | Go to Step 4 | Go to Step 4 | Go to Step 4 | Go to Step 4 |
| None | R4 stream | Go to Step 4 | Go to Step 4 | Go to Step 4 | Go to Step 4 |

The risk for macrophytes is acceptable in the R2 stream scenario when using a no spray buffer zone of 10 m including a 10 m vegetated filter strip.

pH 7.9 – low dose rate – Step 8a

| Macrophytes | |
|---|---|
| ETRmix-CA | |
| R1 Stream | 0.07 |
| R2 Stream | 0.08 |
| R3 Stream | 0.18 |
| R4 Stream | 0.19 |

The risk for macrophytes is acceptable in the R2 stream scenario at Step 3. No risk mitigation measures are needed.

pH 5.1 – high dose rate – banded application – Step 4

| Macrophytes | |
|---|---|
| ETRmix-PPP | |
| R1 Stream | 0.13 |
| R2 Stream | 0.10 |
| R3 Stream | 0.26 |
| R4 Stream | 0.30 |

The risk for macrophytes is acceptable in the R2 stream scenario at Step 3. No risk mitigation measures are needed.
A FOCUS Step 4 risk assessment is needed for the other R scenarios.

| Macrophytes | | | | | |
|---|---|---|---|---|---|
| FOCUS Step 4 | Scenario | ETRmix-PPP | | | |
| | Vegetative strip [m] | None | 10 | 10 vfsmod | 20 |
| Nozzle reduction | No spray buffer [m] | FOCUS default | 10 | 10 | 20 |
| None | R1 stream | 0.13 | 0.06 | | |
| None | R3 stream | 0.26 | 0.12 | 0.01 | 0.06 |
| None | R4 stream | 0.30 | 0.14 | 0.01 | 0.07 |

The risk for macrophytes is acceptable in the R1 stream scenario when using a no spray buffer zone of 10 m including a 10 m vegetated filter strip.
The risk for macrophytes is acceptable in the R3 and R4 stream scenario when using a no spray buffer zone of 20 m including a 20 m vegetated filter strip, unless VFSmod is accepted. In the latter case, a no spray buffer zone of 10 m including a 10 m vegetated filter strip is sufficient to obtain an acceptable risk for macrophytes.

pH 6.5 – high dose rate – banded application – Step 8a

| Macrophytes | |
|---|---|
| ETRmix-CA | |
| R1 Stream | 0.08 |
| R2 Stream | 0.11 |
| R3 Stream | 0.24 |
| R4 Stream | 0.26 |

The risk for macrophytes is acceptable in the R1 stream scenario at Step 3. No risk mitigation measures are needed.
A FOCUS Step 4 risk assessment is needed for the other R scenarios.

| Macrophytes | | | | | |
|---|---|---|---|---|---|
| FOCUS Step 4 | Scenario | ETRmix-CA | | | |
| | Vegetative strip [m] | None | 10 | 10 vfsmod | 20 |
| Nozzle reduction | No spray buffer [m] | FOCUS default | 10 | 10 | 20 |
| None | R2 stream | 0.11 | 0.05 | | |
| None | R3 stream | 0.24 | 0.11 | 0.01 | 0.06 |
| None | R4 stream | 0.26 | 0.12 | 0.01 | 0.06 |

The risk for macrophytes is acceptable in the R2 stream scenario when using a no spray buffer zone of 10 m including a 10 m vegetated filter strip.

The risk for macrophytes is acceptable in the R3 and R4 stream scenario when using a no spray buffer zone of 20 m including a 20 m vegetated filter strip, unless VFSmod is accepted. In the latter case, a no spray buffer zone of 10 m including a 10 m vegetated filter strip is sufficient to obtain an acceptable risk for macrophytes.

pH 7.9 – high dose rate – banded application – Step 4

| Macrophytes | |
|---|---|
| ETRmix-PPP | |
| R1 Stream | Go to Step 5/8 |
| R2 Stream | 0.09 |
| R3 Stream | Go to Step 5/8 |
| R4 Stream | Go to Step 5/8 |

The risk for macrophytes is acceptable in the R2 stream scenario at Step 3. No risk mitigation measures are needed.

pH 7.9 – high dose rate – banded application – Step 8a

| Macrophytes | |
|---|---|
| ETRmix-CA | |
| R1 Stream | 0.06 |
| R2 Stream | Go to Step 4 |
| R3 Stream | 0.14 |
| R4 Stream | 0.15 |

| | | | |
|---|---|---|---|
| FOCUS Step 4 | Scenario | | |
| | Vegetative strip [m] | None | 10 |
| Nozzle reduction | No spray buffer [m] | FOCUS default | 10 |
| None | R3 stream | 0.14 | 0.06 |

The risk for macrophytes is acceptable in the R3 stream scenario when using a no spray buffer zone of 10 m including a 10 m vegetated filter strip.

pH 5.1 – low dose rate – banded application – Step 4

| Macrophytes | |
|---|---|
| ETRmix-PPP | |
| R1 Stream | 0.09 |
| R2 Stream | 0.06 |
| R3 Stream | 0.17 |
| R4 Stream | 0.20 |

| Macrophytes | | | |
|---|---|---|---|
| FOCUS Step 4 | Scenario | ETRmix-PPP | |
| | Vegetative strip [m] | None | 10 |
| Nozzle reduction | No spray buffer [m] | FOCUS default | 10 |
| None | R3 stream | 0.17 | 0.08 |
| None | R4 stream | 0.20 | 0.09 |

The risk for macrophytes is acceptable in the R3 and R4 stream scenario when using a no spray buffer zone of 10 m including a 10 m vegetated filter strip.

pH 6.5 – low dose rate – banded application – Step 4

| Macrophytes | |
|---|---|
| ETRmix-PPP | |
| R1 Stream | 0.06 |
| R2 Stream | Go to Step 5/8 |
| R3 Stream | 0.21 |
| R4 Stream | 0.22 |

| Macrophytes | | | | | |
|---|---|---|---|---|---|
| FOCUS Step 4 | Scenario | ETRmix-PPP | | | |
| | Vegetative strip [m] | None | 10 | 10 vfsmod | 20 |
| Nozzle reduction | No spray buffer [m] | FOCUS default | 10 | 10 | 20 |
| None | R3 stream | 0.21 | 0.10 | | |
| None | R4 stream | 0.22 | 0.10 | 0.00 | 0.05 |

The risk for macrophytes is acceptable in the R3 stream scenario when using a no spray buffer zone of 10 m including a 10 m vegetated filter strip.
The risk for macrophytes is acceptable in the R4 stream scenario when using a no spray buffer zone of 20 m including a 20 m vegetated filter strip, unless VFS-MOD is accepted. In the latter case, a no spray buffer zone of 10 m including a 10 m vegetated filter strip is sufficient to obtain an acceptable risk for macrophytes.

pH 7.9 – low dose rate – banded application

| Macrophytes | |
|---|---|
| ETRmix-CA | |
| R1 Stream | 0.04 |
| R2 Stream | Go to Step 4 |
| R3 Stream | 0.09 |
| R4 Stream | 0.10 |

The risk for macrophytes is acceptable in the R3 stream scenario at Step 3. No risk mitigation measures are needed.

**PECsw from FOCUS Drift Swash Tool**

For completeness, the endpoints obtained in the aquatic studies with GLOB2112dH were also compared to the PECsw of the formulation calculated using the Drift Swash Calculator.

**Table 9.5-15: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for GLOB2112dH for each organism group based on FOCUS drift calculations for the use of GLOB2112dH in maize**

| **Group** | | **Algae** | **Aquatic plants** | |
|---|---|---|---|---|
| **Test species** | | ***Pseudokirchn. subcapitata*** | ***Lemna gibba*** | ***Myriophyllum spicatum*** |
| **Endpoint (µg/L)** | | $E_rC_{50}$ 87100 | $E_rC_{50}$ 18.3 | $E_rC_{50}$ 14.2 |
| **AF** | | 10 | 10 | 10 |
| **RAC (µg/L)** | | 8710 | 1.83 | 1.42 |
| **FOCUS Scenario** | **PEC $_{gl\text{-}max}$ (µg/L)** | | | |
| 0.2 L/ha | | | | |
| 1 m | 1.8811 | < 0.001 | **1.028** | **1.325** |
| 2 m | 1.1138 | - | 0.609 | 0.784 |
| 0.13 L/ha | | | | |

| Group | | Algae | Aquatic plants | |
|---|---|---|---|---|
| 1 m | 1.2227 | < 0.001 | 0.668 | 0.861 |

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

### 9.5.3 Overall conclusions

**Table 9.5-22: Summary table of the aquatic risk assessment**

| RA Tier | Test item | Species | Drift only | FOCUS Step 1&2 | FOCUS Step 3&4 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | D3 | D4 | D5 | D6 | R1 | R2 | R3 | R4 |
| **0.2 L/ha** | | | | | | | | | | | | |
| Tier 1 | All metabolites | Fish, invertebrates, algae, macrophytes | | Resolved Step 1 | | | | | | | | |
| Tier1 | Mesotrione | Fish, invertebrates, algae | | Resolved Step 1 | | | | | | | | |
| Tier1 | Mesotrione | Macrophytes | | | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | pH 5.1 & 7.9: Resolved Step 3 pH 6.5: Resolved Step 4 [10 m vfs] | pH 7.9: Resolved Step 3 pH 5.1 & 6.5: Resolved Step 4 [10 m vfs] |
| Tier1 | Thiencarbazone-methyl | Fish, invertebrates, algae | | Resolved Step 1 | | | | | | | | |
| | | Macrophytes | | | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 4 [10 m vfs] | Resolved Step 4 [10 m vfs] | Resolved Step 4 [10 m vfsmod or 20 m vfs] | Resolved Step 4 [10 m vfsmod or 20 m vfs] |
| Tier 2C | Thiencarbazone-methyl | Macrophytes | | | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 |
| | Formulation | Fish, invertebrates, algae, macrophytes | | | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | pH 5.1: Resolved Step 4 [10 m vfs- | Resolved Step 4 [10 m vfs] | pH 5.1 & 6.5: Resolved Step 4 | pH 5.1 & 6.5: Resolved Step 4 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | mod or 20 m vfs] pH 6.5 & 7.9: Resolved Step 4 [10 m vfs] | | [10 m vfsmod or restriction on sloped areas] pH 7.9: Resolved Step 4 [10 m vfsmod or 20 m vfs] | [10 m vfsmod or restriction on sloped areas] pH 7.9: Resolved Step 4 [10 m vfsmod or 20 m vfs] |
| | Formulation | Algae, macro-phytes | Resolved [2 m bz] | | | | | | | | | |
| **0.13 L/ha** | | | | | | | | | | | | |
| Tier 1 | All metabolites | Fish, inverte-brates, algae, macrophytes | | Resolved Step 1 | | | | | | | | |
| Tier1 | Mesotrione | Fish, inverte-brates, algae | | Resolved Step 1 | | | | | | | | |
| Tier1 | Mesotrione | Macrophytes | | | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 |
| Tier1 | Thiencarbazone-methyl | Fish, inverte-brates, algae | | Resolved Step 1 | | | | | | | | |
| | | Macrophytes | | | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 4 [10 m vfs] | Resolved Step 4 [10 m vfs] |
| Tier 2C | Thiencarbazone-methyl | Macrophytes | | | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 |
| | Formulation | Fish, inverte-brates, algae, macrophytes | | | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | pH 5.1 & 6.5: Resolved Step 4 [10 m vfs] pH 7.9: Resolved Step 3 | pH 5.1 & 6.5: Resolved Step 4 [10 m vfs] pH 7.9: Resolved Step 3 | pH 5.1: Resolved Step 4 [10 m vfsmod or 20 m vfs] pH 6.5: Resolved Step 4 [10 m vfsmod or | pH 5.1: Resolved Step 4 [10 m vfsmod or 20 m vfs] pH 6.5: Resolved Step 4 [10 m vfsmod or |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | restriction on sloped areas] pH 7.9: Resolved Step 4 [10 m vfs] | restriction on sloped areas] pH 7.9: Resolved Step 4 [10 m vfs] |
| | Formulation | Algae, macro-phytes | Resolved [1 m bz] | | | | | | | | | |
| **0.2 L/ha banded application** | | | | | | | | | | | | |
| Tier 1 | All metabolites | Fish, inverte-brates, algae, macrophytes | | Resolved Step 1 | | | | | | | | |
| Tier1 | Mesotrione | Fish, inverte-brates, algae | | Resolved Step 1 | | | | | | | | |
| Tier1 | Mesotrione | Macrophytes | | | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 |
| Tier1 | Thiencarbazone-methyl | Fish, inverte-brates, algae | | Resolved Step 1 | | | | | | | | |
| | | Macrophytes | | | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 4 [10 m vfs] | Resolved Step 4 [10 m vfs] |
| Tier 2C | Thiencarbazone-methyl | Macrophytes | | | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 |
| | Formulation | Fish, inverte-brates, algae, macrophytes | | | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | pH 5.1: Resolved Step 4 [10 m vfs] pH 6.5 & 7.9: Resolved Step 3 | pH 5.1 & 7.9: Resolved Step 3 pH 6.5: Resolved Step 4 [10 m vfs] | pH 5.1 & 6.5: Resolved Step 4 [10 m vfsmod or 20 m vfs] pH 7.9: Resolved Step 4 [10 m vfs] | pH 5.1 & 6.5: Resolved Step 4 [10 m vfsmod or 20 m vfs] pH 7.9: Resolved Step 4 [10 m vfs] |
| | Formulation | Algae, macro-phytes | Resolved [2 m bz] | | | | | | | | | |
| **0.13 L/ha banded application** | | | | | | | | | | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tier 1 | All metabolites | Fish, inverte-brates, algae, macrophytes | | Resolved Step 1 | | | | | | | | |
| Tier1 | Mesotrione | Fish, inverte-brates, algae | | Resolved Step 1 | | | | | | | | |
| Tier1 | Mesotrione | Macrophytes | | | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 |
| Tier1 | Thiencarbazone-methyl | Fish, inverte-brates, algae | | Resolved Step 1 | | | | | | | | |
| | | Macrophytes | | | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 4 [10 m vfs] | Resolved Step 4 [10 m vfs] |
| Tier 2C | Thiencarbazone-methyl | Macrophytes | | | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 |
| | Formulation | Fish, inverte-brates, algae, macrophytes | | | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | Resolved Step 3 | pH 5.1 & 6.5: Resolved Step 4 [10 m vfs] pH 7.9: Resolved Step 3 | pH 5.1 & 7.9: Resolved Step 4 [10 m vfs] pH 6.5: Resolved Step 4 [10 m vfsmod or 20 m vfs] |
| | Formulation | Algae, macro-phytes | Resolved [1 m bz] | | | | | | | | | |

bz = no spray buffer zone, vfs = vegetated filter strip

**zRMS comments:**

**Mesotrine**

For the risk assessment in general the UE agreed endpoints for the active substance were used.

According to the EFSA aquatic guidance (2013): "*According to the data requirements, additional testing may be required by the Member State competent authorities on other macrophyte species depending on the mode of action of the substance, or if clear indications of higher toxicity are apparent to dicotyledonous (for example auxin inhibitor, broad leaf herbicides) or other monocotyledonous (e.g. grass herbicides) plant species from efficacy or testing with terrestrial non-target plants. Additional aquatic macrophyte species tests may be undertaken on a dicotyledonous species, such as Myriophyllum spicatum, Myriophyllum aquaticum or a monocotyledonous species, such as aquatic grass Glyceria maxima, as appropriate*." And "*Growth rate is the preferred endpoint*

*to be used since it is more robust considering varying test conditions*."

In EFSA Journal 2016;14(3):4419, for aquatic macrophyte data only for *Lemna gibba* is available and the EU agreed endpoint is not based on growth rates.

The applicant provided Letter of Access to results of two additional studies on toxicity of mesotrione to *Lemna gibba* (Hengsberger & Wydra, 2015 report amendment 2; Kosak & Wydra, 2016) and *Myriophyllum spicatum* (Gonsior, 2017). These studies were provided in Appendix 2 and assessed and accepted in Core dossier for Callisto 100 SC.

For aquatic macrophytes three approaches in the risk assessment were considered by the Applicant:
- based on $E_bC_{50}$, the EU agreed endpoint (EFSA Journal 2016;14(3):4419),
- based on the lowest $E_rC_{50}$ from additional study with *Lemna gibba* (Hengsberger & Wydra, 2015 report amendment 2; Kosak & Wydra, 2016), in line with EFSA aquatic guidance (2013),
- based on the geometric mean value calculated based on results of two additional studies on toxicity of mesotrione to *Lemna gibba* (Hengsberger & Wydra, 2015 report amendment 2; Kosak & Wydra, 2016) and *Myriophyllum spicatum* (Gonsior, 2017), in line with EFSA aquatic guidance (2013).

Taking into account the risk assessment based on $E_bC_{50}$ value for aquatic macrophytes, calculations performed with consideration of Tier 1 toxicity data were checked by the zRMS. When necessary, the PEC/RAC calculations with FOCUS Step 4 PECsw values were added.

However, it should be noted that the use of $E_bC_{50}$ values is not in line with EFSA aquatic guidance (2013) and should be thus dealt with at the national level.

Based on the PEC/RAC calculations (with $E_rC_{50}$ and geometric mean value) the risk to aquatic organisms is acceptable when:

<u>for the application rate at 75 g a.s./ha:</u>

- pH 5.1
  - acceptable risk is demonstrated with FOCUS Step 3 PECsw values for scenarios D3 ditch, D4 pond, D4 stream, D5 pond, D5 stream, D6 ditch, R1 pond, R1 stream, R2 stream and R3 stream (without risk mitigation measures);
  - acceptable risk is demonstrated with FOCUS Step 4 PECsw values for scenario R4 stream when 10 m VFS and 10 m buffer zone is applied;
- pH 6.5
  - acceptable risk is demonstrated with FOCUS Step 3 PECsw values for scenarios D3 ditch, D4 pond, D4 stream, D5 pond, D5 stream, D6 ditch, R1 pond, R1 stream and R2 stream (without risk mitigation measures);
  - acceptable risk is demonstrated with FOCUS Step 4 PECsw values for scenarios R3 stream and R4 stream when 10 m VFS and 10 m buffer zone is applied;
- pH 7.9
  - acceptable risk is demonstrated with FOCUS Step 3 PECsw values for all scenarios (without risk mitigation measures);

for the application rate at 48.75 g a.s./ha:

- pH 5.1
  - acceptable risk is demonstrated with FOCUS Step 3 PECsw values for all scenarios (without risk mitigation measures);
- pH 6.5
  - acceptable risk is demonstrated with FOCUS Step 3 PECsw values for all scenarios (without risk mitigation measures);
- pH 7.9
  - acceptable risk is demonstrated with FOCUS Step 3 PECsw values for all scenarios (without risk mitigation measures);

for the application rate at 75 g a.s./ha – banded application:

- pH 5.1
  - acceptable risk is demonstrated with FOCUS Step 3 PECsw values for all scenarios (without risk mitigation measures);
- pH 6.5
  - acceptable risk is demonstrated with FOCUS Step 3 PECsw values for all scenarios (without risk mitigation measures);
  -

for the application rate at 48.75 g a.s./ha – banded application:

- pH 5.1
  - acceptable risk is demonstrated with FOCUS Step 3 PECsw values for all scenarios (without risk mitigation measures);
- pH 6.5
  - acceptable risk is demonstrated with FOCUS Step 3 PECsw values for all scenarios (without risk mitigation measures);

**Metabolites of mesotrione**
For the risk assessment the UE agreed endpoints were used.
The risk assessment for metabolites MNBA, AMBA and SYN 5469774 was accepted using FOCUE Step 1 PECsw values.

**Thiencarbazone-methyl**
For the risk assessment in general the UE agreed endpoints for the active substance were used.
For aquatic macrophytes the geometric mean value at 1.87 µg a.s./L, based on $E_rC_{50}$ values from studies evaluated in the DAR were used for the risk assessment by Applicant, what is in line with EFSA aquatic guidance (2013).

However in EFSA Journal 2013;11(7):3270 the geometric mean $EC_{50}$ at 1.35 µg a.s./L was established. According to Addendum Two, April 2013: "As a conservative approach, a worst-case geometric mean endpoint has been calculated based on the lowest $EC_{50}$ value for each of *Lemna gibba* ($E_bC_{50}$ = 0.8 µg a.s./L), *Myriophyllum spicatum* ($E_yC_{50}$ length = 0.58 µg a.s./L) and *Potamogeton pectinatus* ($E_rC_{50}$ length = 5.3 µg a.s./L).

For completeness, the PEC/RAC calculations with geometric mean $EC_{50}$ at 1.35 µg a.s./L and FOCUS Step 1-4 PECsw values were added by zRMS.

Nevertheless, it should be noted that the calculation of geometric mean $EC_{50}$ at 1.35 µg a.s./L values is not in line with EFSA aquatic guidance (2013) and should be thus dealt with at the national level.

Based on the PEC/RAC calculations (with $E_rC_{50}$ geometric mean value) the risk to aquatic organisms is acceptable when:

<u>for the application rate at 15 g a.s./ha:</u>

- acceptable risk is demonstrated with FOCUS Step 3 PECsw values for scenarios D3 ditch, D4 pond, D4 stream, D5 pond, D5 stream, D6 ditch and R1 pond (without risk mitigation measures);
- acceptable risk is demonstrated with FOCUS Step 4 PECsw values for scenario:
  - R1 stream and R2 stream when 10 m VFS and 10 m buffer zone is applied;
  - R3 stream and R4 stream when 20 m VFS and 20 m buffer zone or 10 m vsfmod is applied;

<u>for the application rate at 9.75 g a.s./ha:</u>

- acceptable risk is demonstrated with FOCUS Step 3 PECsw values for scenarios D3 ditch, D4 pond, D4 stream, D5 pond, D5 stream, D6 ditch R1 pond, R1 stream and R2 stream (without risk mitigation measures);
- acceptable risk is demonstrated with FOCUS Step 4 PECsw values for scenario R3 stream and R4 stream when 10 m VFS and 10 m buffer zone is applied;

<u>for the application rate at 15 g a.s./ha – banded application</u>

- acceptable risk is demonstrated with FOCUS Step 3 PECsw values for scenarios D3 ditch, D4 pond, D4 stream, D5 pond, D5 stream, D6 ditch R1 pond, R1 stream and R2 stream (without risk mitigation measures);
- acceptable risk is demonstrated with FOCUS Step 4 PECsw values for scenario R3 stream and R4 stream when 10 m VFS and 10 m buffer zone is applied;

<u>for the application rate at 9.75 g a.s./ha – banded application</u>

- acceptable risk is demonstrated with FOCUS Step 3 PECsw values for all scenarios (without risk mitigation measures);

For the refinement the PEC/RAC, calculations with VFSMod was presented. The use of VFSMod should be considered at the Member State level.

Additionally, in order to refine the risk to aquatic organisms the Applicant provided Tier 2C refined exposure approach, based on results of the pulse exposure studies on *Lemna gibba* and *Myriophyllum spicatum* combined with Exposure Pattern Analysis (EPAT). The Exposure Pattern Analysis has been assessed in Part B8 and can be considered as supporting information.
Regarding Tier 2C studies, it is noted that in general, new Annex II studies should not be evaluated at the zonal level and the risk assessment should be based on the EU agreed endpoints. As no data gap regarding further testing of toxicity of thiencarbazone-methyl on aquatic macrophytes was identified in the EFSA Journal 2013;11(7):3270, newly submitted studies are deemed not necessary to finalisation of the aquatic risk assessment. Moreover, according to *Working document*

*on Risk Assessment of Plant Protection Products in the Central Zone, Ecotoxicology* Version 3.0, December 2024, the most of the Central Zone Member States has concerns regarding reliability the modified exposure studies. Although no final agreement was reached, the most MSs consider that the Tier 2C approach should generally not be supported at zonal level. And if a conclusion of low risk based on a lower tier approach with RMM is possible this should be favoured over a conclusion based on a Tier 2C approach, considering the uncertainties related to such a Tier 2C approach.
For thiencarbazone-methyl, applied as GLOB2112dH / Walkover Trio, acceptable risk to aquatic organisms could be concluded using the geometric mean endpoint calculated based on Tier 1 data, applying standard risk mitigation measures. Taking this into account, no further assessment was deemed necessary at the zonal level and provided refinement based on Tier 2C studies was not evaluated by the zRMS.

This approach should be considered at the Member State level.

**Metabolites of Thiencarbazone-methyl**
For the risk assessment the UE agreed endpoints were used.
The risk assessment for metabolites BYH 18363-carboxylic acid, BYH 18363-sulfonamide, BYH 18363-sulfonamide carboxylic acid, BYH 18363-MMT, BYH 18363-dicarboxysulfonamide and BYH 18363-triazolinone carboxamide was accepted using FOCUE Step 1 PECsw values.

**Formulation GLOB2112dH / Walkover Trio**
For GLOB2112dH, tests on algae *Pseudokirchneriella subcapitata*, and on aquatic plants *Lemna gibba* and *Myriophyllum spicatum* were provided by Applicant, but no tests are reported for fish and daphnia.
According to the Commission Regulation (EU) No 284/2013, point 10.2.1 Acute toxicity:
" *Test shall be carried out on one species from each of the three/four groups of aquatic organisms, that is to say fish, aquatic invertebrates, algae…*"
and
"*Testing shall be performed where:*
*(a) the acute toxicity of the plant protection product cannot be predicted on the basis of the data for the active substance*;.."

**Acute toxicity data for active substance and formulation**

| **Species** | **Mesotrione** | **Thiencarbazone-methyl** | **GLOB1310aH** |
|---|---|---|---|
| | **Endpoint** | **Endpoint** | **Endpoint** |
| *Oncorhynchus mykiss* | > 120 mg a.s./L | > 104 mg/L | - |
| *Daphnia magna* | > 622 mg a.s./L | > 98.6 mg a.s./L | - |

| | | | |
|---|---|---|---|
| *Pseudokirchneriella subcapitata* | 13 mg a.s./L | 1.02 mg a.s./L | 87.1 mg product/L corresponding to 28.09 mg Mesotrione./L and 5.55 mg Thiencarba-zone-methyl /L |
| *Lemna gibba* | 0.0241mg a.s./L | 1.31 mg a.s./L | 0.183 mg product/L corresponding to 0.0059 mg Mesotrione./L and 0.00117 mg Thiencarba-zone-methyl /L |
| *Myriophyllum spicatum* | 0.0287 mg/L | 0.94 mg a.s./L | 0.142 mg product/L corresponding to 0.00458 mg Mesotri-one./L and 0.0009 mg Thiencarbazone-methyl /L |

Based on the aquatic acute data for active substances is not expected that the formulation to be more sensitive to fish and daphnia than algae and macrophytes. Moreover from the combined risk assessment for fish and daphnia the risk is acceptable on a screening level. It can be assumed that acute toxicity to fish and daphnia for formulation can be predicted on the basis of data for the active.

The combined risk assessment for formulation was based on the stepwise approach according to the decision scheme (EFSA Journal 2013;11(7):3290), using the tool AGD_AquaMix_v1.22. It was checked by zRMS using the same tool and Tier 1 tox data in the standard data part, presented in the table below:

| Product data | |
|---|---|
| Product name | GLOB2112dH |
| Density of product [g/cm³] | 1,2153 |
| $LC_{50}$fish [mg prod./L] | |
| $LC_{50}$fish a.s. based [mg sum of a.s./L] | |
| $EC_{50}$invertebrates [mg prod./L] | |
| $LC_{50}$invertebrates a.s. based [mg sum of a.s./L] | |
| $EC_{50}$algae [mg prod./L] | 87,1 |
| $EC_{50}$algae a.s. based [mg sum of a.s./L] | 32,2513 |
| $EC_{50}$macrophytes [mg prod./L] | 0,0142 |
| $EC_{50}$macrophytes a.s. based [mg sum of a.s./L] | 0,0053 |

| Calculated mixture toxicity (Eq. 13) based on Tier 1 data only | [mg sum a.s./L] |
|---|---|
| $ECx_{mix\,CA}$fish | 117 |
| $ECx_{mix\,CA}$invertebrates | 330,0226009 |
| $ECx_{mix\,CA}$algae | 4,39558011 |
| $ECx_{mix\,CA}$macrophytes | 0,004846347 |

| Calculated mixture toxicity (Eq. 13) based also on additional data | [mg sum a.s./L] |
|---|---|
| $ECx_{mix\,CA}$fish | 117 |
| $ECx_{mix\,CA}$invertebrates | 330,0226009 |
| $ECx_{mix\,CA}$algae | 4,39558011 |
| $ECx_{mix\,CA}$macrophytes | 0,004846347 |

**Options**

Show Species

Show In-between calculations (new sheet)

| Active Substance (a.s.) standard data (Tier 1 EP) | | | | |
|---|---|---|---|---|
| Active substance names | TCM | MST pH 5.1 | | |
| Concentration in product [g a.s./L or g a.s./kg] | 75 | 375 | | |
| p(X) (fraction in product) | 0,17 | 0,83 | | |
| $LC_{50}$fish [mg a.s./L] | 104 | 120 | | |
| $LC_{50}$invertebrates [mg a.s./L] | 98,6 | 622 | | |
| $EC_{50}$algae [mg a.s./L] | 1,02 | 13 | | |
| $EC_{50}$macrophytes [mg a.s./L] | 0,00094 | 0,0287 | | |
| Additional a.s. data (i.e. most sensitive species tested as Tier 1 data or refinements Tier 2A/B EP) | | | | |
| $LC_{50}$fish [mg a.s./L] | | | | |
| $LC_{50}$invertebrates [mg a.s./L] | | | | |
| $EC_{50}$algae [mg a.s./L] | | | | |
| $EC_{50}$macrophytes [mg a.s./L] | | | | |
| AF for RAC | | | | |
| Fish | 100 | 100 | 100 | 100 |
| Invertebrates | 100 | 100 | 100 | 100 |
| Algae | 10 | 10 | 10 | 10 |
| Macrophytes | 10 | 10 | 10 | 10 |
| RAC | | | | |
| Fish | 1,04 | 1,2 | | |
| Invertebrates | 0,986 | 6,22 | | |
| Algae | 0,102 | 1,3 | | |
| Macrophytes | 0,000094 | 0,00287 | | |

| Data used for calculation (after Step 3) | | | | |
|---|---|---|---|---|
| Active substances | TCM | MST pH 5.1 | | |
| Concentration in Product [g a.s./L] | 75 | 375 | | |
| p(X) (fraction in product) | 0,17 | 0,83 | | |
| $LC_{50}$fish [mg a.s./L] | 104 | 120 | | |
| $LC_{50}$invertebrates [mg a.s./L] | 98,6 | 622 | | |
| $EC_{50}$algae [mg a.s./L] | 1,02 | 13 | | |
| $EC_{50}$macrophytes [mg a.s./L] | 0,00094 | 0,0287 | | |

The risk is acceptable when the appropriate risk mitigation measures were applied.

Additionally, the risk assessment for formulation was performed based on the comparison of the formulation toxicity values and PECdrift values of the formulation.
For application rate 0.2 L/ha the risk is acceptable when the 2m buffer zone is applied.
For application rate 0.13 L/ha the risk is acceptable without risk mitigation measures.

**Conclusion**

According to the performed risk assessment there is no potential of risk for aquatic organisms resulting from acute and long-term exposure to active substances and their metabolites following use of GLOB2112dH / Walkover Trio in compliance with proposed GAP when the appropriate risk mitigation measures were applied.

The appropriate risk mitigation measures should be considered at national level. If it is necessary Member States will need to further consider the risk to aquatic organisms based on national requirements.

## 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, thiencarbazone-methyl and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on bees of GLOB2112dH were not evaluated as part of the EU assessment of mesotrione and thiencarbazone-methyl. New data submitted with this application are listed in Table 9.6-1 and summarised in Appendix 2.

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

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

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Apis mellifera* | Mesotrione | Acute, oral | **$LD_{50}$ > 11 µg/bee** | EFSA, 2016<br>Jackson D., Gough H.J., 1995 |
| *Apis mellifera* | Mesotrione | Acute, contact | **$LD_{50}$ > 100 µg/bee** | EFSA, 2016<br>Jackson D., Gough H.J., 1995 |
| *Apis mellifera* | Thiencarbazone-methyl | Acute, oral | **48 h $LD_{50}$ > 199 µg/bee** | EFSA, 2013<br>Barth M., 2005 |
| *Apis mellifera* | Thiencarbazone-methyl | Acute, contact | **48 h $LD_{50}$ > 200 µg/bee** | EFSA, 2013<br>Barth M., 2005 |
| *Apis mellifera* | GLOB2112dH | Acute, oral | $LD_{50}$ > 547.5 µg/bee | Schabio S., 2024a |
| *Apis mellifera* | GLOB2112dH | Acute, contact | $LD_{50}$ = 500 µg/bee | Schabio S., 2024a |
| *Bombus terrestris* | GLOB2112dH | Acute, oral | $LD_{50}$ > 564.8 µg/bee | Chiewsko D., 2023 |
| *Bombus terrestris* | GLOB2112dH | Acute, contact | $LD_{50}$ > 500 µg/bee | Chiewsko D., 2023 |
| *Apis mellifera* | GLOB2112dH | Chronic, adult | NOEDD ≥ 98.92 µg/bee/d<br>**$LDD_{50}$ > 98.92 µg/bee/d** | Venturi S., 2023 |
| *Apis mellifera* | GLOB2112dH | Chronic, larvae | **NOED = 33.33 µg/bee** | Venturi S., 2024 |
| **Higher-tier studies (tunnel test, field studies)** | | | | |
| - | | | | |

#### 9.6.1.1 Justification for new endpoints

Effects on bees of GLOB2112dH were not evaluated as part of the EU assessment of mesotrione and thiencarbazone-methyl. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

### 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).
To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group 1 also covers the risk for bees from all other intended uses in group 2 (see 9.1.2).

#### 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 GLOB2112dH in maize**

| | | | |
|---|---|---|---|
| **Intended use**<br>**Active substance**<br>**Application rate (g/ha)** | Maize<br>Mesotrione<br>1 × 75 | | |
| **Test design** | **$LD_{50}$ (lab.) (µg/bee)** | **Single application rate (g/ha)** | **$Q_{HO}$, $Q_{HC}$ criterion: $Q_H \leq 50$** |
| Oral toxicity | 11 | 75 | 6.8 |
| Contact toxicity | 100 | | 0.75 |
| **Active substance**<br>**Application rate (g/ha)** | Thiencarbazone-methyl<br>1 × 15 | | |
| **Test design** | **$LD_{50}$ (lab.) (µg/bee)** | **Single application rate (g/ha)** | **$Q_{HO}$, $Q_{HC}$ criterion: $Q_H \leq 50$** |
| Oral toxicity | > 199 | 15 | 0.075 |
| Contact toxicity | > 200 | | 0.075 |
| **Product**<br>**Application rate (g/ha)** | GLOB2112dH<br>1 × 242 | | |
| **Test design** | **$LD_{50}$ (lab.) (µg/bee)** | **Single application rate (g/ha)** | **$Q_{HO}$, $Q_{HC}$ criterion: $Q_H \leq 50$** |
| Oral toxicity | 547.5 | 242 | 0.44 |
| Contact toxicity | 500 | | 0.48 |

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

**Acute risk assessment to bees according to EFSA 2013**

| **Contact & Oral route of exposure (pollen and nectar)** | | | | |
|---|---|---|---|---|
| | "calculation factor" (linked with dust) | HQ | Trigger | Risk indicator |
| HB – acute: contact | 1 | 0.5 | 42 | OK |
| | "calculation factor" (Ef x SV) | ETR | Trigger | Risk indicator |
| HB – acute: oral | 7.6 | 0.00 | 0.2 | OK |

No unacceptable acute risk is present for honey bees.

### 9.6.3 Chronic risk assessment (KCP 10.3.1.2)

#### 9.6.3.1 Larval chronic risk assessment

A chronic larval study is available and the potential acceptable risk can be further demonstrated by carrying out a worst-case risk assessment through the calculation of a TER value as set out in the modified EPPO 2010 approach according to the ECPA proposal of 9 June 2017 (POS/17/LO/28028).

The default residues, based on the EFSA 2013 bee guidance, can be combined with a measure of consumption in order to estimate the exposure. Worst case data from ***Rortais et al., 2005***[6], as proposed in the EPPO scheme, have been used to estimate the consumption by bee larvae:

Worker larvae consuming 59.4 mg sugar in 5 days Assuming 30% sugar content of nectar the worst-case consumption with worker larvae is:

59.4/0.30 = 198 mg nectar in 5 days.

In addition worker larvae are considered to consume 2 mg pollen during their development phase (EFSA 2013).

Thus considering the mean RUD values for nectar and pollen in EFSA 2013 exposure can be estimated for the whole development period.

Nectar dose: 0.242 x 2.9 x 198/1000 = 0.1390 µg/larva
Pollen dose: 0.242 x 6.1 x 2/1000 = 0.00295 µg/larva

Total exposure ETE = 0.142 µg/larvae (as a default worst-case residue at 0.242 kg a.s./ha)

This can be compared to the larval NOED of 33.33 µg/larva.

TER = NOEDD (µg/larva)/ ETE (µg/larva) = 33.33/0.142 = 235

The EPPO 2010 scheme proposes a trigger of 1 for assessment of the chronic risk to honey bees. It is clear that with a TER value of 78.29, the proposed uses of GLOB2112dH pose an acceptable risk to bee larval development.

The risk assessment was also conducted according to the "EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees)" (EFSA Journal 2013;11(7):3295).

*Chronic oral exposure larvae (liquid formulations):*

Screening step assessment for spray applications:

ETR = AR*SV/NOEL = 0.242*4.4/33.33 = 0.03

The protection goal is met as the calculated value is below the trigger value of 0.2.

[6] Agnès RORTAIS, Gérard ARNOLD, Marie-Pierre HALM, Frédérique TOUFFET-BRIENS (2005). Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. Apidologie 36 (2005) 71–83

### 9.6.3.2 Adult chronic risk assessment

The adult chronic risk assessment is performed using the modified EPPO 2010 approach according to the ECPA proposal of 9 June 2017 (POS/17/LO/28028).

This is based upon the method of EPPO 2010 risk assessment for systemic substances which is cited in the regulation as a current risk assessment scheme. It uses NOEDD values for the endpoint so avoids the issues associated with the generation of $LDD_{50}$ values for substances of low toxicity, and calculates exposure in a similar way to EFSA 2013. The approach is also in line with other chronic risk assessments (e.g. birds and mammals). EPPO 2010 recommended the calculation of a TER using the following equation:

TER = NOEDD/daily dose

Where daily dose (DD) is based on the worst case a sugar need of 128 mg/bee/day (Rortais et al 2005) of a bee feeding exclusively from nectar containing 30% sugar using the following equation:

Daily dose (µg a.i./bee) = A.R. x [128 mg/(1000 x0.3)] x RUD = 0.242 x [128/(1000x0.3)] x 2.9 = 0.2994 µg/bee

A.R. = application rate in kg a.i./ha
RUD = residue per unit dose from the EFSA bee guidance. Mean RUDnectar = 2.9 mg a.i./kg (foliar sprays).

TER = NOEDD/daily dose = 98.92/0.2994 = 330

The EPPO 2010 scheme proposes a trigger of 1 for assessment of the chronic risk to honey bees. It is clear that with a TER value of 330, the proposed uses of GLOB2112dH pose an acceptable chronic risk to adult bees.

The risk assessment was also conducted according to the "EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees)" (EFSA Journal 2013;11(7):3295).

*Chronic oral exposure adult bees (liquid formulations):*

Screening step assessment for spray applications:

ETR = AR*SV/10d $LDD_{50}$ = 0.242*7.6/98.92 = 0.019

The protection goal is met as the calculated value is below the trigger value of 0.03.

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

Not relevant.

### 9.6.4 Effects on bumble bees

The effect of GLOB2112dH on bumble bees was assessed according to the "EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees)" (EFSA Journal 2013;11(7):3295).

*Contact exposure assessment for spray applications*

Screening step assessment for spray applications:

$HQ_{contact} = AR/LD_{50contact} = 242/500 = 0.5$

The protection goal is met as the calculated value is below the trigger value of 7.

*Oral exposure assessment for spray applications*

Screening step assessment for spray applications:

$ETR_{acute\ adult\ oral} = AR*SV/LD_{50oral} = 0.242*11.2/564.8 = 0.005$

The protection goal is met as the calculated value is below the trigger value of 0.036.

### 9.6.5 Effects on solitary bees

Not required.

### 9.6.6 Overall conclusions

The risk to bees when applying GLOB2112dH according to the intended uses is acceptable.

**zRMS Comments:**

The submitted risk assessment is based on the recommendations of the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 rev 2) and new EU guidance (2013).

The EU agreed endpoints for active substance were used in risk assessment.
In addition to that, the Applicant submitted studies on chronic toxicity of formulation GLOB2112dH to adult bees and larvae. New studies were accepted. Therefore, the requirements set out in Regulation 284/2013 are considered fulfilled.

The acute risk assessment performed in accordance with the SANCO guidance presented by the Applicant was accepted.

There is currently no EU agreed chronic risk assessment scheme for bees. However, as agreed in the Central Zone a risk assessment based on the EFSA bee GD is presented below for illustrative purposes.

The ETR values are less than the Tier 1 trigger values for downward sprays indicating that the chronic risk to honeybee larvae and bees is acceptable following use of GLOB2112dH (Walkover Trio) according to the proposed use pattern.

An acceptable risk to bees of the formulation GLOB2112dH (Walkover Trio) can be concluded, based on the risk assessment scheme of the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 rev 2).

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

### 9.7.1 Toxicity data

Studies on the toxicity to non-target arthropods have been carried out with mesotrione, thiencarbazone-

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

Effects on non-target arthropods of GLOB2112dH were not evaluated as part of the EU assessment of mesotrione and thiencarbazone-methyl. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Typhlodromus pyri* (protonymphs) | GLOB2112dH | Laboratory test glass plates (2D) | $LR_{50}$ > 200.1 mL/ha<br>$ER_{50}$ > 200.1 mL/ha | Leopold J., 2023a |
| *Aphidius rhopalosiphi* (adults) | GLOB2112dH | Laboratory test glass plates (2D) | $LR_{50}$ > 200.2 mL/ha<br>$ER_{50}$ > 200.2 mL/ha | Leopold J., 2023b |
| **Field or semi-field tests** | | | | |
| - | | | | |

### 9.7.1.1 Justification for new endpoints

Effects on non-target arthropods of GLOB2112dH were not evaluated as part of the EU assessment of mesotrione and thiencarbazone-methyl. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

## 9.7.2 Risk assessment

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

### 9.7.2.1 Risk assessment for in-field exposure

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group 1 also covers the risk for non-target arthropods from all other intended uses in group 2 (see 9.1.2).

**Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of GLOB2112dH in maize**

| | |
|---|---|
| **Intended use** | Maize |
| **Active substance/product** | GLOB2112dH |
| **Application rate (L/ha)** | 1 × 0.2 |
| **MAF** | - |

| **Test species Tier I** | **$LR_{50}$ (lab.) (mL/ha)** | **$PER_{in\text{-}field}$ (mL/ha)** | **$HQ_{in\text{-}field}$ criterion: HQ ≤ 2** |
|---|---|---|---|
| *Typhlodromus pyri* | 200.1 | 200 | 1 |
| *Aphidius rhopalosiphi* | 200.2 | | 1 |

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient; DALT: Days after last treatment. Criteria values shown in bold breach the relevant trigger.

### 9.7.2.2 Risk assessment for off-field exposure

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group 1 also covers the risk for non-target arthropods from all other intended uses in group 2 (see 9.1.2).

**Table 9.7-3: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of GLOB2112dH in maize**

| | |
|---|---|
| **Intended use** | Maize |
| **Active substance/product** | GLOB2112dH |
| **Application rate (L/ha)** | 1 × 0.2 |
| **MAF** | - |
| **vdf** | 10 (Tier 1)<br>(5 (for information)) |

| **Test species Tier I** | **$LR_{50}$ (lab.) (mL/ha)** | **Drift rate** | **$PER_{off\text{-}field}$ (mL/ha)** | **CF** | **$HQ_{off\text{-}field}$ criterion: HQ ≤ 2** |
|---|---|---|---|---|---|
| *Typhlodromus pyri* | 200.1 | 0.0277 | 0.554<br>(1.108) | 10 | 0.028<br>(0.0554) |
| *Aphidius rhopalosiphi* | 200.2 | | | | 0.028<br>(0.0554) |

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

### 9.7.2.3 Additional higher-tier risk assessment

Not relevant.

### 9.7.2.4 Risk mitigation measures

No risk mitigation needed.

### 9.7.3 Overall conclusions

The risk for non-target arthropods is acceptable when applying GLOB2112dH according to the intended uses.

**zRMS Comments:**
The submitted risk assessment based on the "Guidance Document on Terrestrial Ecotoxicology" (2002) was accepted.

Acceptable risk may be concluded for in-field and off-field populations of non-target arthropods from the intended uses of GLOB2112dH (Walkover Trio).

**Conclusion**
The risk to arthropods other than bees is acceptable if the GLOB2112dH (Walkover Trio) is applied in accordance with proposed use pattern.

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

### 9.8.1 Toxicity data

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

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of GLOB2112dH were not evaluated as part of the EU assessment of mesotrione and thiencarbazone-methyl. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Eisenia fetida* | Mesotrione (based on A12739A) | Chronic, 56 d | **NOEC = 125 mg A12739A/kg dw (equivalent to 10.85 mg a.s./kg d.w. soil)**<br>**$EC_{10}$ = 5.91 mg a.s./kg dw** | EFSA, 2016<br>Friedrich S., 2011 |
| *Eisenia fetida* | AMBA | Chronic, 56 d | **NOEC = 1050 mg/kg dw**<br>$EC_{10}$ = 1050 mg/kg dw | EFSA, 2016<br>Friedrich S., 2013 |
| *Eisenia fetida* | MNBA | Chronic, 56 d | **NOEC = 1050 mg/kg dw**<br>$EC_{10}$ = 1050 mg/kg dw | EFSA, 2016<br>Friedrich S., 2013 |
| *Eisenia fetida* | Thiencarbazone-methyl (based on SC450) | Chronic, 56 d | **NOEC = 7.58 mg/kg dw soil** | EFSA, 2013 |
| *Eisenia fetida* | BYH 18636-carboxylic acid (M01) | Chronic, 56 d | **NOEC = 1000 mg/kg dw soil** | EFSA, 2013<br>Lechelt-Kunze C., 2005 |
| *Eisenia fetida* | BYH 18636-sulfonamide-carboxylic acid | Chronic, 56 d | **NOEC = 100 mg/kg dw soil** | EFSA, 2013<br>Luehrs U., 2006 |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| | (M03) | | | |
| *Eisenia fetida* | BYH 18636-sulfonamide (M15) | Chronic, 56 d | **NOEC = 100 mg/kg dw soil** | EFSA, 2013<br>Friedrich S., 2006 |
| *Eisenia fetida* | BYH 18636-MMT (M21) | Chronic, 56 d | **NOEC = 316 mg/kg dw soil** | EFSA, 2013<br>Luehrs U., 2006 |
| *Folsomia candida* | Mesotrione (based on A12739A) | Chronic, 14 d | **NOEC = 50.54 a.s.mg/kg dw** (equivalent to 556 mg A12739A /kg d.w. soil)<br>$EC_{10}$ = 37.5 mg a.s./kg dw | EFSA, 2016<br>Friedrich S., 2013 |
| *Folsomia candida* | MNBA | Mixed into substrate 28 d, chronic 5 % peat content | NOEC = 180 mg/kg dw<br>$EC_{10}$ = 134 mg pm/kg dw | Dickinson, 2015<br>CA3511_10011 |
| *Folsomia candida* | Thiencarbazone-methyl | Chronic, 28 d | **NOEC = 1000 mg/kg dw soil** | EFSA, 2013<br>Frommholz U., 2006 |
| *Folsomia candida* | BYH 18636-carboxylic acid (M01) | Chronic, 28 d | **NOEC = 1000 mg/kg dw soil** | EFSA, 2013<br>Frommholz U., 2005 |
| *Folsomia candida* | BYH 18636-sulfonamide-carboxylic acid (M03) | Chronic, 28 d | **NOEC = 1000 mg/kg dw soil** | EFSA, 2013<br>Friedrich S., 2006 |
| *Folsomia candida* | BYH 18636-triazolinone-carboxamide (M20) | Chronic, 28 d | **NOEC = 1000 mg/kg dw soil** | EFSA, 2013<br>Friedrich S., 2006 |
| *Folsomia candida* | BYH 18636-MMT (M21) | Chronic, 28 d | **NOEC = 1000 mg/kg dw soil** | EFSA, 2013<br>Friedrich S., 2006 |
| *Hypoaspis aculeifer* | Mesotrione (based on A12739A) | Chronic, 28 d | **NOEC = 90.9 mg/kg dw**<br>$EC_{10}$ > 90.9 mg a.s./kg dw | EFSA, 2016<br>Schulz L., 2013 |
| *Hypoaspis aculeifer* | MNBA | Mixed into substrate 14 d, chronic 5 % peat content | NOEC = 1050 mg/kg dw<br>$EC_{10}$ could not be calculated | Ramsden, 2015<br>CA3511_10010 |
| *Eisenia fetida* | GLOB2112dH | Mixed into substrate 56 d, chronic 10 % peat content | $NOEC_{mortality} \geq 25$ mg/kg dw<br>**$NOEC_{reproduction}$ = 1.32 mg/kg dw**<br>$EC_{10}$ = 1.49 mg/kg dw<br>$EC_{20}$ = 1.99 mg/kg dw<br>$EC_{50}$ = 3.48 mg/kg dw | Hübner S., 2024 |
| *Folsomia candida* | GLOB2112dH | Mixed into substrate 28 d, chronic 5 % peat content | $NOEC_{mortality} \geq 1000$ mg/kg dw<br>$NOEC_{reproduction}$ = 309 | Hübner S., 2023a |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| | | | mg/kg dw<br>**$EC_{10}$ = 306.7 mg/kg dw**<br>$EC_{20}$ = 454.3 mg/kg dw<br>$EC_{50}$ = 963.6 mg/kg dw | |
| *Hypoaspis aculeifer* | GLOB2112dH | Mixed into substrate<br>14 d, chronic<br>5 % peat content | $NOEC_{mortality}$ = 95.4 mg/kg dw<br>**$NOEC_{reproduction}$ = 95.4 mg/kg dw**<br>$EC_{10}$ = 105.6 mg/kg dw<br>$EC_{20}$ = 152.2 mg/kg dw<br>$EC_{50}$ = 306.0 mg/kg dw | Hübner S., 2023b |
| **Field studies** | | | | |
| - | | | | |
| **Litter bag test** | | | | |
| - | | | | |

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

#### 9.8.1.1 Justification for new endpoints

Studies with Hypoaspis for thiencarbazone-methyl and metabolites have not been performed for the Annex I inclusion and thus no studies peer-reviewed on EU level are available.

### 9.8.2 Risk assessment

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

#### 9.8.2.1 First-tier risk assessment

The relevant $PEC_{soil}$ for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3. According to the assessment of environmental-fate data, multi-annual accumulation in soil does not need to be considered for mesotrione and thiencarbazone-methyl.
To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group 1 also covers the risk for earthworms and other non-target soil organisms (meso- and macrofauna) from all other intended uses in group 2 (see 9.1.2).

**Table 9.8-2: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of GLOB2112dH in maize**

| **Intended use** | Maize | | |
|---|---|---|---|
| **Chronic effects on earthworms** | | | |
| **Product/active substance** | **NOEC (mg/kg dw)** | **$PEC_{soil}$ (mg/kg dw)** | **$TER_{lt}$ (criterion TER ≥ 5)** |
| Mesotrione | ~~125~~ 5.91 | 0.0750 | ~~1667~~ 78.8 |
| AMBA | 1050 | 0.0046 | 228261 |
| MNBA | 1050 | 0.0310 | 33871 |
| Thiencarbazone-methyl | 7.58 | 0.0150 | 505 |
| BYH 18636-carboxylic acid (M01) | 1000 | ~~0.0064~~ 0.0103** | ~~156250~~ 97087 |
| BYH 18636-sulfonamide-carboxylic acid (M03) | 100 | 0.0018 | 55556 |
| BYH 18636-sulfonamide (M15) | 100 | 0.0014 | 71429 |
| BYH 18636-MMT (M21) | 316 | 0.0010 | 316000 |
| GLOB2112dH | 1.32 | 0.2430 | 5.4 |
| **Chronic effects on other soil macro- and mesofauna** | | | |
| **Product/active substance** | **NOEC (mg/kg dw)** | **$PEC_{soil}$ (mg/kg dw)** | **$TER_{lt}$ (criterion TER ≥ 5)** |
| *Folsomia candida* | | | |
| Mesotrione | ~~50.54~~ 37.5 | 0.0750 | ~~674~~ 500 |
| MNBA | 134 | 0.0310 | 4323 |
| AMBA | 3.75* | 0.0046 | 815 |
| Thiencarbazone-methyl | 1000 | 0.0150 | 66667 |
| BYH 18636-carboxylic acid (M01) | 1000 | ~~0.0064~~ 0.0103** | ~~156250~~ 97087 |
| BYH 18636-sulfonamide-carboxylic acid (M03) | 1000 | 0.0018 | 555556 |
| BYH 18636-triazolinone-carboxamide (M20) | 1000 | 0.0005 | 2000000 |
| BYH 18636-MMT (M21) | 1000 | 0.0010 | 1000000 |
| GLOB2112dH | 306.7 | 0.2430 | 1262 |
| *Hypoaspis aculeifer* | | | |
| Mesotrione | 90.9 | 0.0750 | 1212 |
| MNBA | 1050 | 0.0310 | 33871 |
| AMBA | 9.09* | 0.0046 | 1976 |
| GLOB2112dH | 95.4 | 0.2430 | 393 |

TER values shown in bold fall below the relevant trigger.

*Tests with AMBA have not been carried out, and the risk assessment has been performed on the basis of the end-point for the parent divided by 10.

**Corrected according to Part B8

### 9.8.2.2 Higher-tier risk assessment

Not relevant.

### 9.8.3 Overall conclusions

The risk for soil meso- and macro-organisms is acceptable when applying GLOB2112dH according to the intended uses.

**zRMS comments:**

$PEC_{soil}$ values were calculated considering GAP of Walkover Trio. The highest predicted environmental concentrations ($PEC_{soil}$) of the active substances, major metabolites and formulation were taken into account for the risk assessment.

**Mesotrione**
For the risk assessment generally the EU agreed endpoints were used.

The Letters of Access for Mesotrione and Callisto 100 SC data were provided by applicant. Two additional studies on toxicity of metabolite MNBA to *Folsomia candida* and *Hypoaspis aculeifer*, which were assessed and accepted for the risk assessment, were presented in dRR for Callisto 100 SC (2020). For completeness, studies summary were added in Appendix 2 and studies results were used in the risk assessment in this submission by zRMS.

No additional studies on toxicity of metabolite AMBA to *Folsomia candida* and *Hypoaspis aculeifer* were provided. Therefore the risk assessment should be performed with the assumption that this metabolite is 10 times more toxic than the parent.

The lower of NOEC and $EC_{10}$ value should be used in the risk assessment.

All TER values are above trigger value of 5.

**Thiencarbazone-methyl**
For the risk assessment the EU agreed endpoints were used.
All TER values are above trigger value of 5.

**Formulation GLOB2112dH / Walkover Trio**
The risk assessment was performed based on the endpoint from the formulation studies.
All TER values are above trigger value of 5.

**Conclusion**:
According to the performed risk assessment there is low chronic risk to earthworms and other non-target organisms resulting from long-term exposure to active substances and their metabolites following use of GLOB2112dH / Walkover Trio in compliance with proposed GAP.

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

### 9.9.1 Toxicity data

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

Effects on soil microorganisms of GLOB2112dH were not evaluated as part of the EU assessment of mesotrione and thiencarbazone-methyl. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

| Endpoint | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| N-mineralisation | Mesotrione (based on A12739A) | 28 d, aerobic natural soil | **7.8 % effect at day 28 at 0.53 mg a.s./kg soil dw** (equivalent to 5.84 mg A12739A/ kg soil dw) | EFSA, 2016 Schulz L., 2013 |
| N-mineralisation | AMBA | 28 d, aerobic natural soil | **-7.6 % effect at day 28 at 1.13 mg/kg soil dw** | EFSA, 2016 Schulz L., 2013 |
| N-mineralisation | MNBA | 28 d, aerobic natural soil | **-4.8 % effect at day 28 at 1.13 mg/kg soil dw** | EFSA, 2016 Schulz L., 2013 |
| N-mineralisation | Thiencarbazone-methyl | 28 d | **2% at 0.3 mg/kg dw soil** | EFSA, 2013 Lechelt-Kunze C., 2005 |
| N-mineralisation | BYH 18636-carboxylic acid (M01) | 28 d | **0% at 0.29 mg/kg dw soil** | EFSA, 2013 Lechelt-Kunze C., 2005 |
| N-mineralisation | BYH 18636-sulfonamide-carboxylic acid (M03) | 28 d | **12% at 0.17 mg/kg dw soil** | EFSA, 2013 Heimbach F., 2006 |
| N-mineralisation | BYH 18636-sulfonamide (M15) | 28 d | **18% at 0.18 mg/kg dw soil** | EFSA, 2013 Heimbach F., 2006 |
| N-mineralisation | BYH 18636-MMT (M21) | 28 d | **13% at 0.10 mg/kg dw soil** | EFSA, 2013 Heimbach F., 2006 |
| N-mineralisation | GLOB2112dH | 28 d, aerobic loamy sand | **No effects > 25% at 0.32 and 1.62 mg/kg soil dw** | Hammesfahr U., 2023 |

#### 9.9.1.1 Justification for new endpoints

Effects on soil microorganisms of GLOB2112dH were not evaluated as part of the EU assessment of mesotrione and thiencarbazone-methyl. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

### 9.9.2 Risk assessment

The evaluation of the risk for soil microorganisms was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).
The relevant $PEC_{soil}$ for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.8).
To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group 1 also covers the risk for the soil microorganisms from all other intended uses in group 2 (see 9.1.2).

**Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of GLOB2112dH in maize**

| **Intended use** | Maize | | |
|---|---|---|---|
| **N-mineralisation** | | | |
| **Product/active substance** | **Max. conc. with effects ≤ 25 % (mg/kg dw)** | **$PEC_{soil}$ (mg/kg dw)** | **Risk acceptable?** |
| Mesotrione | 0.53 (at 28 d) | 0.0750 | yes |
| AMBA | 1.13 (at 28 d) | 0.0046 | yes |
| MNBA | 1.13 (at 28 d) | 0.0310 | yes |
| Thiencarbazone-methyl | 0.3 (at 28 d) | 0.0150 | yes |
| BYH 18636-carboxylic acid (M01) | 0.29 (at 28 d) | ~~0.0064~~ 0.0103* | yes |
| BYH 18636-sulfonamide-carboxylic acid (M03) | 0.17 (at 28 d) | 0.0018 | yes |
| BYH 18636-sulfonamide (M15) | 0.18 (at 28 d) | 0.0014 | yes |
| BYH 18636-MMT (M21) | 0.10 (at 28 d) | 0.0010 | yes |
| GLOB2112dH | 1.62 (at 28 d) | 0.2430 | yes |

*Corrected according to Part B8

### 9.9.3 Overall conclusions

The risk to soil micro-organisms is acceptable when applying GLOB2112dH according to the intended uses.

**zRMS comments:**

For the risk assessment the EU agreed endpoints for active substances and major metabolites were used. For GLOB2112dH / Walkover Trio the endpoints from the formulation studies were used.

The worst-case predicted environmental concentrations in soil ($PEC_{soil}$) of the active substances, major metabolites and formulation were taken into account for the risk assessment.

**Conclusion**:
Since no effects (> 25%) were seen at application rates far higher than the values of PECsoil for active substances, their metabolites and formulation it can be concluded that application of GLOB2112dH / Walkover Trio, according to the GAP, will not cause any detrimental effect to soil mi-cro-organisms

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

### 9.10.1 Toxicity data

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

Effects on non-target terrestrial plants of GLOB2112dH were not evaluated as part of the EU assessment of mesotrione and thiencarbazone-methyl. 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 is in line with the results of the EU review process.

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

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Brassica napus*$_d$ [1)]<br>*Lactuca sativa*$_d$ [2)]<br>*Cucumis sativus*$_d$ [3)]<br>*Beta vulgaris*$_d$ [4)]<br>*Lolium perenne*$_m$ [5)]<br>*Allium cepa*$_m$ [6)] | GLOB2112dH | 21 d<br>Seedling emergence | Fresh weigth:<br>[1)] **$ER_{50}$ > 28 mL/ha**<br>[2)] $ER_{50}$ = 48.9 mL/ha<br>[3)] $ER_{50}$ = 139 mL/ha<br>[4)] $ER_{50}$ = 36.4 mL/ha<br>[5)] $ER_{50}$ = 43.9 mL/ha<br>[6)] $ER_{50}$ = 43.7 mL/ha<br><br>Heigth:<br>[1)] $ER_{50}$ > 28 mL/ha<br>[2)] $ER_{50}$ = 113 mL/ha<br>[3)] $ER_{50}$ > 84 mL/ha<br>[4)] $ER_{50}$ = 108 mL/ha<br>[5)] $ER_{50}$ = 39.6 mL/ha<br>[6)] $ER_{50}$ = 78.8 mL/ha<br><br>Phytotoxicity:<br>[1)] $ER_{50}$ > 28 mL/ha<br>[2)] $ER_{50}$ = 84.6 mL/ha<br>[3)] $ER_{50}$ = n.d.*<br>[4)] $ER_{50}$ = n.d.*<br>[5)] $ER_{50}$ = 66.8 mL/ha<br>[6)] $ER_{50}$ > 84.0 mL/ha | Dommes A.B., 2024a |
| *Brassica oleracea*$_d$ [1)]<br>*Cucumis sativus*$_d$ [2)]<br>*Solanum lycopersicum*$_d$ [3)] | GLOB2112dH | 21 d<br>Vegetative vigour | Fresh weigth:<br>[1)] $ER_{50}$ = 102 mL/ha<br>[2)] $ER_{50}$ = 132 mL/ha<br>[3)] $ER_{50}$ = 29.2 mL/ha | Dommes A.B., 2024b |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Helianthus annuus*$_d$ [4)]<br>*Lolium perenne*$_m$ [5)]<br>*Allium cepa*$_m$ [6)] | | | [4)] **$ER_{50}$ = 17.1 mL/ha**<br>[5)] $ER_{50}$ = 69.3 mL/ha<br>[6)] $ER_{50}$ = 131 mL/ha<br><br>Heigth:<br>[1)] $ER_{50}$ > 200 mL/ha<br>[2)] $ER_{50}$ = 85.5 mL/ha<br>[3)] $ER_{50}$ = 24.6 mL/ha<br>[4)] $ER_{50}$ = 38.3 mL/ha<br>[5)] $ER_{50}$ = 142 mL/ha<br>[6)] $ER_{50}$ > 200 mL/ha<br><br>Phytotoxicity:<br>[1)] $ER_{50}$ = 77.8 mL/ha<br>[2)] $ER_{50}$ = 36.8 mL/ha<br>[3)] $ER_{50}$ = 21.8 mL/ha<br>[4)] $ER_{50}$ = 32.1 mL/ha<br>[5)] $ER_{50}$ = 64.1 mL/ha<br>[6)] $ER_{50}$ > 200 mL/ha | |

m: monocotyledonous; d: dicotyledonous
* n.d. = not determined due to mathematical reasons (too much variation between replicates)

### 9.10.1.1 Justification for new endpoints

Effects on non-target terrestrial plants of GLOB2112dH were not evaluated as part of the EU as-sessment of mesotrione and thiencarbazone-methyl. New data submitted with this application are listed in Appendix 1 summarised in Appendix 2.

## 9.10.2 Risk assessment

### 9.10.2.1 Tier-1 risk assessment (based screening data)

Not relevant.

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

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

**Table 9.10-2: Assessment of the risk for non-target plants due to the use of GLOB2112dH in maize**

| | | | | |
|---|---|---|---|---|
| **Intended use** | Maize | | | |
| **Active substance/product** | GLOB2112dH | | | |
| **Application rate (L/ha)** | 1 × 0.2 | | | |
| **MAF** | - | | | |
| **Test species** | **$ER_{50}$ (mL/ha)** | **Drift rate** | **$PER_{off\text{-}field}$ (mL/ha)** | **TER criterion: TER ≥ 5** |

| | | | | |
|---|---|---|---|---|
| *Brassica napus* - Seedling emergence | > 28 | 0.0277 | 5.54 | > 5.05 |
| *Helianthus annuus* - vegetative vigour | 17.1 | 0.0277 | 5.54 | **3.09** |
| **Intended use** | Maize | | | |
| **Active substance/product** | GLOB2112dH | | | |
| **Application rate (L/ha)** | 1 × 0.13 | | | |
| **MAF** | - | | | |
| **Test species** | **$ER_{50}$ (mL/ha)** | **Drift rate** | **$PER_{off\text{-}field}$ (mL/ha)** | **TER criterion: TER ≥ 5** |
| *Brassica napus* - *Seedling emergence* | > 28 | 0.0277 | 3.60 | > 7.78 |
| *Helianthus annuus* | 17.1 | 0.0277 | 3.60 | **4.75** |

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

### 9.10.2.3 Higher-tier risk assessment

Not relevant.

### 9.10.2.4 Risk mitigation measures

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

**Table 9.10-3: Risk assessment for non-target terrestrial plants due to the use of GLOB2112dH in maize (considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)**

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | | Maize | | | |
| **Active substance/product** | | GLOB2112dH | | | |
| **Application rate (L/ha)** | | 1 × 0.2 | | | |
| **MAF** | | - | | | |
| **Buffer strip (m)** | **Drift rate (%)** | **$PER_{off\text{-}field}$ (mL/ha)** | **$PER_{off\text{-}field}$ 50 % drift red. (mL/ha)** | **$PER_{off\text{-}field}$ 75 % drift red. (mL/ha)** | **$PER_{off\text{-}field}$ 90 % drift red. (mL/ha)** |
| 1 | 2.77 | 5.54 | 2.77 | 1.39 | 0.55 |
| 5 | 0.57 | 1.14 | 0.57 | 0.29 | 0.11 |
| 10 | 0.29 | 0.58 | 0.29 | 0.15 | 0.058 |
| **Toxicity value** | | **TER** | | | |
| $ER_{50}$ = 17.1 mL/ha | | **criterion: TER ≥ 5** | | | |
| 1 | | **3.09** | 6.17 | - | - |
| 5 | | 15 | - | - | - |
| 10 | | - | - | - | - |

| **Intended use** | | Maize | | | |
|---|---|---|---|---|---|
| **Active substance/product** | | GLOB2112dH | | | |
| **Application rate (L/ha)** | | 1 × 0.13 | | | |
| **MAF** | | - | | | |
| **Buffer strip (m)** | **Drift rate (%)** | **PER$_{off-field}$ (mL/ha)** | **PER$_{off-field}$ 50 % drift red. (mL/ha)** | **PER$_{off-field}$ 75 % drift red. (mL/ha)** | **PER$_{off-field}$ 90 % drift red. (mL/ha)** |
| 1 | 2.77 | **3.60** | 1.80 | 0.90 | 0.36 |
| 5 | 0.57 | 0.74 | 0.37 | 0.19 | 0.07 |
| 10 | 0.29 | 0.38 | 0.19 | 0.09 | 0.04 |
| **Toxicity value** | | **TER** | | | |
| $ER_{50}$ = 17.1 mL/ha | | **criterion: TER ≥ 5** | | | |
| 1 | | **4.75** | 9.50 | - | - |
| 5 | | 23 | - | - | - |
| 10 | | - | - | - | - |

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

### 9.10.3 Overall conclusions

A buffer zone of 1 m in combination with 50% drift reducing techniques or a buffer zone of 5 m is needed to protect non-target plants.

**zRMS comments:**

The risk assessment was based on results of studies for formulation presented in Appendix 2 (vegetative vigour and on seedling emergence). *Helianthus annuus* was found to be the most sensitive species in the vegetative vigour test, and *Brassica napus* was found to be the most sensitive species in the seedling emergence test.

The TER is above the trigger value of 5 when 5 m buffer zone or 50% drift reduction is applied.

**Conclusion**:
According to the performed risk assessment there is low risk to non-target terrestrial plants resulting from exposure to active substances following use of GLOB2112dH / Walkover Trioin compliance with proposed GAP when:

- 5 m buffer zone or
- 50% drift reduction is applied.

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

Not required.

## 9.12 Monitoring data (KCP 10.8)

Not required.

## 9.13 Classification and Labelling

Classification of GLOB2112dH was performed according to the EU Regulation 1272/2008 (CLP labelling).
Acute toxicity tests were performed with the formulation GLOB2112dH. Reference is made to the table 9.5-2 provided under point 9.5 of section B9 for a summary table of the acute toxicity studies. No chronic toxicity data with the formulation is available.
The $EC_{50}$ value for Lemna is below 1 mg product/L. Therefore, the formulation is classified as category 1 for aquatic acute toxicity; H400.
For chronic classification, the summation method in accordance with EU Regulation 1272/2008 (CLP labelling) was applied. GLOB2112dH should be classified as category 1 for aquatic chronic toxicity; H410. For more details, reference is made to the Part C.

**zRMS comments:**

zRMS agree with the classification proposal. For details see Part C.

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

Tables considered not relevant can be deleted as appropriate.

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

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

| **Data point** | **Author(s)** | **Year** | **Title**<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 10.1.2.2 | Hazlerigg C. & Garrat J. | 2016 | A kinetic analysis of the dissipation of mesotrione in maize<br>Report No E2016-13<br>Enviresearch Limited<br>Not GLP<br>Unpublished | N | Globachem NV |
| KCP 10.1.2.2 | Grimm & Katzschner | 2019 | Generic monitoring of European hares to determine proportion of time spend foraging in early maize in Central Europe.<br>Rifcon GmbH<br>GLP<br>Unpublished | N | Syngenta<br>*Globachem access* |
| KCA 8.2.7 | Minati R. | 2024 | Thiencarbazone-methyl: Toxicity to the Aquatic Plant Lemna gibba in a Pulsed Exposure Growth Inhibition Test<br>178651240<br>Ibacon GmbH<br>GLP<br>Unpublished | N | Globachem NV |
| KCA 8.2.7 | Bebon R. | 2024 | Thiencarbazone-methyl: Toxicity to the aquatic plant Myriophyllum spicatum in a pulsed exposure growth inhibition test with a prior rooting phase<br>178651215<br>Ibacon GmbH<br>GLP | N | Globachem NV |

| 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 |
|---|---|---|---|---|---|
| | | | Unpublished | | |
| KCP 10.2.1 | Bauer J. | 2024a | GLOB2112dH: Toxicity to *Pseudokirchneriella subcapitata* in an Algal Growth Inhibition Test<br>177011210<br>Ibacon GmbH<br>GLP<br>Unpublished | N | Globachem NV |
| KCP 10.2.1 | Bauer J. | 2024b | GLOB2112dH: Toxicity to the Aquatic Plant *Lemna gibba* in a Static Growth Inhibition Test 177011240<br>Ibacon GmbH<br>GLP<br>Unpublished | N | Globachem NV |
| KCP 10.2.1 | Bauer J. | 2024c | GLOB2112dH: Toxicity to the aquatic plant *Myriophyllum spicatum* in a static growth inhibition test with a prior rooting phase<br>177011215<br>Ibacon GmbH<br>GLP<br>Unpublished | N | Globachem NV |
| KCP 10.2.1 | Kosak & Wydra | 2016 | Mesotrione Wet Paste (ZA1296) - Toxicity to the Aquatic Plant *Lemna gibba* in a Semi-Static Growth Inhibition Test with a Subsequent Recovery Period<br>105732240<br>Ibacon GmbH<br>GLP<br>Unpublished | N | Syngenta *Globachem access* |
| KCP 10.2.1 | Gonsior G. | 2017 | Mesotrione – Growth Inhibition of *Myriophyllum spicatum* in a Water/Sediment System<br>Eurofins Agroscience Services EcoChem GmbH<br>S16-06273<br>GLP<br>Unpublished | N | Syngenta *Globachem access* |
| KCP 10.3.1.1 | Schabio S. | 2024 | GLOB2112dH: effects (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory<br>177011035 | N | Globachem NV |

| 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<br>Y/N | Owner |
|---|---|---|---|---|---|
| | | | Ibacon GmbH<br>GLP<br>Unpublished | | |
| KCP 10.3.1.1 | Chwiesko D. | 2024 | GLOB2112dH: acute contact and oral toxicity to bumblebees (*Bombus terrestris* L.) in the laboratory<br>177011105<br>Ibacon GmbH<br>GLP<br>Unpublished | N | Globachem NV |
| KCP 10.3.1.2 | Venturi S. | 2023 | Chronic oral effects of GLOB2112dH to adult worker honeybees (Apis mellifera L.) in a 10-day feeding laboratory test<br>BT215/23<br>BioTecnologie BT S.r.l.<br>GLP<br>Not published | N | Globachem NV |
| KCP 10.3.1.3 | Venturi S. | 2024 | Effects of GLOB2112dH on honeybees (*Apis mellifera L.*) 22-day larval toxicity test with repeated exposure<br>BT131/23<br>BioTechnologie BT<br>GLP<br>Unpublished | N | Globachem NV |
| KCP 10.3.2 | Leopold J. | 2023a | GLOB2112dH: Effects on the predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae) in the laboratory. A dose response test on glass plates.<br>177011063<br>Ibacon GmbH<br>GLP<br>Not published | N | Globachem NV |
| KCP 10.3.2 | Leopold J. | 2023b | GLOB2112dH: Effects on the parasitoid *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) in the laboratory. A dose response test on glass plates.<br>177011001<br>Ibacon GmbH | N | Globachem NV |

| 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>Not published | | |
| KCP 10.4.1.1 | Hübner S. | 2024 | GLOB2112dH: Effects on reproduction and growth of earthworms Eisenia andrei in artificial soil<br>177011022<br>Ibacon GmbH<br>GLP<br>Not published | N | Globachem NV |
| KCP 10.4.2.1 | Hübner S. | 2023a | GLOB2112dH: Effects on reproduction of Collembola (*Folsomia candida*) in artificial soil<br>177011016<br>Ibacon GmbH<br>GLP<br>Not published | N | Globachem NV |
| KCP 10.4.2.1 | Hübner S. | 2023b | GLOB2112dH: Effects on the reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil.<br>177011089<br>Ibacon GmbH<br>GLP<br>Not published | N | Globachem NV |
| KCP 10.4.2.1 | Dickinson R. | 2015 | R169649 - Collembola (Folsomia candida) Reproduction Test in Soil<br>Syngenta Crop Protection AG, Basel, Switzerland<br>AgroChemex Ltd, Manningtree, United Kingdom, ENV-14-015<br>GLP<br>not published<br>Syngenta File No CA3511_10011 | N | Syngenta *Globachem access* |
| KCP 10.4.2.1 | Ramsden C. | 2015 | R169649 - Predatory Mite (Hypoaspis (Geolaelaps) aculeifer) Reproduction Test in Soil<br>Syngenta Crop Protection AG, Basel, Switzerland<br>AgroChemex Ltd, Manningtree, United Kingdom, ENV-14-012<br>GLP<br>not published<br>Syngenta File No CA3511_10010 | N | Syngenta Globachem access |

| 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 10.5 | Hammesfahr U. | 2023 | GLOB2112dH: Effects on the Activity of the Soil Microflora in the Laboratory (Nitrogen Transformation).<br>177011080<br>Ibacon GmbH<br>GLP<br>Not published | N | Globachem NV |
| KCP 10.6 | Dommes A.B. | 2024a | GLOB2112dH: Effects on terrestrial (non-target) plants: seedling emergence and seedling growth test<br>177011086<br>Ibacon GmbH<br>GLP<br>Not published | N | Globachem NV |
| KCP 10.6 | Dommes A.B. | 2024b | GLOB2112dH: Effects on terrestrial (non-target) plants : vegetative vigour test<br>177011087<br>Ibacon GmbH<br>GLP<br>Not published | N | Globachem NV |

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

| Data point | Author(s) | Year | Title<br>Company Report No.<br>Source (where different from company)<br>GLP or GEP status<br>Published or not | Vertebrate study Y/N | Owner |
|---|---|---|---|---|---|
| KCA 8.1.1.1 | [redacted] | 1995 | ZA 1296: Acute oral toxicity ($LD_{50}$) of mesotrione to Bobwhite quail<br>[redacted]<br>GLP<br>Unpublished | Y | Syngenta<br>*Data out of protection* |

| 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 |
|---|---|---|---|---|---|
| KCA 8.1.1.1 | [redacted] | 2005 | Acute oral toxicity for bobwhite quail (Colinius virgianus) with BYH 18636 a.s.<br>[redacted]<br>GLP<br>Unpublished | Y | Bayer CropScience<br>*Data out of protection* |
| KCA 8.1.1.2 | [redacted] | 1995 | ZA 1296: Sub-acute dietary toxicity ($LC_{50}$) to the Bobwhite quail<br>[redacted]<br>GLP<br>Unpublished | Y | Syngenta<br>*Data out of protection* |
| KCA 8.1.1.2 | [redacted] | 1995 | ZA 1296: Sub-acute dietary toxicity ($LC_{50}$) to the Mallard duck<br>[redacted]<br>GLP<br>Unpublished | Y | Syngenta<br>*Data out of protection* |
| KCA 8.1.1.3 | [redacted] | 1997 | ZA 1296: Effects on reproduction in Bobwhite quail<br>[redacted]<br>GLP<br>Unpublished | Y | Syngenta<br>*Data out of protection* |
| KCA 8.1.1.3 | [redacted] | 1997 | ZA 1296: Effects on reproduction of Mallard duck.<br>[redacted]<br>GLP<br>Unpublished | Y | Syngenta<br>*Data out of protection* |
| KCA 8.1.1.3 | [redacted] | 2007 | Effect of technical BYH 18636 on mallard reproduction<br>[redacted]<br>GLP<br>Unpublished | Y | Bayer CropScience<br>*Data out of protection* |
| KCA 8.1.2.1 | [redacted] | 1994 | ZA 1296: Acute oral toxicity to the rat<br>[redacted] | Y | Syngenta |

| 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 | | *Data out of protection* |
| KCA 8.1.2.1 | [redacted] | 1996 | 2-nitro-4-methylsulfonyl benzoic acid: Acute oral toxicity to the rat<br>[redacted]<br>GLP<br>Unpublished | Y | Syngenta<br><br>*Data out of protection* |
| KCA 8.1.2.1 | [redacted] | 1996 | AMBA (2-amino-4-methylsulfonyl benzoic acid): Acute oral toxicity to the rat<br>[redacted]<br>GLP<br>Unpublished | Y | Syngenta<br><br>*Data out of protection* |
| KCA 8.1.2.1 | [redacted] | 2004 | BYH 18636 – Acute toxicity in the rat after oral administration<br>[redacted]<br>GLP<br>Unpublished | Y | Bayer CropScience<br><br>*Data out of protection* |
| KCA 8.1.2.2 | [redacted] | 1997 | ZA 1296: Multigeneration study in the rat<br>[redacted]<br>GLP<br>Unpublished | Y | Syngenta<br><br>*Data out of protection* |
| KCA 8.1.2.2 | [redacted] | 2005 | BYH 18636 – Two-generation reproduction study in the Wistar rat by administration in the diet<br>[redacted]<br>GLP<br>Unpublished | Y | Bayer CropScience<br><br>*Data out of protection* |
| KCA 8.2.1 | [redacted] | 1994 | ZA 1296: Acute Toxicity to Rainbow Trout (Oncorhynchus mykiss)<br>[redacted]<br>GLP<br>Unpublished | Y | Syngenta<br><br>*Data out of protection* |
| KCA 8.2.1 | [redacted] | 1994 | ZA 1296: Acute Toxicity to Bluegill Sunfish (Lepomis macrochirus) | Y | Syngenta |

| 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 |
|---|---|---|---|---|---|
| | | | [redacted]<br>GLP<br>Unpublished | | *Data out of protection* |
| KCA 8.2.1 | [redacted] | 1997 | MNBA: Acute Toxicity to Rainbow Trout (Oncorhynchus mykiss)<br>[redacted]<br>GLP<br>Unpublished | Y | Syngenta<br>*Data out of protection* |
| KCA 8.2.1 | [redacted] | 1998 | R044276 (AMBA): Acute Toxicity to Rainbow Trout (Onchorhynchus mykiss)<br>[redacted]<br>GLP<br>Unpublished | Y | Syngenta<br>*Data out of protection* |
| KCA 8.2.1 | [redacted] | 2005 | Acute toxicity of BYH 18636 technical to the rainbow trout (Oncorhyncus mykiss) under static conditions<br>[redacted]<br>GLP<br>Unpublished | Y | Bayer CropScience<br>*Data out of protection* |
| KCA 8.2.1 | [redacted] | 2005 | Acute toxicity of BYH 18636 sulfonamide to the rainbow trout (Oncorhyncus mykiss) under static conditions<br>[redacted]<br>GLP<br>Unpublished | Y | Bayer CropScience<br>*Data out of protection* |
| KCA 8.2.2 | [redacted] | 1997 | ZA 1296: Chronic Toxicity to Fathead Minnow (Pimephales promelas) Embryos and Larvae<br>[redacted]<br>GLP<br>Unpublished | Y | Syngenta<br>*Data out of protection* |
| KCA 8.2.2 | [redacted] | 2006 | Early life stage toxicity of BYH 18636 technical to the fathead minnow (Pimephales promelas) under flow-through conditions<br>[redacted] | Y | Bayer CropScience |

| 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** |
|---|---|---|---|---|---|
| | | | [redacted]<br>GLP<br>Unpublished | | *Data out of protection* |
| KCA 8.2.4.1 | Gentle W. E., Hamer M. J. | 1995 | ZA 1296: Acute Toxicity of the Technical Material to First Instar Daphnia magna<br>RJ1872B<br>Zeneca Agrochemicals<br>GLP<br>Unpublished | N | Syngenta<br>*Data out of protection* |
| KCA 8.2.4.1 | Kent S. J., Shillabeer N. | 1997 | MNBA: Acute Toxicity to Daphnia magna<br>BL6108/B<br>Zeneca Brixham Laboratory<br>GLP<br>Unpublished | N | Syngenta<br>*Data out of protection* |
| KCA 8.2.4.1 | Magor S. E., Gore N. R. | 1998 | R044276 (AMBA): Acute Toxicity to Daphnia magna<br>BL6392/B<br>Zeneca Brixham Laboratory<br>GLP<br>Unpublished | N | Syngenta<br>*Data out of protection* |
| KCA 8.2.4.1 | Banman C.S.; Lam C.V. | 2005 | Acute toxicity of BYH 18636 technical to the Daphnia magna under static conditions<br>EBGSM007<br>Bayer CropScience<br>GLP<br>Unpublished | N | Bayer CropScience<br>*Data out of protection* |
| KCA 8.2.4.1 | Bruns E. | 2007 | Acute toxicity of BYH 18636-sulfonamide to the water flea Daphnia magna in a static laboratory test system – limit test<br>EBGSP087<br>Bayer CropScience<br>GLP<br>Unpublished | N | Bayer CropScience<br>*Data out of protection* |

| 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 |
|---|---|---|---|---|---|
| KCA 8.2.5.1 | Morris D.S. *et al.* | 1996 | ZA 1296: Chronic Toxicity to Daphnia magna<br>BL5832/B<br>Zeneca Brixham Laboratory<br>GLP<br>Unpublished | N | Syngenta<br><br>*Data out of protection* |
| KCA 8.2.5.1 | Kern M.E., Lam C.V. | 2006 | Chronic toxicity of BYH 18636 technical to the Daphnia magna under static renewal conditions<br>EBGSM008-1<br>Bayer CropScience<br>GLP<br>Unpublished | N | Bayer CropScience<br><br>*Data out of protection* |
| KCA 8.2.5.3 | Bruns E. | 2006 | Acute toxicity of BYH 18636 (tech.) to larvae of Chironomus riparius in a 48 h static laboratory test system (Limit-Test)<br>EBGSP03-7<br>Bayer CropScience<br>GLP<br>Unpublished | N | Bayer CropScience<br><br>*Data out of protection* |
| KCA 8.2.5.3 | Bruns E. | 2006 | Acute toxicity of BYH 18636-carboxylic acid to larvae of Chironomus riparius in a 48 h static laboratory test system (Limit-Test)<br>EBGSP07-9<br>Bayer CropScience<br>GLP<br>Unpublished | N | Bayer CropScience<br><br>*Data out of protection* |
| KCA 8.2.5.3 | Bruns E. | 2006 | Acute toxicity of BYH 18636-sulfonamide-carboxylic acid to larvae of Chironomus riparius in a 48 h static laboratory test system (Limit-Test)<br>EBGSP07-8<br>Bayer CropScience<br>GLP<br>Unpublished | N | Bayer CropScience<br><br>*Data out of protection* |
| KCA 8.2.6.1 | Shillabeer N., Kent S.J., Smyth D.V. | 1997 | ZA 1296: Toxicity to the green alga, Selenastrum capricornutum<br>BL6113/B | N | Syngenta |

| 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** |
|---|---|---|---|---|---|
| | | | Brixham Environmental Laboratory, Zeneca Limited<br>GLP<br>Unpublished | | *Data out of protection* |
| KCA 8.2.6.1 | Smyth D.V. *et al.* | 1997 | MNBA: Toxicity to the green alga, Selenastrum capricornutum<br>BL6066/B<br>Brixham Environmental Laboratory, Zeneca Limited<br>GLP<br>Unpublished | N | Syngenta<br>*Data out of protection* |
| KCA 8.2.6.1 | Smyth, D. V., Magor, S. E., Shillabeer, N. | 1998 | R044276 (AMBA): Toxicity to Green Alga (Selenastrum capricornutum)<br>BL6354/B<br>Brixham Environmental Laboratory, Zeneca Limited<br>GLP<br>Unpublished | N | Syngenta<br>*Data out of protection* |
| KCA 8.2.6.1 | Kern M.E., Banman C.S., Lam C.V. | 2005 | Toxicity of BYH 18636 technical to the green algae – Pseudokirchneriella subcapitata<br>EBGSM001<br>Bayer CropScience<br>GLP<br>Unpublished | N | Bayer CropScience<br>*Data out of protection* |
| KCA 8.2.6.1 | Banman C.S., Lam C.V. | 2005 | Toxicity of BYH 18636 sulfonamide to the green algae Pseudokirchneriella subcapitata<br>EBGSP003<br>Bayer CropScience<br>GLP<br>Unpublished | N | Bayer CropScience<br>*Data out of protection* |
| KCA 8.2.6.2 | Kern M.E., Roberts J.A., Lam C.K. | 2005 | Toxicity of BYH 18636 technical to the freshwater diatom Navicula pelliculosa<br>EBGSPM015<br>Bayer CropScience<br>GLP<br>Unpublished | N | Bayer CropScience<br>*Data out of protection* |
| KCA | Kern M.E., Lam C.V. | 2006 | Toxicity of BYH 18636 technical to the blue-green alga Anabaena flos-aquae | N | Bayer |

| 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 |
|---|---|---|---|---|---|
| 8.2.6.2 | | | EBGSP012-1<br>Bayer CropScience<br>GLP<br>Unpublished | | CropScience<br><br>*Data out of protection* |
| KCA 8.2.7 | Smyth D.V. *et al.* | 1997 | ZA 1296: Toxicity to the Duckweed (Lemna gibba)<br>BL5849/B<br>Brixham Environmental Laboratory, Zeneca Limited<br>GLP<br>Unpublished | N | Syngenta<br><br>*Data out of protection* |
| KCA 8.2.7 | Liedtke A. | 2013 | R169649 - Toxicity to the aquatic higher plant Lemna gibba in a 7-day growth inhibition test<br>D55592<br>Harlan Laboratories Ltd.<br>GLP<br>Unpublished | N | Syngenta<br><br>*Matching data provided* |
| KCA 8.2.7 | Renner P. | 2016 | Effects of MNBA on lemna gibba in a growth inhibition test under semi-static test conditions.<br>16 10 48 034 W<br>BioChem agrar<br>GLP<br>Unpublished | N | Globachem NV<br><br>*Matching data* |
| KCA 8.2.7 | Liedtke A. | 2013 | R44276 - Toxicity to the aquatic higher plant Lemna gibba in a 7-day growth inhibition test<br>D55614<br>Harlan Laboratories Ltd.<br>GLP<br>Unpublished | N | Syngenta<br><br>*Matching data provided* |
| KCA 8.2.7 | Renner P. | 2016 | Effects of AMBA on *lemna gibba* in a growth inhibition test under semi-static test conditions.<br>16 10 48 035 W<br>BioChem agrar<br>GLP<br>Unpublished | N | Globachem NV<br><br>*Matching data* |

| 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 |
|---|---|---|---|---|---|
| KCA 8.2.7 | Liedtke A. | 2013 | SYN546974 - Toxicity to the aquatic higher plant Lemna gibba in a 7-day growth inhibition test<br>D77394<br>Harlan Laboratories Ltd.<br>GLP<br>Unpublished | N | Syngenta<br><br>*Matching data provided* |
| KCA 8.2.7 | Renner P. | 2016 | Effects of SYN546974 on lemna gibba in a growth inhibition test under semi-static test conditions.<br>16 10 48 036W<br>BioChem agrar<br>GLP<br>Unpublished | N | Globachem NV<br><br>*Matching data* |
| KCA 8.2.7 | Kern M.E., Lam C.V. | 2006 | Toxicity of BYH 18636 technical to duckweed (Lemna gibba G3) under static-renewal conditions<br>EBGSM016<br>Bayer CropScience<br>GLP<br>Unpublished | N | Bayer CropScience<br><br>*Data out of protection* |
| KCA 8.2.7 | Christ M. T., Lam C.V. | 2007 | Toxicity of BYH 18636 technical to the aquatic macrophyte, Myriophyllum spicatum, during a 14-day exposure and 14-day recovery period<br>EBGSP077<br>Bayer CropScience<br>GLP<br>Unpublished | N | Bayer CropScience<br><br>*Data out of protection* |
| KCA 8.2.7 | Hoberg J.R. | 2007 | BYH 18636 – Comparative toxicity to three aquatic macrophytes during a 14-day exposure followed by a 14-day recovery period<br>EBGSP086<br>Bayer CropScience<br>GLP<br>Unpublished | N | Bayer CropScience<br><br>*Data out of protection* |
| KCA 8.2.7 | Banman C.S., Lam C.V. | 2005 | Toxicity of BYH 18636 carboxylic acid to duckweed (Lemna gibba G3) under static renewal conditions<br>EBGSP019<br>Bayer CropScience | N | Bayer CropScience |

| 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 | | *Data out of protection* |
| KCA 8.2.7 | Dorgerloh M. | 2006 | Lemna gibba G3 growth inhibition test with BYH 18636 – sulfonamide-carboxylic acid under static conditions<br>EBGSP042<br>Bayer CropScience<br>GLP<br>Unpublished | N | Bayer CropScience<br><br>*Data out of protection* |
| KCA 8.2.7 | Christ M.T., Lam C.V. | 2006 | Toxicity of BYH 18636 sulfonamide (a metabolite of BYH 18636) to duckweed (Lemna gibba G3) under static-renewal conditions<br>EBGSP029<br>Bayer CropScience<br>GLP<br>Unpublished | N | Bayer CropScience<br><br>*Data out of protection* |
| KCA 8.2.7 | Christ M.T., Lam C.V. | 2007 | Toxicity of BYH 18636 MMT (a metabolite of BYH 18636) to duckweed (Lemna gibba G3) under static-renewal conditions<br>EBGSP040<br>Bayer CropScience<br>GLP<br>Unpublished | N | Bayer CropScience<br><br>*Data out of protection* |
| KCA 8.2.7 | Christ M.T., Hoffmann J.M., Lam C.V. | 2007 | Toxicity of BYH 18636-dicarboxy-sulfonamide (a metabolite of BYH 18636) to duckweed (Lemna gibba G3) under static-renewal conditions<br>EBGSP045<br>Bayer CropScience<br>GLP<br>Unpublished | N | Bayer CropScience<br><br>*Data out of protection* |
| KCA 8.2.8 | [redacted] | 2005 | Acute toxicity of BY 18636 technical to the sheepshead minnow (Cyprinodon variegatus) under static conditions<br>[redacted] | Y | Bayer CropScience<br><br>*Data out of* |

| 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 | | *protection* |
| KCA 8.2.8 | Putt A.E. | 2006 | BYH 18636 technical – acute toxicity to Mysids (Americamysis bahia) under flow-through conditions<br>EBGSP011<br>Bayer CropScience<br>GLP<br>Unpublished | N | Bayer CropScience<br><br>*Data out of protection* |
| KCA 8.2.8 | Putt A.E. | 2006 | BYH18636 technical – life-cycle toxicity test with mysids (americamysis bahia) under flow-through conditions<br>EBGSP004<br>Bayer CropScience<br>GLP<br>Unpublished | N | Bayer CropScience<br><br>*Data out of protection* |
| KCA 8.2.8 | Cafarella M.A. | 2006 | BYH 18636 technical – acute toxicity to Eastern Oyster (Crassostrea virginica) under flow-through conditions<br>EBGSP010<br>Bayer CropScience<br>GLP<br>Unpublished | N | Bayer CropScience<br><br>*Data out of protection* |
| KCA 8.2.8 | Christ M.T., Lam C.V. | 2006 | Toxicity of BYH 18636 technical to the saltwater diatom Skeletonema costatum<br>EBGSM017<br>Bayer CropScience<br>GLP<br>Unpublished | N | Bayer CropScience<br><br>*Data out of protection* |
| KCA 8.3.1.1 | Jackson D., Gough H.J. | 1995 | ZA 1296: Acute Contact and Oral Toxicity to the Honey Bees (Apis mellifera) of Technical Material<br>RJ1959B<br>Zeneca Agrochemicals<br>GLP<br>Unpublished | N | Syngenta<br><br>*Data out of protection* |

| 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 |
|---|---|---|---|---|---|
| KCA 8.3.1.1 | Barth M. | 2005 | Acute toxicity of BYH 18636 a.i. tech. to the honeybee Apis mellifera L. under laboratory conditions<br>05 10 48 030<br>BioChem agrar<br>GLP<br>Unpublished | N | Bayer CropScience<br><br>*Data out of protection* |
| KCA 8.4.1 | Friedrich S. | 2011 | Mesotrione SC (A12739A) - Sublethal toxicity to the earthworm Eisenia fetida in artificial soil<br>11 10 48 003 S<br>BioChem agrar<br>GLP<br>Unpublished | N | Syngenta<br><br>*Matching data provided* |
| KCA 8.4.1 | Friedrich S. | 2016 | Sublethal effects of Mesotrione 100 SC on the earthworm Eisenia fetida in artificial soil.<br>16 10 48 112 S<br>BioChem agrar<br>GLP<br>Unpublished | N | Globachem NV<br><br>*Matching data* |
| KCA 8.4.1 | Friedrich S. | 2013 | R44276 – Sublethal Toxicity to the Earthworm Eisenia fetida in Artificial Soil with 5% Peat<br>13 10 48 111 S<br>BioChem agrar<br>GLP<br>Unpublished | N | Syngenta<br><br>*Matching data provided* |
| KCA 8.4.1 | Friedrich S. | 2016 | Effects of AMBA on the earthworm Eisenia fetida in artificial soil.<br>16 10 48 144 S<br>BioChem agrar<br>GLP<br>Unpublished | N | Globachem NV<br><br>*Matching data* |
| KCA 8.4.1 | Friedrich S. | 2013 | R169649 – Sublethal Toxicity to the Earthworm Eisenia fetida in Artificial Soil with 5 % Peat<br>13 10 48 086 S<br>BioChem agrar<br>GLP<br>Unpublished | N | Syngenta<br><br>*Matching data provided* |

| 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** |
|---|---|---|---|---|---|
| KCA 8.4.1 | Friedrich S. | 2016 | Effects of MNBA on the earthworm *Eisenia fetida* in artificial soil.<br>16 10 48 145 S<br>BioChem agrar<br>GLP<br>Unpublished | N | Globachem NV<br><br>*Matching data* |
| KCA 8.4.1 | Lechelt-Kunze C. | 2005 | BYH 18636-carboxylic acid (technical): Effects on survival, growth and reproduction on the earthworm Eisenia fetida tested in artificial soil<br>LKC-RG-R-17/05<br>Bayer CropScience<br>GLP<br>Unpublished | N | Bayer CropScience<br><br>*Data out of protection* |
| KCA 8.4.1 | Friedrich S. | 2006 | BYH 18636-sulfonamide: Sublethal toxicity to the earthworm Eisenia fetida in artificial soil<br>06 10 48 063<br>BioChem agrar GmbH<br>GLP<br>Unpublished | N | Bayer CropScience<br><br>*Data out of protection* |
| KCA 8.4.1 | Luehrs U. | 2006 | BYH 18636-sulfonamide-carboxylic acid: effects on reproduction and growth of earthworms Eisenia fetida in artificial soil<br>28471022<br>Ibacon GmbH<br>GLP<br>Unpublished | N | Bayer CropScience<br><br>*Data out of protection* |
| KCA 8.4.1 | Luehrs U. | 2006 | BYH 18636-MMT: effects on reproduction and growth of earthworms Eisenia fetida in artificial soil<br>28461022<br>Ibacon GmbH<br>GLP<br>Unpublished | N | Bayer CropScience<br><br>*Data out of protection* |
| KCA 8.4.2 | Friedrich S. | 2013 | Mesotrione SC (A12739A) - Effects on the Reproduction of the Collembolan Folsomia candida<br>13 10 48 009 S<br>BioChem agrar | N | Syngenta<br><br>*Matching data* |

| 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 | | *provided* |
| KCA 8.4.2 | Friedrich S. | 2016 | Effects of Mesotrione 100 SC on the reproduction of the collembolan *Folsomia candida*<br>16 10 48 111 S<br>BioChem agrar<br>GLP<br>Unpublished | N | Globachem NV<br><br>*Matching data* |
| KCA 8.4.2 | Schulz L. | 2013 | Mesotrione SC (A12739A) - Effects on the Reproduction of the Predatory Mite Hypoaspis aculeifer<br>13 10 48 010 S<br>BioChem agrar<br>GLP<br>Unpublished | N | Syngenta<br><br>*Matching data provided* |
| KCA 8.4.2 | Schulz L. | 2016 | Effects of Mesotrione 100 SC on the reproduction of the predatory mite *Hypoaspis aculeifer*<br>16 10 48 058 S<br>BioChem agrar<br>GLP<br>Unpublished | N | Globachem NV<br><br>*Matching data* |
| KCA 8.4.2 | Frommholz U. | 2006 | BYH 18636 tech.: Influence on the reproduction of the collembola species Folsomia candida tested in artificial soil<br>FRM-COLL-46/06<br>Bayer CropScience<br>GLP<br>Unpublished | N | Bayer CropScience<br><br>*Data out of protection* |
| KCA 8.4.2 | Frommholz U. | 2005 | BYH 18636-carboxylic acid: Influence on the reproduction of the collembola species Folsomia candida tested in artificial soil<br>LKC-COLL-44/05<br>Bayer CropScience<br>GLP<br>Unpublished | N | Bayer CropScience<br><br>*Data out of protection* |

| 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 |
|---|---|---|---|---|---|
| KCA 8.4.2 | Friedrich S. | 2006 | BYH 18636-sulfonamide-carboxylic acid: Effects on the reproduction of the collembolans Folsomia candida<br>06 10 48 168<br>BioChem agrar GmbH<br>GLP<br>Unpublished | N | Bayer CropScience<br><br>*Data out of protection* |
| KCA 8.4.2 | Friedrich S. | 2006 | BYH 18636-MMT: Effects on the reproduction of the collembolans Folsomia candida<br>06 10 48 167<br>BioChem agrar GmbH<br>GLP<br>Unpublished | N | Bayer CropScience<br><br>*Data out of protection* |
| KCA 8.4.2 | Friedrich S. | 2006 | BYH 18636-triazolinone-carboxamide: Effects on the reproduction of the collembolans Folsomia candida<br>06 10 48 169<br>BioChem agrar GmbH<br>GLP<br>Unpublished | N | Bayer CropScience<br><br>*Data out of protection* |
| KCA 8.5 | Schulz L. | 2013<br>2014 | Mesotrione SC (A12739A) – Effects on the Activity of Soil Microflora (Nitrogen and Carbon Transformation Tests)<br>13 10 48 006 C/N<br>BioChem agrar<br>GLP<br>Unpublished | N | Syngenta<br><br>*Matching data provided* |
| KCA 8.5 | Servajean E. | 2013 | Soil micro-organisms: nitrogen transformation test with Mesotrione 100 SC (OECD 216, January 2000).<br>16-99-053-ES<br>Phytosafe s.a.r.l.<br>GLP<br>Unpublished | N | Globachem NV<br><br>*Matching data* |
| KCA 8.5 | Schulz L. | 2013 | R169649 and R44276 – Effects on the Activity of Soil Microflora (Nitrogen and Carbon Transformation Tests)<br>12 10 48 045 C/N | N | Syngenta<br><br>*Matching data* |

| 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 |
|---|---|---|---|---|---|
| | | | BioChem agrar<br>GLP<br>Unpublished | | *provided* |
| KCA 8.5 | Schulz L. | 2016 | Effects of AMBA on the activity of soil microflora (Nitrogen transformation test)<br>16 10 48 035 N<br>BioChem agrar<br>GLP<br>Unpublished | N | Globachem NV<br><br>*Matching data* |
| KCA 8.5 | Schulz L. | 2016 | Effects of MNBA on the activity of soil microflora (Nitrogen transformation test).<br>16 10 48 036 N<br>BioChem agrar<br>GLP<br>Unpublished | N | Globachem NV<br><br>*Matching data* |
| KCA 8.5 | Lechelt-Kunze C. | 2005 | BYH 18636 tech.: determination of effects on nitrogen transformation in soil<br>LKC-N-55/05<br>Bayer CropScience<br>GLP<br>Unpublished | N | Bayer CropScience<br><br>*Data out of protection* |
| KCA 8.5 | Lechelt-Kunze C. | 2005 | Metabolite BYH 18636-carboxylic acid: determination of effects on nitrogen transformation in soil<br>LKC-N-56/05<br>Bayer CropScience<br>GLP<br>Unpublished | N | Bayer CropScience<br><br>*Data out of protection* |
| KCA 8.5 | Heimbach F. | 2006 | Metabolite BYH 18636-sulfonamide: determination of effects on nitrogen transformation in soil<br>LKC-N-66/06<br>Bayer CropScience<br>GLP<br>Unpublished | N | Bayer CropScience<br><br>*Data out of protection* |
| KCA 8.5 | Heimbach F. | 2006 | Metabolite BYH 18636-sulfonamide-carboxylic acid: determination of effects on nitrogen transformation | N | Bayer |

| 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** |
|---|---|---|---|---|---|
| | | | in soil<br>LKC-N-67/06<br>Bayer CropScience<br>GLP<br>Unpublished | | CropScience<br><br>*Data out of protection* |
| KCA 8.5 | Heimbach F. | 2006 | Metabolite BYH 18636-MMT: determination of effects on nitrogen transformation in soil<br>LKC-N-65/06<br>Bayer CropScience<br>GLP<br>Unpublished | N | Bayer CropScience<br><br>*Data out of protection* |

The following tables are to be completed by MS

**List of data submitted by the applicant and not relied on**

| Data point | Author(s) | Year | **Title**<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 XX | Author | YYYY | Title<br>Company Report N<br>Source<br>GLP/non GLP/GEP/non GEP<br>Published/Unpublished | Y/N | Owner |
| | | | | | |

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

| Data point | Author(s) | Year | Title<br>Company Report No.<br>Source (where different from company)<br>GLP or GEP status<br>Published or not | Vertebrate study<br>Y/N | Owner |
|---|---|---|---|---|---|
| KCP XX | Author | YYYY | Title<br>Company Report N<br>Source<br>GLP/non GLP/GEP/non GEP<br>Published/Unpublished | Y/N | Owner |
| | | | | | |

# Appendix 2 Detailed evaluation of the new studies

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

### A 2.1.1 KCP 10.1.1 Effects on birds

No new studies were 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

No new studies were submitted.

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

| | |
|---|---|
| Comments of zRMS: | In general, the kinetic evaluation is considered acceptable.<br>As results from 5 trials performed in only 1 country is available (4 are Northern France not belonging to the Central Zone, although conditions in Northern France are similar to the Central Europe and 1 is Southern France), it is proposed by the zRMS to use the worst case DT50 of 21.9 hours for purposes of the risk refinement. |

| | |
|---|---|
| Reference: | KCP 10.1.2.2 |
| Report | A kinetic analysis of the dissipation of mesotrione in maize, Hazlerigg C. & Garrat J, 2016, E2016-13 |
| Guideline(s): | Yes, FOCUS guidance |
| Deviations: | No |
| GLP: | / |
| Acceptability: | Yes/No/Supplementary |
| Duplication (if vertebrate study) | / |

**Materials and methods**

Residue trials were undertaken and reported by Schneider (2016) at 5 sites in France. Mesotrione 100 g/L was applied as a foliar spray to maize and the whole plant was analysed at intervals. An overview of the trials and the measured residues are given in the Appendix of this study and are also described in dRR Part B7 (and the relevant study report (Schneider, 2016).

FOCUS (2006, 2014) degradation kinetics guidance was applied to calculate $DT_{50}$ endpoints for mesotrione modelling from residues measured in five plant residue trials in Europe. The data were described reasonably well by either SFO kinetics or bi-phasic FOMC kinetics and acceptable end-points were derived for all five studies.

**Results and discussions**

The calculated $DT_{50}$ values and statistics for the decline of mesotrione in maize are shown in the table below. The $DT_{50}$ values ranged from 10.1 to 21.9 hours. The final $DT_{50}$ recommended for modelling is the geomean of 14 hours.

**Summary of fitted parameters for the decline of mesotrione.**

| Study | Kinetic model | t-test | $\chi$2-error | Visual fit | DT50 (hours) |
|---|---|---|---|---|---|
| B5116 AN1 | FOMC | n/a | 7.93 | Good | 13.3 * |
| B5116 MA1 | SFO | Pass | 6.92 | Good | 21.9 |
| B5116 BM1 | SFO | Pass | 14.3 | Medium | 10.1 |
| B5116 ND1 | FOMC | n/a | 6.74 | Good | 15.2 * |
| B5116 EF1 | FOMC | n/a | 10.9 | Good | 12.1 * |
| **Geomean of all trials** | | | | | **14.0** |

* pseudo first-order $DT_{50}$ calculated as FOMC DT90/3.32 (FOCUS 2006, 2014

**Conclusion**

FOCUS (2006, 2014) guidance was applied to calculate $DT_{50}$ endpoints for mesotrione modelling from residues measured in four plant residue trials in Europe. The data were described reasonably well by either SFO kinetics or bi-phasic FOMC kinetics. The $DT_{50}$ values ranged from 10.1 to 21.9 hours with a geomean of 14 hours. This refined DT50 of 14 hours (instead of the default value of 10 days) was used in the higher tier risk assessment for the reproductive risk to mammals.

| | |
|---|---|
| Comments of zRMS: | The study was evaluated and accepted (Callisto, 2020). |

| | |
|---|---|
| Reference: | KCP 10.1.2.3 |
| Report | Generic monitoring of European hares to determine proportion of time spent foraging in early maize in Central Europe, Grimm T. & Katzschner I., 2019, R1740045 |
| Guideline(s): | Conducted under consideration of the EFSA Guidance Document on Risk Assessment for Birds & Mammals (EFSA 2009). |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | / |

**Executive Summary**

The aim of this generic study was to investigate hares (Lepus europaeus) that use maize fields as foraging habitat in Central Europe in order to determine respective PT values (i.e. proportion of diet obtained in treated area, calculated as proportion of active time spent potentially foraging in maize fields) during the early growing period of maize via continuous 24-hour radio-tracking sessions of multiple individuals. In total, radio-tracking sessions of 21 individual hares at five study sites were performed during the early crop development of maize in Central Europe. The number of conducted 24h telemetry sessions was 23 (17 in Germany, six in Hungary), since two individuals were radio tracked twice. Thereof, one session had to be excluded from analysis, as this session was no 'consumer session' according to EFSA (2009), i.e. the animal was never located being active (i.e. showed behaviour categorized as potentially foraging) in a maize field during the session or at least had maize in the 24h home range. The calculated PT value ranged from 0.02 to 0.94. The mean PT was 0.36, and the 90th percentile PT was 0.62.

**Materials**

| | |
|---|---|
| **Test Material** | No substance was tested. |
| **Test organisms** | |
| **Species:** | European brown hare (*Lepus europaeus*) |
| **Crop:** | Maize, BBCH 00-19 |
| **Test design** | |
| **Replication:** | 5 study sites |
| **Duration of study:** | 3 months |

**Study Design and Methods**

Experimental dates: April - June 2018
The study was conducted in five study sites in Central Europe in two typical areas for maize growing. Three study sites were located in Lower Saxony (Germany), near Holtrop, Rastede and Burhafe, and two study sites were located in the administrative county of Győr-Moson-Sopron (Hungary), one near Szany and one close to Bösarkany.

Initial site selection was based on the presence of sufficient numbers of the European hare and the suitability for performing radio-tracking. A suitable landscape for radio-tracking should be accessible by car and should not contain steep slopes that could lead to disturbances in radio-tracking signals.

These conditions are very important for site selection concerning hares, where observers need to follow the animals over large distances within short time periods.
The vegetation status of maize in the study sites was recorded by BBCH growth stages (Meier 2001). The whole study sites, comprising of the locations around the trapping locations of the tagged animals and the positions in which they were recorded during single check and 24h telemetry, was mapped for habitat types.
The majority of animals were trapped and fitted with radio tags at the beginning of the Field Phase before the drilling period of maize. In Bösarkany trapping of three individuals was conducted on 23.03.2018, before drilling of maize fields took place. In Germany as well trapping in all three study sites in Germany (16 individuals) took place prior to the drilling period and was carried out from 08.04.2018 to 21.04.2018. In the study site near Szany, trapping and tagging of four hares took place after the first maize fields were already drilled (25.04.2018 and 28.04.2018). To assure the availability of maize within the home range of the tracked individuals, hares were captured either on maize fields/future maize fields or nearby (. e.g. in adjacent off-crop structures or neighbouring fields).
Hares were trapped using series of nets of 1.3 m height, 100 m lengths and a mesh size of 5 cm. After trapping, hares were handled directly or (in case of the capture of several individuals at the same time) hares were transferred to a wooden box where animal mobility and light radiation were low, reducing the stress level for the animal. During the handling procedure, animals were sexed, weighed, equipped with a radio tag and released at the trapping site. After installation of the transmitter, animals were given time to acclimatize to normal behaviour. In order to exclude any bias, animals were not tracked on the day of trapping, and radio-tracking started not earlier than two days after tagging. No mortalities occurred during

the Field Phase of the study. In order to confirm the animal's status based on the radio signals, animals were observed with binoculars, scopes and night observation devices ('visual contact') whenever possible. Animals were radio-tracked with Yagi antennas for 24 consecutive hours. Each change of habitat (if possible) and/or each change of behaviour (active/inactive) was recorded with the time (exact to the minute).
Drilled maize fields which were not yet emerged were checked in each study site for the occurrence of weeds at the beginning of the field phase.
The calculation of PT assumes a correlation between the time spent by an active hare in a particular habitat and the amount of food it ingested in that habitat. Hence, it is assumed that the amount of food taken by a hare in a certain time span will be the same in any habitat or crop within its home range. At each telemetry session the proportion of active time of an individual hare in maize (PT) was calculated as the proportion of time the hare spends 'potentially foraging' in that crop. Thus the 'time potentially foraging' is the sum of the time periods covered by behavioural categories when foraging could not be excluded, such as 'foraging', 'moving around' and 'stationary'. All instances when the animal was definitely known to be performing non-foraging activities (e.g. fighting) were excluded from PT calculation. For each 24h telemetry the 'time potentially foraging' within the crop of concern was compared with the total 'time potentially foraging' in all other habitat (see below).

Definition of behavioural categories used for calculation of PT:

| | |
|---|---|
| **Potentially foraging** | all instances when the animal was definitely foraging, or might have been foraging |
| Foraging | animal was definitely foraging (visually observed during radio-tracking while searching for food) |
| Moving around | animal classified as 'moving around' (short distance movement) by radio-tracking signal (and no visual contact) |
| Stationary | animal recorded as not moving (but active signal and no visual contact) |
| **Travelling far** | animal classified as 'travelling far' (long distance at speed) by radio-tracking signal |
| Resting | inactive signal or animal visually observed to be resting |
| Others (excluding foraging) | only for visual contact: active behaviour that can be assigned as non-foraging |
| Behaviour unknown | behaviour cannot be specified due to lack of the signal |

**Results and Discussion**

It was aspired to conduct at least 20 tracking sessions with no less than 15 individuals to guarantee representative tracking results. However, during trapping 23 individuals could be equipped with a radio tag (16 in Germany, seven in Hungary), whereof 21 individuals were tracked for full 24 hour-tracking sessions (15 in Germany, six in Hungary). One individual could never be found after tagging again (animal no. 16 H). One individual was found at the end of May far outside of the study site (animal no. 2 G). These two animals were not radio-tracked by 24h telemetry. To increase the number of radio-tracking sessions and to cover a wider range of BBCH growth stages, two individuals (both in Germany) were radiotracked twice, resulting in 23 radio-tracking sessions.

**Information on trapped individuals**

| Country | Study site | Animal no. | Date of trapping (dd.mm.yyyy) | Body weight (kg) | Sex | Reproductive status |
|---|---|---|---|---|---|---|
| Germany | Rastede | 1_G | 08.04.2018 | -[1] | female | inactive |
| | | 4_G | 15.04.2018 | 3.80 | male | inactive |
| | | 5_G | 15.04.2018 | 3.50 | male | inactive |
| | Burhafe | 2_G | 14.04.2018 | 4.30 | female | inactive |
| | | 3_G | 14.04.2018 | 4.60 | female | active |
| | Holtrop | 13_G | 21.04.2018 | 4.10 | male | inactive |
| | | 14_G | 21.04.2018 | 4.80 | female | active |
| | | 15_G | 21.04.2018 | 4.60 | male | inactive |
| | | 16_G | 21.04.2018 | -[1] | female | inactive |
| | | 17_G | 21.04.2018 | 3.80 | male | active |
| | | 18_G | 21.04.2018 | 3.90 | female | inactive |
| | | 19_G | 21.04.2018 | 3.70 | male | inactive |
| | | 20_G | 21.04.2018 | 4.60 | female | active |
| | | 21_G | 21.04.2018 | 3.60 | female | inactive |
| | | 22_G | 21.04.2018 | 4.60 | female | active |
| | | 23_G | 21.04.2018 | 3.40 | male | inactive |

| | | | | | | |
|---|---|---|---|---|---|---|
| Hungary | Bösarkany | 1_H | 23.03.2018 | 4.60 | male | inactive |
| | | 2_H | 23.03.2018 | .60 | female | inactive |
| | | 3_H | 23.03.2018 | 4.30 | female | inactive |
| | Szany | 13_H | 25.04.2018 | 4.50 | male | active |
| | | 14_H | 25.04.2018 | 4.10 | male | inactive |
| | | 15_H | 25.04.2018 | [1] | male | inactive |
| | | 16_H* | 28.04.2018 | 4.20 | male | active |

* No 24h telemetry performed for this individual (animal no. 2_G was located far outside the study site at the end of May and animal no. 16 H was never located again after tagging)

[1] no weight taken in order to reduce stress for the animal

Drilled maize fields which were not yet emerged were checked in each study site for the occurrence of weeds at the beginning of the Field Phase (except of Szany, where all maize fields, except one, were already emerged). Fields that were still not emerged prior to the start of 24h radio-tracking were checked for weed occurrence again (one field in Holtrop, one field in Szany and two fields in Bösarkany). Each survey showed that no weeds occurred on not yet emerged maize fields in the five study sites. Therefore since hares on those fields could not be foraging, those results were excluded from the PT analysis.

The calculated PT values ranged from 0.02 to 0.94. Calculated PT values did not differ substantially between different study sites, although mean values were slightly higher in Germany.

**Calculated PT values of hares in maize fields in early BBCH growth stages in Central Europe**

| Country | Session ID | PT | Date (dd.mm.2018) | |
|---|---|---|---|---|
| Germany | 398_GER_01 | 0.56 | 18.05. | 14_G |
| | 398_GER_02 | 0.40 | 19.05. | 18_G |
| | 398_GER_03 | 0.17 | 20.05. | 23_G |
| | 398_GER_04 | 0.08 | 23.05. | 20_G |
| | 398_GER_05 | 0.50 | 22.05. | 17_G |
| | 398_GER_06 | 0.09 | 23.05. | 13_G |
| | 398_GER_07 | 0.08 | 22.05. | 19_G |
| | 398_GER_08 | 0.02 | 24.05. | 21_G |
| | 398_GER_09 | 0.44 | 25.05. | 15_G |
| | 398_GER_10 | 0.41 | 26.05. | 16_G |
| | 398_GER_12 | 0.18 | 27.05. | 1_G |
| | 398_GER_13 | 0.89 | 28.05. | 5_G |
| | 398_GER_14 | 0.37 | 30.05. | 4_G |
| | 398_GER_15 | 0.26 | 01.06. | 3_G |
| | 398_GER_16 | 0.94 | 04.06. | 5_G |
| | 398_GER_17 | 0.63 | 05.06. | 1_G |
| Hungary | 398_HU_01 | 0.42 | 29.04. | 15_H |

| Country | Session ID | PT | Date (dd.mm.2018) | |
|---|---|---|---|---|
| | 398_HU_02 | 0.38 | 30.04. | 14_H |
| | 398_HU_03 | 0.56 | 02.05. | 13_H |
| | 398_HU_04 | 0.28 | 13.05. | 1_H |
| | 398_HU_05 | 0.21 | 14.05. | 2_H |
| | 398_HU_06 | 0.02 | 15.05. | 3_H |
| **Mean** | | **0.36** | - | - |
| **90th percentile** | | **0.62** | - | - |
| **SD** | | **0.26** | - | - |

The proportions of all habitats within the outermost locations during 24h telemetry of all individuals per study site are presented in the table below.

**Habitat proportions**

| Country | Study site | Habitat (%) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Arable crop other than maize * | Forest/ Hedges | Others** | Meadow | Maize | | |
| | | | | | | Total maize | emerged maize fields | pre-emerged maize fields |
| Germany | Burhafe | 0.26 | 07.02 | 1.21 | 76.74 | 14.76 | 14.76 | 0.00 |
| | Holtrop | 2.87 | 16.53 | 6.60 | 42.61 | 31.39 | 29.81 | 1.59 |
| | Rastede | 0.00 | 16.85 | 5.75 | 30.95 | 46.45 | 46.45 | 0.00 |
| | Total | 1.75 | 16.33 | 6.12 | 39.54 | 36.26 | 35.29 | 0.96 |
| Hungary | Bösarkany | 34.02 | 8.36 | 1.83 | 1.32 | 54.48 | 44.91 | 9.57 |
| | Szany | 63.74 | 9.26 | 2.83 | 0.63 | 23.54 | 22.27 | 1.27 |
| | Total | 51.71 | 8.89 | 2.42 | 36.06 | 0.91 | 31.44 | 4.63 |
| Total | | 23.83 | 13.05 | 4.48 | 22.47 | 36.17 | 33.59 | 2.58 |

* cereal, lucerne, sunflower, oilseed rape, other crops (e.g. potatoe), stubble and ploughed fields

**streets, settlements, water

The PT values between the sexes did not differ substantially, even though mean values were slightly higher in male hares (0.43) than in female hares (0.28).

**Calculated PT values per sex**

| Species | Sex | Radio-tracking session [N] | PT |
|---|---|---|---|
| Hare | Male | 12 | 0.43 |
| | Female | 10 | 0.28 |

PT*= mean value of all single PT values calculated per sex, unknown habitat excluded from the analyses

**Calculated PT values depending on plant development**

| Species | BBCH growth stages of maize fields inside MCP | Radio-tracking session | PT |
|---|---|---|---|

| | 100% | [N] | |
|---|---|---|---|
| Hare | <15[1] | 16 | 0.35 |
| | 15 or higher[2] | 6 | 0.39 |

PT*= mean value of all single PT values, unknown habitat excluded from the analyses
[1] i.e. only maize fields with BBCH growth stage <15 within the home range of the respective sessions
[2] i.e. maize fields with BBCH growth stage 15 or higher within the home range of the respective sessions

Regarding BBCH growth stages, no clear trend was detectable, although hares tend to spend slightly more active time inside maize fields during 24h radio-tracking sessions containing fields with BBCH growth stages 15 or higher inside the home range. In radio-tracking session in which maize fields of later stages (i.e. BBCH growth stage ≥15) were part of the home range, the mean PT value was 0.39,

whereas the mean PT value in sessions including only maize fields in early stages (i.e. BBCH growth stage < 15) within the home range was 0.35.

**Conclusion**
This study demonstrated that maize fields, at pre-emergence growth stage, are in general not relevant foraging habitats for hares. In total, radio-tracking sessions of 21 individual hares at five study sites were performed during the early crop development of maize in Central Europe. Radio-tracking sessions were performed from late April until early June 2018 and covered the maize BBCH growth stages number of conducted 24h telemetry sessions was 23 (17 in Germany, six in Hungary), since two individuals were radio tracked twice. Thereof, one session had to be excluded from analysis, as this session was no 'consumer session'. The calculated PT value ranged from 0.02 to 0.94, with a mean of .36 and a 90th percentile of 0.62. Calculated PT values did not differ substantially between different study sites; mean values were slightly higher in Germany. Also PT values between sex did not differ substantially. Regarding BBCH growth stages, no clear trend was detectable, although hares tent to spend slightly more active time inside maize fields during 24h tracking sessions containing fields with BBCH growth stages 15 or higher inside the home range. In tracking session in which maize fields in later stages (i.e. BBCH ≥15) were part of the home range, the mean PT value was 0.39, whereas the mean PT value of sessions including only fields in early stages (i.e. BBCH ≥15) were part of the home range, the mean PT value was 0.39, whereas the mean PT value of sessions including only fields in early stages (i.e. BBCH < 15) within the home range was 0.35.

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

No new studies were 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

| | |
|---|---|
| Comments of zRMS: | The study has been assessed and accepted in dRR Callisto, 2020 (Finalization date).<br><br>lowest 7-d $E_rC_{50}$ = 0.028 mg a.s./L |

| | |
|---|---|
| | Corrected for purity:<br>$E_rC_{50}$ frond no or biomass = 0.0241 mg/L nom |

| | |
|---|---|
| Reference: | KCP 10.2.1 |
| Report | Mesotrione Wet Paste (ZA1296) - Toxicity to the Aquatic Plant Lemna gibba in a Semi-Static Growth Inhibition Test with a Subsequent Recovery Period, Kosak & Wydra, 2016, 105732240 |
| Guideline(s): | Yes, OECD 221 (2016) and US EPA OPPTS 850.4400 (1996) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes/No/Supplementary |

**Executive Summary**

The toxicity of ZA1296 to the aquatic plant *Lemna gibba* was determined in a 7-day static test. The *Lemna* were exposed to nominal concentrations of 64, 32, 16, 8, 4 and 2 µg test item/L alongside a dilution water control.

For frond number, the 7-day $EC_{50}$ for yield ($EyC_{50}$) and growth rate ($ErC_{50}$) for ZA1296 to *Lemna gibba* were 6.0 and 28 µg test item /L respectively, based on nominal concentrations. For dry weight, the 7-day $EC_{50}$ for yield ($EyC_{50}$) and growth rate ($ErC_{50}$) were 5.2 and 28 µg test item /L respectively, based on nominal concentrations.

**Materials**

| | |
|---|---|
| **Test Material** | ZA1296<br>Mesotrione Wet Paste |
| **Actual content of active ingredients:** | 631795 (SMO7F333) |
| **Purity:** | 86.1 % (wt/wt) |
| **Description:** | Brown solid |
| **Stability of test compound:** | Stable under standard conditions |
| **Reanalysis/expiry date:** | End of February 2016 |
| **Density:** | n/a |
| **Treatments** | |
| **Test concentrations:** | Dilution water control; nominal concentration of 64, 32, 16, 8, 4 and 2 µg test item/L |
| **Solvent:** | None |
| **Vehicle and/or positive control:** | Potassium dichromate is used as a positive control at least twice a year. |
| **Analysis of test concentrations:** | Yes, analysis of fresh and aged medium at each renewal ~~on days 0 and 7~~ |
| **Test organisms** | |
| **Species:** | *Lemna gibba* |
| **Source:** | In-house cultures |
| **Test design** | |
| **Test vessels:** | 250 mL glass flasks filled with 200 mL test medium covered with glass dishes |
| **Test medium:** | 20X AAP-Growth Medium |
| **Replication:** | Three vessels for the control and each test concentration |
| **Initial frond number:** | 4 fronds per plant, total 12 fronds per replicate |
| **Exposure regime:** | Semi-static (renewal at days 3 and 5) |
| **Duration:** | 7 days |
| **Environmental conditions** | |
| **Temperature:** | 23 - 24 °C |

**pH:** Fresh media:7.5 – 7.9
Aged media: 8.5 – 9.0

**Lighting:** Continuous illumination, 7300 - 7770 Lux (mean 7467 Lux).

**Study Design and Methods**

Experimental dates: 21 August to 28 October 2015

Before test start and before the test medium renewal a concentrated stock solution was prepared by dissolving 10.2, 10.0 and 10.3 mg test item in 1020-, 1000- and 1030-mL test water, respectively. The stock solution was intensively stirred for 40 minutes, and short ultrasonic treatment was used for 15 minutes. Then, adequate volumes were mixed into test water to obtain the desired test concentrations. Appropriate volumes of the stock solution were diluted to give the test concentration series. The control consisted of culture medium only.

200 mL of the test solutions were transferred into 250 mL glass flasks and inoculated with *Lemna* plants. Cultures were then transferred to a temperature-controlled room where they were maintained under the conditions indicated above.

Assessments of frond number were made on days 0, 3, 5 and 7. Fronds were harvested for measurement of dry weight after 7 days, and the initial dry weight was determined using a sample of 12 fronds identical to that used to inoculate the test.

Temperature was measured continuously, light intensity was recorded once at test start and pH was recorded on days 0, 3, 5 and 7 days.

The test concentrations were verified by chemical analysis of ZA1296 at days 0 and 7, using high performance liquid chromatography with ultraviolet-visible detection

**Results**

At the start of the test, the concentrations of the test item were found to be in the range 93 to 107 % of the nominal values and at the end of the test were in the range 87 to 122 % (see table below). Nominal concentrations were used for the calculation and reporting of results.

**Analytical results**

| Nominal concentrations (µg test item /L) | % of nominal measured at 0 days | % of nominal measured at 3 days (aged) | % of nominal measured at 3 days (fresh) | % of nominal measured at 5 days (aged) | % of nominal measured at 5 days (fresh) | % of nominal measured at 7 days |
|---|---|---|---|---|---|---|
| 2 | 107 | 100 | 110 | 101 | 110 | 103 |
| 4 | 119 | 108 | 110 | 109 | 113 | 122 |
| 8 | 97 | 97 | 113 | 108 | 102 | 103 |
| 16 | 97 | 92 | 99 | 94 | 100 | 87 |
| 32 | 93 | 88 | 97 | 93 | 95 | 94 |
| 64 | 101 | 91 | 102 | 95 | 98 | 91 |

Data for frond number and dry weight was used to calculate growth rates and yield for the control and each exposure concentration. Non-linear regression was used to calculate the 7-day $ErC_{50}$ and $EyC_{50}$, based on percent inhibition relative to the control. For the No Observed Effect Concentration and Lowest Observed Effect Concentration, a Williams test was used to determine values significantly different to the control.

Mean frond numbers are presented below along with the growth rate, yield and respective inhibition values, alongside estimated $EC_{50}$ values:

**Effect of ZA1296 on growth rate and yield (frond number) of *Lemna gibba***

| Nominal concentration (µg/L)** | Mean No. fronds/replicate (day 7) | Based on Frond Number (0-7 days) | | | |
|---|---|---|---|---|---|
| | | Growth Rate | Inhibition of Growth Rate (%) | Yield | Inhibition of Yield (%) |
| Control | 250.0 | 0.434 | - | 238.0 | - |
| 2 | 246.3 | 0.431 | 0.6 | 234.3 | 1.5 |
| 4 | 138.7 | 0.349* | 19.6 | 126.7* | 46.8 |
| 8 | 107.0 | 0.312* | 27.9 | 95.0* | 60.1 |
| 16 | 66.0 | 0.243* | 43.9 | 54.0* | 77.3 |
| 32 | 49.0 | 0.200* | 53.8 | 37.0* | 84.5 |
| 64 | 43.7 | 0.184* | 57.7 | 31.7* | 86.7 |
| **$EC_{50}$ µg/L** | | 28 | | 6.0 | |
| **95% confidence limits** | | 20 - 37 | | 4.3 – 8.4 | |
| **NOEC** | | 2.0 | | 2.0 | |
| **LOEC** | | 4.0 | | 4.0 | |

Inoculum = 12 fronds
* mean value significantly different from the control (tested with Williams Test, α = 0.05, one-sided)
** Given as the test item not corrected for purity

Mean dry weights are presented below along with the growth rate, yield and respective inhibition values, alongside estimated $EC_{50}$ values:

**Effect of ZA1296 on growth rate and yield (dry weight) of *Lemna gibba***

| Nominal concentration (µg/L)** | Dry Weight (mg) (day 7) | Based on Dry Weight (0-7 days) | | | |
|---|---|---|---|---|---|
| | | Growth Rate | Inhibition of Growth Rate (%) | Yield | Inhibition of Yield (%) |
| Control | 32.8 | 0.440 | - | 31.3 | - |
| 2 | 32.5 | 0.439 | 0.3 | 31.0 | 0.7 |
| 4 | 16.9 | 0.345* | 21.7 | 15.4* | 50.9 |
| 8 | 11.2 | 0.287* | 34.8 | 9.7* | 69.0 |
| 16 | 8.0 | 0.238* | 45.9 | 6.5* | 79.2 |
| 32 | 7.5 | 0.230* | 47.8 | 6.0* | 80.8 |
| 64 | 5.6 | 0.188* | 57.3 | 4.1* | 86.9 |
| **$EC_{50}$ µg/L** | | 28 | | 5.2 | |
| **95% confidence limits** | | 19 - 42 | | 3.5 – 7.7 | |
| **NOEC** | | 2.0 | | 2.0 | |
| **LOEC** | | 4.0 | | 4.0 | |

Inoculum = 1.5 mg dry weight per vessel
* mean value significantly different from the control (tested with Williams Test, α = 0.05, one-sided)
** Given as the test item, not corrected for purity

## Validity criteria

- Doubling time of frond number was 1.6 (must be less than 2.5 days (60 h))

## Conclusions

For frond number, the 7-day $EC_{50}$ for yield ($EyC_{50}$) and growth rate ($ErC_{50}$) for ZA1296 to *Lemna gibba* were 6.0 and 28 µg test item /L respectively, based on nominal concentrations.
For dry weight, the 7-day $EC_{50}$ for yield ($EyC_{50}$) and growth rate ($ErC_{50}$) for were 5.2 and 28 µg test item ~~ai~~/L respectively, based on nominal concentrations.
The 7-day NOEC was determined to be 2.0 µg test item/L and the 7-day LOEC was determined to be 4.0 µg test item/L

| | |
|---|---|
| Comments of zRMS: | The study has been assessed and accepted in dRR Callisto, 2020 (Finalization date) without pulse-exposure part.<br><br>According to zRMS (Callisto, 2020): "*It should be noted that most of the Central Zone Member States has concerns regarding reliability the modified exposure studies due to uncertainties related to the exposure profiles modelled using FOCUS. Extensive discussion regarding this issue took place during the Central Zone harmonisation meetings and it was concluded that results of Tier 2C studies should be considered only when no acceptable risk may be demonstrated using standard approach (i.e. standard toxicity endpoints and exposure calculated with consideration of the risk mitigation measures).*<br><br>*For mesotrione applied as Callisto acceptable risk to aquatic organisms could be concluded using the endpoint required by EFSA, 2013 (i.e. ErC50) and applying standard risk mitigation measures. Taking this into account, the pulsed-exposure part of the summarised below was not necessary to finalise the risk assessment at the zonal level and in consequence was not evaluated by the zRMS.*"<br><br>14-d $E_rC_{50}$ = 0.0339 mg a.s./L<br>14-d $E_yC_{50}$ = 0.00301 mg a.s./L |

| | |
|---|---|
| Reference: | KCP 10.2.1 |
| Report | Mesotrione – Growth Inhibition of *Myriophyllum spicatum* in a Water/Sediment System, Gonsior G., 2017, S16-06273 |
| Guideline(s): | Yes, OECD 239 (2014) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes/No/Supplementary |

**Executive Summary**

The toxicity of Mesotrione technical to the rooted aquatic macrophyte *Myriophyllum spicatum* was determined in a 14-day semi-static test. The plants were exposed to nominal concentrations of 4.77, 15.3, 48.8, 156 and 500 µg Mesotrione tech./L alongside a dilution water control. Additionally, *Myriophyllum spicatum* was exposed to 70.0 and 120 µg Mesotrione tech./L for 24 hours followed by 13 days growth in untreated medium in two single pulse dose tests.

Based on nominal concentrations, the 14-day $EC_{50}$ values for growth rate ($E_rC_{50}$) and yield ($E_yC_{50}$) for Mesotrione technical to *Myriophyllum spicatum* were 33.9 and 3.01 µg Mesotrione tech./L, respectively, based on total shoot length. The $E_rC_{50}$ and $E_yC_{50}$ values based on biomass (fresh weight) were 108 and 6.90 µg Mesotrione tech./L, respectively, and were 53.3 and 5.81 µg Mesotrione tech./L, respectively, based on biomass (dry weight). No significant effects were observed due to a 24-hour pulse of exposure at rates up to and including 120.0 µg Mesotrione tech./L.

## Materials

| | |
|---|---|
| **Test Material** | Mesotrione Technical<br>ZA1296 |
| **Lot/Batch #:** | 765385<br>SMO0H028 |
| **Purity:** | 84.6 % wt/wt |
| **Description:** | Brown powder |
| **Stability of test compound:** | Stable under standard conditions |
| **Reanalysis/expiry date:** | 28 February 2019 |
| **Density:** | Not applicable |
| **Treatments** | |
| **Test concentrations:** | Toxicity test: Dilution water control; nominal concentration of 4.77, 15.3, 48.8, 156 and 500 µg Mesotrione tech./L<br>~~Pulse dose test 1: 120 µg Mesotrione tech./L~~<br>~~Pulse dose test 2: 70.0 µg Mesotrione tech./L~~ |
| **Solvent:** | None |
| **Analysis of test concentrations:** | Yes, analysis of Mesotrione in overlying water at the start, day 4 (aged and fresh), day 8 (aged and fresh), day 11 (aged and fresh) and at day 14 (aged) in the toxicity test ~~and at test start, day 1 (aged and fresh) and day 4 (aged and fresh) in pulse dose tests~~, using HPLC-MS/MS |
| **Test organisms** | |
| **Species:** | *Myriophyllum spicatum* L. |
| **Source:** | In-house cultures, originally obtained from the Federal Environment Agency Berlin, Germany |
| **Test design** | |
| **Test vessels:** | 300 mL glass vessels (9 cm diameter, 5 cm height) placed in 2 L glass-beakers (12 cm diameter, 24 cm height) containing approximately 350 g moist sediment and 1.5 L growth medium |
| **Test medium:** | SMART AND BARKO growth medium |
| **Replication:** | Toxicity test: five replicates for each test concentration and ten for the control<br>~~Pulse dose tests: ten replicates for each test concentration and ten for the control~~ |
| **Number of shoots per vessel:** | 1 rooted apical shoot |
| **Exposure regime:** | Semi-static |
| **Duration:** | 14 days |
| **Environmental conditions** | |
| **Temperature:** | Toxicity test: 19.2 ± 1.0 °C (18.0 – 21.7 °C)<br>~~Pulse dose test 1: 19.7 ± 1.2 °C (18.0 – 21.9 °C)~~<br>~~Pulse dose test 2: 19.5 ± 1.1 °C (18.0 – 21.6 °C)~~ |
| **pH:** | Toxicity test: 7.93 ± 0.47 (7.48 – 9.84)<br>~~Pulse dose test 1: 8.25 ± 0.72 (7.55 – 9.83)~~<br>~~Pulse dose test 2: 8.23 ± 0.71 (7.55 – 9.90)~~ |
| **Dissolved oxygen:** | Toxicity test: 100 ± 14 % (78 – 156 %)<br>~~Pulse dose test 1: 116 ± 20 % (96 – 171 %)~~<br>~~Pulse dose test 2: 114 ± 19 % (94 – 172 %)~~ |

**Lighting:** 16 hour day length, approximately 120 – 160 µE $m^{-2}$ $s^{-1}$

**Study Design and Methods**

Experimental dates: 03 November 2016 to 02 December 2016

A semi-static toxicity test ~~and two single 24-hour pulse dose tests~~ were performed. A stock solution with a nominal concentration of 500 µg Mesotrione tech./L was prepared by adding the required amount of the test item to a volumetric flask and adding test medium up to the benchmark. The solution was homogenised by shaking and ultrasonication for 4 hours. Appropriate volumes of the stock solution and preceding test solutions were diluted to give the test concentrations. The control consisted of test medium only.

Three days before the start of the test, 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. The test vessels were placed in 2 L glass beakers and filled carefully with 1.5 L of growth medium to a depth of 14 cm. On the day of the test, one rooted apical shoot per vessel was planted carefully, ensuring the plant was rooted into the sediment. Only plants of the same size (e.g., ± 10 – 20 % of mean shoot length) were used for the test. The test item solution was then added and mixed in with gentle stirring. The test solution renewal was performed at day 4, 8 and 11 in the toxicity test ~~and at day 1 and 4 in the pulse dose tests~~. The test vessels were maintained in a controlled environment under the conditions indicated above.

Assessments of plant growth were made on days 0, 7 and 14. Plants were harvested for measurement of biomass (plant fresh weight and plant dry weight), shoot length and number and length of side shoots on day 14, and observations on shoot and root development (e.g., necrosis, deformation) were documented. The initial biomass (plant fresh weight and plant dry weight) and shoot length were determined using a sample of 15 additional plants, representative of those used in the test.

Water temperature, pH and dissolved oxygen saturation were recorded on days 0, 4 (aged and fresh), 8 (aged and fresh), 11 (aged and fresh) and 14 (aged) in the toxicity test and on days 0, 1 (aged and fresh), 4 (aged and fresh), 8 (aged and fresh), 11 (aged and fresh) and 14 (aged) in the pulse dose tests. Light intensity on the water surface was measured at test start.

The test concentrations were verified by analyses of Mesotrione at all concentration levels by analysing the overlaying water at test start, day 4 (aged and fresh), 8 (aged and fresh), 11 (aged and fresh) and 14 (aged) in the toxicity test ~~and at test start, at day 1 (aged and fresh) and 4 (aged and fresh) in the pulse dose tests~~, using high performance liquid chromatography with tandem mass spectrometry.

**Results**

The concentrations of the test item in the freshly prepared solutions were found to be in the range 83 to 110 % of the nominal values and in the aged solutions in the range 87 to 108 %. ~~At the start of the pulse dose tests, the concentrations of the test item in the freshly prepared solutions were found to be in the range 103 to 109 % of the nominal values and after 1 day were 98-99% of nominal; at day 4 of the test concentrations in the aged solution were 1 % of the nominal values (see table below).~~

The limit of quantification in this study was 0.4 µg Mesotrione/L (in water) corresponding to 0.473 µg Mesotrione tech./L. Nominal concentrations were used for the calculation and reporting of results.

**Analytical results**

| Test | Nominal concentrations | | % of nominal Mesotrione concentration measured in overlaying water | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | (µg Meso-trione tech./L) | (µg Mesotri-one/L) | Day 0 | Day 1 | | Day 4 | | Day 8 | | Day 11 | | Day 14 |
| | | | fresh | aged | fresh | aged | fresh | aged | fresh | aged | fresh | aged |
| Toxicity test | Control | 0 | - | n.a. | n.a. | - | - | - | - | - | - | - |
| | 4.77 | 4.04 | 102 | n.a. | n.a. | 108 | 91 | 101 | 83 | 87 | 84 | 91 |
| | 15.3 | 12.9 | 97 | n.a. | n.a. | 102 | 95 | 98 | 84 | 87 | 95 | 95 |
| | 48.8 | 41.3 | 99 | n.a. | n.a. | 100 | 93 | 95 | 86 | 88 | 94 | 96 |
| | 156 | 132 | 105 | n.a. | n.a. | 98 | 94 | 96 | 90 | 92 | 98 | 102 |
| | 500 | 423 | 108 | n.a. | n.a. | 101 | 96 | 100 | 98 | 99 | 110 | 105 |
| ~~Pulse dose test~~ | ~~Control~~ | ~~0~~ | ~~-~~ | ~~-~~ | ~~-~~ | ~~-~~ | ~~-~~ | ~~n.a.~~ | ~~n.a.~~ | ~~n.a.~~ | ~~n.a.~~ | ~~n.a.~~ |
| | ~~70.0~~ | ~~59.2~~ | ~~109~~ | ~~99~~ | ~~-~~ | ~~1~~ | ~~-~~ | ~~n.a.~~ | ~~n.a.~~ | ~~n.a.~~ | ~~n.a.~~ | ~~n.a.~~ |
| | ~~120~~ | ~~102~~ | ~~103~~ | ~~98~~ | ~~-~~ | ~~1~~ | ~~-~~ | ~~n.a.~~ | ~~n.a.~~ | ~~n.a.~~ | ~~n.a.~~ | ~~n.a.~~ |

- Not detectable
n.a. – not analysed

## Biological Results 'Toxicity Test'

Data for total shoot length and biomass was used to calculate growth rates and yield for the control and each exposure concentration. Non-linear analysis was used to calculate the 14-day $E_rC_{10, 20, 50}$ and $E_yC_{10, 20, 50}$. For the No Observed Effect Concentration and Lowest Observed Effect Concentration, all data were subjected to ANOVA. Normality was tested using Shapiro-Wilk's test and homogeneity of variances across treatment groups were tested using a Bartlett's or Levene's test. Normally distributed and homogeneous data were analysed using a Dunnett's test and a Bonferroni-U Exact Test was used to analyse non-normal distribution data to determine significant differences from controls.

Mean total shoot length are presented below along with the growth rate, yield and respective inhibition values, alongside calculated $EC_{10, 20, 50}$ values:

**Effect of Mesotrione technical on growth rate and yield (mean total shoot length) of *Myriophyllum spicatum* in the 'toxicity test'**

| Test type | Nominal concentration (µg Mesotri-one tech./L) | Mean total shoot length (cm) | | Based on mean total shoot length (0-14 days) | | | |
|---|---|---|---|---|---|---|---|
| | | Day 0[1] | Day 14 | Growth Rate (1/day) | Reduction of Growth Rate (%) | Yield (cm) | Reduction of Yield (%) |
| Toxicity test | Control | 5.6 | 44.0 | 0.1466 | - | 38.44 | - |
| | 4.77 | 5.6 | 23.2 | 0.1013* | 30.9* | 17.6* | 54.2* |
| | 15.3 | 5.6 | 20.5 | 0.0915* | 37.6* | 14.9* | 61.2* |
| | 48.8 | 5.6 | 12.5 | 0.0562* | 61.7* | 6.9* | 82.0* |
| | 156 | 5.6 | 12.1 | 0.0540* | 63.2* | 6.5* | 83.1* |
| | 500 | 5.6 | 10.4 | 0.0429* | 70.7* | 4.8* | 87.5* |
| **$EC_{10}$ µg Mesotrione tech./L[2]** | | | | 0.149 | | (-) | |
| **95 % confidence limits** | | | | 0.024 – 0.930 | | (-) | |
| **$EC_{20}$ µg Mesotrione tech./L[2]** | | | | 0.958 | | (-) | |
| **95 % confidence limits** | | | | 0.164 – 5.78 | | (-) | |
| **$EC_{50}$ µg Mesotrione tech./L[2]** | | | | 33.9 | | 3.01 | |
| **95 % confidence limits** | | | | 3.69 – 294 | | 0.117 – 90.3 | |
| **NOEC** | | | | n.d. | | n.d. | |

| Test type | Nominal concentration (µg Mesotrione tech./L) | Mean total shoot length (cm) | | Based on mean total shoot length (0-14 days) | | | |
|---|---|---|---|---|---|---|---|
| | | Day 0[1] | Day 14 | Growth Rate (1/day) | Reduction of Growth Rate (%) | Yield (cm) | Reduction of Yield (%) |
| **LOEC** | | | | 4.77 | | 4.77 | |

[1] Based on 15 additional plants, representative of those used in the test
[2] Calculation based on 3-param. Normal CDF (cumulative distribution function)
(-) Values not reliable, control CV exceeded the effect level
* Significantly different reduction compared to the control
n.d.- not detectable; no NOEC could be determined

Mean fresh weights are presented below along with the growth rate, yield and respective inhibition values, alongside calculated $EC_{10, 20, 50}$ values:

**Effect of Mesotrione technical on growth rate and yield (mean fresh weight) of *Myriophyllum spicatum* in the 'toxicity test'**

| Test | Nominal concentration (µg Mesotrione tech./L) | Mean fresh weight (g) | | Based on mean fresh weight (0-14 days) | | | |
|---|---|---|---|---|---|---|---|
| | | Day 0[1] | Day 14 | Growth Rate (1/day) | Reduction of Growth Rate (%) | Yield (g) | Reduction of Yield (%) |
| Toxicity test | Control | 0.1069 | 1.1573 | 0.1685 | - | 1.0504 | - |
| | 4.77 | 0.1069 | 0.7195 | 0.1357 | 19.5* | 0.6126* | 41.7* |
| | 15.3 | 0.1069 | 0.5317 | 0.1140 | 32.3* | 0.4248* | 59.6* |
| | 48.8 | 0.1069 | 0.3545 | 0.0847* | 49.7* | 0.2476* | 76.4* |
| | 156 | 0.1069 | 0.3261 | 0.0769* | 54.4* | 0.2192* | 79.1* |
| | 500 | 0.1069 | 0.2896 | 0.0709* | 57.9* | 0.1827* | 82.6* |
| **$EC_{10}$ µg Mesotrione tech./L[2]** | | | | 0.300 | | (-) | |
| **95 % confidence limits** | | | | 0.044 – 2.03 | | (-) | |
| **$EC_{20}$ µg Mesotrione tech./L[2]** | | | | 2.26 | | (-) | |
| **95 % confidence limits** | | | | 0.341 – 15.3 | | (-) | |
| **$EC_{50}$ µg Mesotrione tech./L[2]** | | | | 108 | | 6.90 | |
| **95 % confidence limits** | | | | 8.97 - 1174 | | 0.267 - 200 | |
| **NOEC** | | | | n.d. | | n.d. | |
| **LOEC** | | | | 4.77 | | 4.77 | |

[1] Based on 15 additional plants, representative of those used in the test
[2] Calculation based on 3-param. Normal CDF (cumulative distribution function)
(-) Values not reliable, control CV exceeded the effect level
* Significantly different reduction compared to the control
n.d.- not detectable; no NOEC could be determined

Mean dry weights are presented below along with the growth rate, yield and respective inhibition values, alongside calculated $EC_{10, 20, 50}$ values:

**Effect of Mesotrione technical on growth rate and yield (dry weight) of *Myriophyllum spicatum* in the 'toxicity test'**

| Test | Nominal concentration (µg Mesotrione tech./L) | Mean dry weight (g) | | Based on mean dry weight (0-14 days) | | | |
|---|---|---|---|---|---|---|---|
| | | Day 0[1] | Day 14 | Growth Rate (1/day) | Reduction of Growth Rate (%) | Yield (g) | Reduction of Yield (%) |
| Toxicitytest | Control | 0.0116 | 0.0740 | 0.1311 | - | 0.0624* | - |

| Test | Nominal concentration (µg Mesotrione tech./L) | Mean dry weight (g) | | Based on mean dry weight (0-14 days) | | | |
|---|---|---|---|---|---|---|---|
| | | Day 0[1] | Day 14 | Growth Rate (1/day) | Reduction of Growth Rate (%) | Yield (g) | Reduction of Yield (%) |
| | 4.77 | 0.0116 | 0.439 | 0.0943* | 28.1* | 0.0323* | 48.2* |
| | 15.3 | 0.0116 | 0.0415 | 0.0900* | 31.4* | 0.0299* | 52.1* |
| | 48.8 | 0.0116 | 0.0243 | 0.0509* | 61.2* | 0.0127* | 79.6* |
| | 156 | 0.0116 | 0.0278 | 0.0595* | 54.6* | 0.0162* | 74.0* |
| | 500 | 0.0116 | 0.0208 | 0.0412* | 68.6* | 0.0092* | 85.3* |
| **$EC_{10}$ µg Mesotrione tech./L[2]** | | | | (-) | | (-) | |
| **95 % confidence limits** | | | | (-) | | (-) | |
| **$EC_{20}$ µg Mesotrione tech./L[2]** | | | | 1.42 | | (-) | |
| **95 % confidence limits** | | | | 0.124 – 17.1 | | (-) | |
| **$EC_{50}$ µg Mesotrione tech./L[2]** | | | | 53.3 | | 5.81 | |
| **95 % confidence limits** | | | | 2.37 - 1087 | | 0.067 - 533 | |
| **NOEC** | | | | n.d. | | n.d. | |
| **LOEC** | | | | 4.77 | | 4.77 | |

[1] Based on 15 additional plants, representative of those used in the test
[2] Calculation based on 3-param. Normal CDF (cumulative distribution function)
(-) Values not reliable, control CV exceeded the effect level
* Significantly different reduction compared to the control
n.d - no NOEC could be determined

In the toxicity test, visible effects of the test material on shoot development were observed after 7 days at 48.8 µg Mesotrione tech./L and 14 days at 15.3 µg Mesotrione tech./L and above.

~~***Biological Results 'Pulse Dose Tests'***~~

~~Following exposure to Mesotrione Technical for 24 hours in a pulse dose design, no significant differences from the controls were seen in either the shoot length nor biomass (fresh weight and dry weight) results for either of the concentrations tested, as indicated in the tables below.~~

~~**Effect of Mesotrione technical on growth rate and yield (mean total shoot length and shoot fresh weight) of *Myriophyllum spicatum* in the 'pulsed dose test'**~~

| ~~Nominal conc. for 24h pulse [µg/L]~~ | ~~Total shoot length after 14 days~~ | | | | ~~Shoot fresh weight after 14 days~~ | | | |
|---|---|---|---|---|---|---|---|---|
| | ~~yield [g]~~ | ~~reduction in yield [%]~~ | ~~growth rate [1/day]~~ | ~~reduction in growth rate [%]~~ | ~~yield [g]~~ | ~~reduction in yield [%]~~ | ~~growth rate [1/day]~~ | ~~reduction in growth rate [%]~~ |
| ~~Control~~ | ~~42.6~~ | ~~-~~ | ~~0.1532~~ | ~~-~~ | ~~1.2139~~ | ~~-~~ | ~~0.1789~~ | ~~-~~ |
| ~~70.0~~ | ~~40.5~~ | ~~4.9 n.s.~~ | ~~0.1502~~ | ~~2.0 n.s.~~ | ~~1.1425~~ | ~~5.9 n.s.~~ | ~~0.1749~~ | ~~2.2 n.s.~~ |
| ~~120~~ | ~~41.4~~ | ~~2.8 n.s.~~ | ~~0.1517~~ | ~~1.0 n.s.~~ | ~~1.3112~~ | ~~-8 n.s.~~ | ~~0.1843~~ | ~~-3 n.s.~~ |

~~n.s. = not significantly different from control~~

~~**Effect of Mesotrione technical on growth rate and yield (mean total shoot dry weight) of *Myriophyllum spicatum* in the 'pulsed dose test'**~~

| ~~**Nominal conc. for 24h pulse [µg/L]**~~ | ~~**Shoot dry weight after 14 days**~~ | | | |
|---|---|---|---|---|
| | ~~**yield [g]**~~ | ~~**reduction in yield [%]**~~ | ~~**growth rate [1/day]**~~ | ~~**reduction in growth rate [%]**~~ |
| ~~Control~~ | ~~0.0748~~ | ~~-~~ | ~~0.1429~~ | ~~-~~ |
| ~~70.0~~ | ~~0.0764~~ | ~~-2.1 n.s.~~ | ~~0.1440~~ | ~~-0.8 n.s.~~ |
| ~~120~~ | ~~0.0831~~ | ~~-11.1 n.s.~~ | ~~0.1497~~ | ~~-4.8 n.s.~~ |

~~n.s. = not significantly different from control~~

~~In the pulse dose tests, no visible effects of the test material on shoot development were apparent after 7 and 14 days.~~

**Validity**

Control plants had no visual symptoms of chlorosis and were visibly free from contamination by other organisms such as algae and/or bacterial films on the plants, at the surface of the sediment and in the aqueous growth medium. Since the coefficient of variations (CV) for fresh weight and shoot length yield were below 35 % (actual: 24.3 and 16.0 %, respectively, in the toxicity test and 15.5 and 14.1 %, respectively, in the pulse dose tests) and a doubling of shoot biomass and length was reached within the test duration (actual: > 6-fold), the mean control growth rates and variability were considered acceptable.

**Conclusions**

Based on nominal concentrations, the 14-day $EC_{50}$ values for growth rate ($E_rC_{50}$) and yield ($E_yC_{50}$) for Mesotrione technical to *Myriophyllum spicatum* were 33.9 and 3.01 µg Mesotrione tech./L, respectively, based on total shoot length. The $E_rC_{50}$ and $E_yC_{50}$ values based on biomass (fresh weight) were 108 and 6.90 µg Mesotrione tech./L, respectively, and were 53.3 and 5.81 µg Mesotrione tech./L, respectively, based on biomass (dry weight). The 14-day NOEC for growth rate and yield based on total shoot length and biomass could not be determined. The 14-day LOEC for growth rate and yield was 4.77 µg Mesotrione tech./L, based on total shoot length and biomass (fresh weight and dry weight).

~~No significant effects were observed due to a 24-hour pulse of exposure at rates up to and including 120.0 µg Mesotrione tech./L.~~

| | |
|---|---|
| Comments of zRMS: | The study was performed according to OECD TG 221 and principles of GLP.<br>The validity criteria are met: the doubling time of frond numbers in the con-trol was less than 2.5 days (actually 1.6 days – 1st week and 1.4 days – 2nd week), as required by OECD 221 Guideline.<br><br>There were no deviations to the study plan.<br><br>The study was performed with two successive 24-hour pulses. Toxicological (in)dependence of the two pulses were described.<br><br>However for Thiencarbazone-methyl, applied as GLOB2112dH / Walkover Trio, acceptable risk to aquatic organisms could be concluded using the EU agreed end-points and applying standard risk mitigation measures. Taking this into account, the Tier 2C study was not necessary to finalise the risk assessment at the zonal |

| | |
|---|---|
| | level.<br><br>**The suitability of the study results for the risk assessment should be considered at the MS level.** |

| | |
|---|---|
| Reference: | KCA 8.2.7 |
| Report | Thiencarbazone-methyl: Toxicity to the aquatic plant *Lemna gibba* in a pulsed exposure growth inhibition test, Minati R., 2024, 178651240 |
| Guideline(s): | Yes, OECD 221, 2006 and SANTE/2020/12830 Rev.2 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes/No/Supplementary |

**Executive summary**

The purpose of this study was to determine the inhibitory effect of the test item Thiencarbazone-methyl on the growth of the freshwater aquatic plant *Lemna gibba* under pulsed exposure conditions.
For this purpose, cultures of *Lemna gibba* were exposed in two pulses to various concentrations under defined conditions. The inhibition of growth in relation to control cultures was determined over a test period of 14 days with a first 24-hour pulse on day 0-1 followed by a non-exposure phase till day 7 (1st week) and a second successive 24-hour pulse on day 7-8 followed by a non-exposure phase till day 14 (2nd week).
The purpose of the analytical part of this study was to verify the concentrations of the test item in the test medium.

**Materials and methods**

| | |
|---|---|
| Test Item: | Thiencarbazone-methyl; batch no.: HERF00-H220901; purity: 98.8 % |
| Test Species: | *Lemna gibba* G 3 |
| Test Design: | This study encompassed 7 treatment groups (6 dose rates of the test item and a control) with 3 replicates per test concentration and control.<br>A pulsed exposure study was performed with two successive 24-hour pulses:<br>- First 24-hour pulse from day 0 to day 1 followed by non-exposure phase in fresh test water from day 1 to day 7 (1st week)<br>- Second 24-hour pulse from day 7 to day 8 followed by non-exposure phase in fresh test water from day 8 until test end at day 14 (2nd week)<br>At test start (day 0) 12 fronds were introduced in each replicate.<br>To avoid nutrient depletion and space limitations, only 12 fronds of each replicate were transferred into the exposure media at start of the second pulse (day 7).<br>The frond numbers were determined on days 2, 5, 7, 9, 12 and 14.<br>Initial dry weight of a sample of fronds identical to that used to inoculate the test vessels was determined at day 0. Dry weights were determined at the end of the first non-exposure period on day 7 and at test termination (day 14).<br>Sublethal symptoms were recorded at test start (day 0), on days 2, 5, 7, 9 and 12 and at test end on day 14. |
| Endpoints: | Inhibition of growth expressed in terms of yield and growth rate based on frond number and dry weight. |
| Test Concentrations: | 60, 19, 6.01, 1.9, 0.6 and 0.19 µg test item/L (spacing factor 3.16) and |

a control.

Test Conditions: Light regime (1st and 2nd week): continuous illumination:
observed mean light intensity: 7820 lux (7260 to 8090 lux);

1st week:
Water temperature: 23.3 to 23.8°C
pH values of the freshly prepared control medium at test start (day 0) and at day 1: 7.5
pH values of the aged control medium at day 1 and day 7: 8.0 to 8.7
pH values of the freshly prepared media in the test item treatment at test start (day 0) and at day 1: 7.5
pH values of the aged media in the test item treatments at day 1 and day 7: 8.0 to 8.6;
2nd week:
Water temperature: 23.0 to 23.4 C
pH values of the freshly prepared control medium at day 7 and at day 8: 7.5
pH values of the aged control medium at day 8 and test end (day 14): 8.1 to 9.5
pH values of the freshly prepared media in the test item treatments media at day 7 and at day 8: 7.5
pH values of the aged test media at day 8 and test end (day 14): 8.0 to 9.4;

**Results**

**1st week:**

Validity Criteria: Doubling Time of fronds in the control:
Achieved: 1.6 days (criteria: < 2.5d)
Thus, the validity criterion was met.

Biological Results: Observed effects on plant growth followed a concentration-response-relationship. Phytotoxic symptoms were observed after 7 days at the test item concentrations of 1.9, 6.01, 19 and 60 µg test item/L and included chlorosis, gibbous growth, shortened roots and smaller fronds.

**Summary of Biological Results (1st week)**

| Parameter | Yield (frond number) [µg test item/L] | Growth rate (frond number) [µg test item/L] | Yield (dry weight) [µg test item/L] | Growth rate (dry weight) [µg test item/L] |
|---|---|---|---|---|
| $EC_{50}$ (7-day) | 3.94 | 27.5 | 4.91 | 47.0 |
| 95 % conf. limits | 2.68 - 5.23 | 19.9 - 35.2 | 3.65 - 6.60 | 35.8 - 58.4 |
| $EC_{20}$ (7-day) | 0.826 | 2.54 | 0.923 | 4.18 |
| 95 % conf. limits | 0.462 - 1.21 | 1.32 - 3.99 | 0.617 - 1.40 | 2.29 - 6.37 |
| $EC_{10}$ (7-day) | 0.331 | 0.631 | 0.386 | 1.02 |
| 95 % conf. limits | < 0.19 - 0.559 | 0.241 - 1.21 | 0.229 - 0.654 | 0.418 - 1.87 |
| 7-day NOEC | 1.9 | 0.6 | 1.9 | 0.6 |
| 7-day LOEC | 6.01 | 1.9 | 6.01 | 1.9 |

conf. limits: confidence limits, Values refer to nominal test concentrations

**2nd week:**

Validity Criteria: Doubling Time of fronds in the control:
Achieved: 1.4 days (criteria: < 2.5d)

Thus, the validity criterion was met.

Biological Results: Observed effects on plant growth followed a concentration-response-relationship. Phytotoxic symptoms were observed after 14 days at the test item concentrations of 1.9, 6.01, 19 and 60 µg test item/L and included chlorosis, gibbous growth, shortened roots, smaller fronds, overlapping fronds and necrosis.

**Summary of Biological Results (2nd week)**

| Parameter | Yield (frond number) [µg test item/L] | Growth rate (frond number) [µg test item/L] | Yield (dry weight) [µg test item/L] | Growth rate (dry weight) [µg test item/L] |
|---|---|---|---|---|
| $EC_{50}$ (14-day) | 3.30 | 12.2 | 5.33 | 52.2 |
| 95 % conf. limits | 2.26 - 4.35 | 10.5 - 13.9 | 3.45 - 8.24 | 37.6 - > 60 |
| $EC_{20}$ (14-day) | 1.13 | 3.67 | 1.20 | 4.86 |
| 95 % conf. limits | 0.594 - 1.65 | 2.89 - 4.42 | 0.664 - 2.22 | 2.33 - 7.90 |
| $EC_{10}$ (14-day) | 0.607 | 1.82 | 0.553 | 1.21 |
| 95 % conf. limits | 0.250 - 1.00 | 1.30 - 2.35 | 0.252 - 1.21 | 0.402 - 2.46 |
| 14-day NOEC | 0.6 | 0.6 | 0.6 | 0.6 |
| 14-day LOEC | 1.9 | 1.9 | 1.9 | 1.9 |

conf. limits: confidence limits
Values refer to nominal test concentrations

Toxicological (in)dependence of the two pulses:
For the number of fronds, the ANOVA results indicate that the explanatory variable pulse showed a significant impact on the inhibition in both yield and growth rate. A further post-hoc investigation based on the paired one-sided t-test for each concentration shows that only the inhibition in yield and in growth rate for the two highest concentrations of 19 and 60 µg test item/L are significantly greater after pulse 2 than after pulse 1

For the dry weight, the ANOVA results indicate that the explanatory variable pulse does not show a significant impact on the inhibition in both yield and growth rate. A further post-hoc investigation based on the paired one-sided t-test for each concentration supports the conclusion that there is no significant increase in inhibition after pulse 2 relative to pulse 1

Analytical results:

The quantification of the of the test item Thiencarbazone-methyl in the test samples was performed using liquid chromatography with MS/MS detection.
The concentrations of the test item were determined in the test media of the nominal test concentrations of 0.19, 0.60, 1.90, 6.01, 19.0 and 60.0 µg test item/L and in the control.
At the start of the test (start of the first exposure peak) 101% (97%-104%) of the nominal test concentrations were found in the test media (average of all test concentrations). At the end of the first exposure peak 104% (102%-106%) of the nominal test concentrations were found (average of all test concentrations). At the beginning of the first non-exposure phase the test item concentration was below the LOD for all test concentrations.
At the start of the second exposure peak 107% (96%-137%) of the nominal test concentrations were found in the test media (average of all test concentrations). At the end of the second exposure peak 105% (102%-118%) of the nominal test concentrations were found (average of all test concentrations).
Thus, the test item was dosed correctly and stable during the exposure periods. The transfer of the plants from the test media to the fresh test water was performed without any contamination.

**Conclusion**

The study is valid since all required validity criteria were fulfilled.

The influence of Thiencarbazone-methyl on the growth of the freshwater plant *Lemna gibba* was assessed in a pulsed exposure concentration-response test.

1st week:
The 7-day $E_yC_{50}$ value was calculated to be 3.94 and 4.91 µg test item/L for frond number and dry weight, respectively.
The 7-day $E_rC_{50}$ value was calculated to be 27.5 and 47.0 µg test item/L for frond number and dry weight.
The 7-day $NOE_yC$ and the $LOE_yC$ were determined to be 1.9 and 6.01 µg test item/L for frond number and dry weight.
The 7-day $NOE_rC$ and the $LOE_rC$ were determined to be 0.6 and 1.9 µg test item/L for frond number and dry weight.

2nd week:
The 14-day $E_yC_{50}$ value was calculated to be 3.30 and 5.33 µg test item/L for frond number and dry weight, respectively.
The 14-day $E_rC_{50}$ value was calculated to be 12.2 and 52.2 µg test item/L for frond number and dry weight.
The 14-day $NOE_yC$ and the $LOE_yC$ were determined to be 0.6 and 1.9 µg test item/L for frond number and dry weight.
The 14-day $NOE_rC$ and the $LOE_rC$ were determined to be 0.6 and 1.9 µg test item/L for frond number and dry weight.

Additional statistical analysis shows that the two pulses are independent for the parameter dry weight for all test concentration. For fronds number, inhibitions in yield and in growth rate for the two highest concentrations of 19 and 60 µg test item/L are significantly greater after pulse 2 than after pulse 1.

The measurement of the samples taken at the start of the non-exposure phases demonstrated that the transfer of the plants from the test media to fresh test water was performed without significant contamination. The concentrations of the test item were below the LOD in all samples.
All reported results refer to nominal values since the concentrations of the test item were mainly within ± 20% of the nominal concentrations during the pulse exposure phases.

| | |
|---|---|
| Comments of zRMS: | The study was performed according to OECD TG 239 and principles of GLP.<br><br>The study was performed with two exposure regimes (scenarios A and B). Scenar-io A: 24-hour pulse (± 30 min) from day 0 to day 1 followed by non-exposure phase in fresh test water (Smart & Barko only) from day 1 until test end at day 14. Toxicological (in)dependence of the two pulses were described. Scenario B: First 24-hour pulse (± 30 min) from day 0 to day 1 followed by non-exposure phase in fresh test water (Smart & Barko only) from day 1 to day 7 and second 24-hour pulse (± 30 min) from day 7 to day 8 followed by non-exposure phase in fresh test water (Smart & Barko only) from day 8 until test end at day 14<br><br>The validity criteria are met<br>Scenario A<br>- Control plants did not show any sign of sublethal effects and were visibly free from contamination by other organisms such as algae and/or bac-terial film.<br>- The total shoot length increased by a factor of 6.4 after 14 days of expo-sure (criteria: factor of ≥ 2).<br>- The fresh weight increased by a factor of 5.4 after 14 days of expo-sure |

| | |
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| | (criteria: factor of ≥ 2).<br>- The coefficient of variation of yield fresh weight was 12.6 % (criteria: ≤ 35%). as required by OECD 221 Guideline. There were no deviations to the study plan.<br><br>Scenario B<br>- Control plants did not show any sign of sublethal effects and were visibly free from contamination by other organisms such as algae and/or bacterial film.<br>- The total shoot length in control plants increased by a factor of 5.0 during the 14-days study period (criteria: factor of ≥ 2).<br>- The fresh weight in control plants increased by a factor of 4.7 during the 14-days study period (criteria: factor of ≥ 2).<br>- The coefficient of variation of yield fresh weight in control plants was 20.1 % (criteria: ≤ 35%).<br>-<br>The following deviation to the study plan, regarding analysis of test item concentration was noted:<br>According to Study Plan: fresh samples from both non-exposure periods will be taken.<br>Deviation to the Study Plan: fresh samples were taken only from the start of the 1st non-exposure period. From the 2nd non-exposure period no samples were taken.<br>Presumed Effect on the Study:<br>None, the measurements at the start of non-exposure phase after the 1st peak confirm that the method for the medium exchange is appropriate and show no transfer of test substance after medium-renewal. This is in line with the results of the range finder, in which samples from start of both non-exposure periods were taken and measured (all samples <LOD).<br><br>Toxicological (in)dependence of the two pulses were described.<br><br>However for Thiencarbazone-methyl, applied as GLOB2112dH / Walkover Trio, acceptable risk to aquatic organisms could be concluded using the EU agreed endpoints and applying standard risk mitigation measures. Taking this into account, the Tier 2C study was not necessary to finalise the risk assessment at the zonal level.<br><br>The suitability of the study results for the risk assessment should be considered at the MS level. |

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| Reference: | KCA 8.2.7 |
| Report | Thiencarbazone-methyl: Toxicity to the aquatic plant *Myriophyllum spicatum* in a pulsed exposure growth inhibition test with a prior rooting phase, Bebon R., 2024, 178651215 |
| Guideline(s): | Yes, OECD 239, 2014 and SANTE/2020/12830 Rev.2 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes/No/Supplementary |

**Executive summary**

The purpose of this test was to determine the inhibitory effect of the test item Thiencarbazone-methyl on the vegetative growth of the freshwater aquatic plant *Myriophyllum spicatum* under pulsed exposure conditions. Plants were exposed in two different pulsed exposure scenarios to various concentrations of the test item under defined conditions. The inhibition of growth in relation to control cultures was determined over a test period of 14 days with a single 24-hour pulse on day 0-1 followed by a non-exposure phase till day 14 (Scenario A) and over a test period of 14 days with two successive 24-hour pulses on days 0-1 and 7-8 followed by non-exposure phases till day 7 and day 14, respectively (Scenario B).
The purpose of the analytical part of this study was to verify the concentrations of the test item in the test medium.

**Materials and methods**

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| Test Item: | Thiencarbazone-methyl; Batch No.: HERF00-H220901; Purity: 98.8 %. |
| Test Species: | *Myriophyllum spicatum* |
| Test Design: | This study encompassed 6 treatment groups (5 dose rates of the test item and a control) with five replicates per test concentration and ten replicates for the control in each scenario. Each replicate (test beaker) contained one pot with one plant (shoot).<br>After a pre-rooting phase of 7 days, 1 plant per replicate was incubated for 14 days under pulsed exposure conditions.<br>For destructive measurements of the fresh and dry weight on day 7 in Scenario B, five additional replicates per test concentration and ten additional replicates for the control, each containing one plant, were prepared.<br><u>Scenario A:</u><br>- 24-hour pulse (± 30 min) from day 0 to day 1 followed by non-exposure phase in fresh test water (Smart & Barko only) from day 1 until test end at day 14<br><br><u>Scenario B:</u><br>- First 24-hour pulse (± 30 min) from day 0 to day 1 followed by non-exposure phase in fresh test water (Smart & Barko only) from day 1 to day 7<br>Second 24-hour pulse (± 30 min) from day 7 to day 8 followed by non-exposure phase in fresh test water (Smart & Barko only) from day 8 until test end at day 14<br>At the start of the test and at each test medium renewal, the aged test water or test medium was removed completely and replaced by the appropriate freshly prepared untreated (control) or treated test media.<br>The shoot length was determined at test start (day 0) and at test end (day 14) in both exposure regimes (scenarios A and B). In Scenario B, additional length measurements were performed at day 7.<br>Sublethal parameters were assessed at test start, once during the test (e.g. day 7) and at test end in both scenarios.<br>At test end, fresh and dry weight of each replicate was determined in both exposure regimes (scenarios A and B). In Scenario B, additional weight determination was performed at day 7.<br>The samples of fresh and aged test media collected at the start and end of each of the pulses, respectively, and the fresh samples taken at the start of the 1st recovery phases (from both scenarios) were analysed. |
| Endpoints: | Inhibition of growth expressed in terms of yield and growth rate, based on total shoot length, fresh and dry weight |
| Test Concentrations: | Nominally 27.3, 9.1, 3.0, 1.0 and 0.34 µg test item/L (spacing factor |

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| | 3) and a control. |
| Test Conditions: | Water temperature: observed: 20.9 - 22.3 °C (target: 20 ± 2 °C)<br>Light regime: 16 h light : 8 h dark; observed mean light intensity: 8771 lux (8020 - 9660 lux) (target: 8000-10000 lux)<br><br><u>Scenario A:</u><br>Before usage of the untreated test water the pH was measured: 7.9 - 8.0<br>pH values of the control in the freshly prepared test media: 7.8 - 7.9, in the aged test media: 7.8 - 10.0*;<br>pH values of test item treatments in the freshly prepared test media: 7.7 - 8.0, in the aged test media: 7.8 - 10.1;<br>Oxygen concentrations in the freshly prepared test media: 8.5 - 9.3 mg/L, in the aged test media: 8.5 - 14.7 mg/L.<br><br><u>Scenario B:</u><br>Before usage of the untreated test water the pH was measured: 7.9 - 8.0<br>pH values of the control in the freshly prepared test media: 7.9 - 8.0, in the aged test media: 8.0 - 10.0*;<br>pH values of test item treatments in the freshly prepared test media: 7.8 - 8.0, in the aged test media: 7.8 - 10.0;<br><br>Oxygen concentrations in the freshly prepared test media: 8.5 - 9.3 mg/L, in the aged test media: 8.7 - 13.9 mg/L.<br><br>* According to the OECD guideline 239 the increase of pH of >1.5 does not invalidate the study.<br>The pH increases because $HCO_3^-$ from the medium is metabolised by growing plants. They need the $CO_2$ for their cell growth and release $OH^-$, which in consequence increases the pH of the test medium. This is a natural reaction called biogenic decalcification. |

**Results**

<u>Scenario A:</u>

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| Validity Criteria: | Control plants did not show any sign of sublethal effects and were visibly free from contamination by other organisms such as algae and/or bacterial film.<br>The mean total shoot length in control plants increased by a factor of 6.4 during the 14-days study period (criteria: factor of ≥ 2).<br>The mean fresh weight in control plants increased by a factor of 5.4 during the 14 days study period (criteria: factor of ≥ 2).<br>The coefficient of variation of yield fresh weight in control plants was 12.6 % (criteria: ≤ 35%).<br>Thus, all validity criteria were met. |
| Biological Results: | Observed effects on plant growth followed a concentration-response-relationship. Phytotoxic symptoms were observed at 1.0, 3.0, 9.1 and 27.3 µg test item/L and number and extent of the symptoms increased with increasing test concentration. Symptoms observed included shortened shoot tips, lack of buoyancy, chlorosis, shortened roots and decrease in root number. |

**Summary of Biological Results (Scenario A)**

| Parameter | Yield (total shoot length) [µg test item/L] | Growth rate (total shoot length) [µg test item/L] | Yield (fresh weight) [µg test item/L] | Growth rate (fresh weight) [µg test item/L] | Yield (dry weight) [µg test item/L] | Growth rate (dry weight) [µg test item/L] |
|---|---|---|---|---|---|---|
| $EC_{50}$ (14-day) | 7.01 | 15.4 | 6.92 | 18.8 | >27.3 | >27.3 |
| 95 % conf. limits | 5.26 - 9.23 | 12.9 - 18.6 | 5.42 - 9.05 | 14.9 - 25.1 | n.d. | n.d. |
| $EC_{20}$ (14-day) | 1.13 | 4.44 | 1.05 | 3.23 | 4.74 | >27.3 |
| 95 % conf. limits | 0.572 - 1.76 | 2.92 - 5.89 | 0.653 - 1.48 | 2.23 - 4.26 | 1.09 - 13.0 | 14.3 - >27.0 |
| $EC_{10}$ (14-day) | 0.338* | 1.95 | 0.390 | 1.29 | 0.476 | 2.83 |
| 95 % conf. limits | 0.120* - 0.648 | 1.02 - 2.96 | 0.197* - 0.629 | 0.722 - 1.92 | 0.003* - 1.68 | 0.155* - 7.41 |
| 14-day NOEC | <0.34 | 0.34 | 0.34 | 0.34 | 0.34 | 0.34 |
| 14-day LOEC | ≤0.34 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |

n.d.: could not be determined
Values refer to nominal test concentrations
*extrapolated by Probit analysis (fresh weight and dry weight) and Weibull analysis (total shoot length)

Scenario B:

Validity Criteria: Control plants did not show any sign of sublethal effects and were visibly free from contamination by other organisms such as algae and/or bacterial film.
The total shoot length in control plants increased by a factor of 5.0 during the 14-days study period (criteria: factor of ≥ 2).
The fresh weight in control plants increased by a factor of 4.7 during the 14-days study period (criteria: factor of ≥ 2).
The coefficient of variation of yield fresh weight in control plants was 20.1 % (criteria: ≤ 35%).
Thus, all validity criteria were met.

Biological Results: Observed effects on plant growth followed a concentration-response-relationship. Phytotoxic symptoms were observed at 1.0, 3.0, 9.1 and 27.3 µg test item/L and number and extent of the symptoms increased with increasing test concentration. Symptoms observed included shortened shoot tips, lack of buoyancy, chlorosis, necrosis and shortened roots.

**Summary of Biological Results (Scenario B)**

| Parameter | Yield (total shoot length) [µg test item/L] | Growth rate (total shoot length) [µg test item/L] | Yield (fresh weight) [µg test item/L] | Growth rate (fresh weight) [µg test item/L] | Yield (dry weight) [µg test item/L] | Growth rate (dry weight) [µg test item/L] |
|---|---|---|---|---|---|---|
| $EC_{50}$ (14-day) | 4.98 | 7.04 | 3.60 | 6.30 | >27.3 | >27.3 |
| 95 % conf. limits | 4.21 - 5.77 | 6.03 - 8.12 | 2.93 - 4.42 | 5.34 - 7.46 | n.d. | n.d. |
| $EC_{20}$ (14-day) | 2.28 | 3.24 | 1.29 | 2.07 | 5.76 | 19.4 |
| 95 % conf. limits | 1.62 - 2.86 | 2.17 - 4.08 | 0.880 - 1.68 | 1.53 - 2.60 | 3.09 - 8.94 | 12.7 - >27.0 |
| $EC_{10}$ (14-day) | 1.36 | 1.94 | 0.751 | 1.16 | 1.46 | 4.18 |
| 95 % conf. limits | 0.827 - 1.86 | 1.05 - 2.71 | 0.447 - 1.06 | 0.765 - 1.56 | 0.398 - 2.80 | 1.77 - 6.74 |
| 14-day NOEC | 1.0 | 1.0 | 1.0 | 1.0 | 3.0 | 3.0 |
| 14-day LOEC | 3.0 | 3.0 | 3.0 | 3.0 | 9.1 | 9.1 |

n.d.: could not be determined
Values refer to nominal test concentrations

Toxicological (in)dependence of the two pulses:

The ANOVA results indicate that the explanatory variable pulse showed a significant impact on the inhibition in dry weight yield. A further pairwise comparison (t-test) of the two pulses for each concentration revealed no significantly greater differences for pulse 2 compared to pulse 1.
For the inhibition in dry weight growth rate, fresh weight yield and fresh weight growth rate the ANOVA did not show any significant impact of the explanatory variable pulse. A further pairwise t-test for each concentration shows that the inhibition in dry weight growth rate, fresh weight yield and fresh weight growth rate was not significantly greater after pulse 2 compared to pulse 1.
For the total shoot length, as not all the assumptions were met to run a mixed ANOVA for both inhibition in yield and inhibition in growth rate, only paired one-sided greater t-test were run for each concentration. The results of the paired t-tests indicate that, in each concentration, inhibition in both yield and growth rate was not significantly greater in pulse 2 compared to pulse 1.

Analytical results:

The quantification of the of the test item Thiencarbazone-methyl in the test samples was performed using liquid chromatography with MS/MS detection.
The concentrations of the test item were determined in the test media of the nominal test concentrations of 27.3, 9.1, 3.0, 1.0 and 0.34 µg test item/L and in the control.
At the start of the test (start of the first exposure peak) 95 % (90 % - 99 %) of the nominal test concentrations were found in the overlying test media (average of all test concentrations, pooled replicates of both scenarios). At the end of the first exposure peak 96 % (92 % - 104 %) of the nominal test concentrations were found (average of all test concentrations, pooled replicates of both scenarios). At the beginning of the first non-exposure phase the test item concentration was below the LOD for all test concentrations in both scenarios.
At the start of the second exposure peak (scenario B) 98 % (93 % - 101 %) of the nominal test concentrations were found in the overlying test media (average of all test concentrations). At the end of the second exposure peak (scenario B) 97 % (94 % - 101 %) of the nominal test concentrations were found (average of all test concentrations).
Thus, the test item was dosed correctly and stable during the exposure periods. The transfer of the plants from the test media to the fresh test water was performed without any contamination.

**Conclusion**

The study is valid since all required validity criteria were fulfilled.

The influence of Thiencarbazone-methyl on the growth of the dicotyledonous freshwater plant *Myriophyllum spicatum* was assessed in a pulsed concentration-response test.

Scenario A:
The 14-day $NOE_yC$ and the $LOE_yC$ were determined to be <0.34 and ≤0.34 µg test item/L for total shoot length.
The 14-day $NOE_yC$ and the $LOE_yC$ were determined to be 0.34 and 1.0 µg test item/L for fresh weight and dry weight, respectively.
The 14-day $NOE_rC$ and the $LOE_rC$ were determined to be 0.34 and 1.0 µg test item/L for total shoot length, fresh weight and dry weight, respectively.
The 14-day $E_yC_{50}$ were calculated to be 7.01, 6.92 and >27.3 µg test item/L for total shoot length, fresh weight and dry weight, respectively.
The 14-day $E_rC_{50}$ were calculated to be 15.4, 18.8 and >27.3 µg test item/L for shoot length, fresh weight and dry weight, respectively.

Scenario B:

The 14-day NOEC value and the corresponding LOEC value for yield and growth rate based on total shoot length and fresh weight were calculated to be 1.0 and 3.0 µg test item/L.
The 14-day NOEC value and the corresponding LOEC value for yield and growth rate based on dry weight were calculated to be 3.0 and 9.1 µg test item/L.
The 14-day $E_yC_{50}$ was calculated to be 4.98, 3.60 and >27.3 µg test item/L for total shoot length, fresh weight and dry weight, respectively.
The 14-day $E_rC_{50}$ was calculated to be 7.04, 6.30 and >27.3 µg test item/L for shoot length, fresh weight and dry weight, respectively.

The correct application of the test item and the maintenance of the exposure concentrations during the pulses were demonstrated by measurement of the test media taken at the start and end of both pulses. The measured concentrations of the test item were close to the nominal test concentrations in all samples.

The measurement of the samples taken at the start of the 1st non-exposure phase (from both scenarios) demonstrated that the transfer of the plants from the test media of the 1st pulse to fresh test water was performed without significant contamination. The concentrations of the test item were below the LOD in all samples. From the 2nd non-exposure period no samples were taken.

All reported results refer to nominal values since the concentrations of the test item were within ± 20 % of the nominal concentrations during the exposure peaks.

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| Comments of zRMS: | The study was performed according to OECD TG 201 and principles of GLP. The validity criteria are met:<br>- Cell Density Increase in Control Cultures: 85.4-fold increase within 72 hours (required: ≥ 16-fold),<br>- Coefficient of Variation of Sectional (Daily) Growth Rates in Control Cultures: 15.8 % (required: ≤ 35 %),<br>- Coefficient of Variation of Average Growth between Control Replicates: 1.5 % (required: ≤ 7 %) and thus, the validity criterion was met.<br>There were no deviations to the study plan.<br><br>The study is considered acceptable and suitable for the risk assessment.<br><br>72h $E_yC_{50}$ = 13.3 mg test item/L,<br>72h $E_bC_{50}$ = 14.5 mg test item/L<br>72h $E_rC_{50}$ = 87.1 mg test item/L. |

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| Reference: | KCP 10.2.1 |
| Report | GLOB2112dH: Toxicity to *Pseudokirchneriella subcapitata* in an algal growth inhibition test, Bauer J., 2024a, 177011210 |
| Guideline(s): | Yes, OECD 201 (2011) and SANTE/2020//12830 Rev. 2 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes/No/Supplementary |

**Executive Summary**

The purpose of this test was to determine the inhibitory effect of the test item GLOB2112dH on the growth of the freshwater unicellular green algal species *Pseudokirchneriella subcapitata*. For this purpose, exponentially growing cultures of this unicellular green algal species were exposed to various concentrations of the test item under defined conditions. The inhibition of growth in relation to control cul-

tures was determined over a test period of 72 hours, and thus over several algal generations. The purpose of the analytical part of this study was to verify the concentration of the test item in the test medium.
The 72-hour $E_yC_{50}$ was calculated to be 13.3 mg test item/L, the 72-hour $E_bC_{50}$ was calculated to be 14.5 mg test item/L and the 72-hour $E_rC_{50}$ value was calculated to be 87.1 mg test item/L.

**Materials and methods**

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| Test Item: | GLOB2112dH; Batch No.: MAM 107683; content of a.i.: Mesotrione 375 g/L, Cyprosulfamide (safener) 112 g/L and Thiencarbazone-methyl 75 g/L, according to certificate of analysis. |
| Test Species: | *Pseudokirchneriella subcapitata*, Strain No. 61.81 SAG formerly known as *Selenastrum capricornutum*, and recently renamed as *Raphidocelis subcapitata* (KORSHIKOV).<br>Cultivated in the laboratories of the test facility; original source: "Culture Collection of Algae at Goettingen University (SAG)", 37073 Göttingen, Germany. |
| Test Design: | This study encompassed 6 treatment groups (5 concentrations of the test item and a control) with three replicates per test concentration and six replicates for the control. At test start 50 mL of the test media were inoculated with 5000 algal cells per mL test medium and defined volumes of the algal suspensions were sampled after 24, 48 and 72 hours for determination of cell densities by spectrophotometric measurement. |
| Endpoints: | Yield and growth rate of the algae |
| Test Concentrations: | 100, 31.6, 10, 3.2 and 1.0 mg test item/L (spacing factor 3.16), and a control. |
| Test Conditions: | Water temperature: observed: 21.8 to 22.3°C (target: 21 – 24°C, controlled at ± 2°C);<br>pH value in the control at test start: 8.0,<br>pH value in the control at test end: 8.0 ;<br>pH values in the test item treatments at test start: 7.8 to 7.9,<br>pH values in the test item treatments at test end: 7.6 to 8.0 ~~7.8~~;<br>(target pH: 8.1 ± 0.1 at test start in untreated test water)<br>continuous illumination:<br>observed mean light intensity: 6897 lux (6230 to 7250 lux)<br>(target: 4440 – 8880 lux, ± 15 % of mean value) |
| Statistical analysis: | Based on the calculated cell densities, the 72-hour $E_rC_{50}$, $E_bC_{50}$ and the 72-hour $E_yC_{50}$, the corresponding $EC_{20}$ and $EC_{10}$ values and where possible their 95 %-confidence limits were calculated by probit analysis.<br>For the determination of the 72-hour LOEC and the 72-hour NOEC, the calculated growth rates and yields at each test concentration were tested for significant differences compared to the control values by Williams t-test (yield and growth rate).<br>The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat® Solutions GmbH. |

**Results**

*Biological results*

Validity criteria:

Cell density in the control:
Achieved: 85.4-fold increase (criterion: ≥ 16) within 72 hours;
Coefficient of Variation (CV) of sectional (daily) growth rate of the control:
Achieved: 15.8% (criterion: ≤ 35%);

CV of average growth of control replicates:
Achieved: 1.5% (criterion: ≤ 7%);
and thus, the validity criteria were met.

The biological results are summarized in the table below.

**Biological Results**

| Parameter (0-72 h) | Yield [mg test item/L] | Growth rate [mg test item/L] | Biomass [mg test item/L] |
|---|---|---|---|
| 72-hour $EC_{50}$ | 13.3 | 87.1 | 14.5 |
| 95 % conf. interval | 11.9 - 14.9 | 80.1 - 95.4 | 12.9 – 16.3 |
| 72-hour $EC_{20}$ | 4.37 | 18.0 | 4.35 |
| 95 % conf. interval | 3.60 -5.13 | 16.1 - 19.8 | 3.58 – 5.12 |
| 72-hour $EC_{10}$ | 2.44 | 7.87 | 2.31 |
| 95 % conf. interval | 1.88 - 3.01 | 6.68 -9.08 | 1.78 – 2.87 |
| 72-hour NOEC | 1 | 1 | 1 |
| 72-hour LOEC | 3.2 | 3.2 | 3.2 |

*Analytical results*

The quantification of the active ingredients Mesotrione and Thiencarbazone-methyl and the safener Cyprosulfamide of the test item GLOB2112dH in the test samples was performed using liquid chromatography with MS/MS detection.

Cyprosulfamide:
At the start of the test 99% of the nominal test concentrations were found (average of all test concentrations). After 72 hours test duration, 98% of the nominal value was determined (average of all test concentrations). During the test the algae were exposed to a mean of 99% of the nominal test concentrations.

Mesotrione:
At the start of the test 95% of the nominal test concentrations were found (average of all test concentrations). After 72 hours test duration, 95% of the nominal value was determined (average of all test concentrations). During the test the algae were exposed to a mean of 95% of the nominal test concentrations.

Thiencarbazone-methyl:
At the start of the test 101% of the nominal test concentrations were found (average of all test concentrations). After 72 hours test duration, 101% of the nominal value was determined (average of all test concentrations). During the test the algae were exposed to a mean of 101% of the nominal test concentrations.

**Conclusion**

The study is valid since all required validity criteria were fulfilled.
The influence of GLOB2112dH on the growth of the freshwater green algae *Pseudokirchneriella subcapitata* was assessed in a static concentration-response test.
The 72-hour $E_yC_{50}$ was calculated to be 13.3 mg test item/L, the 72-hour $E_bC_{50}$ was calculated to be 14.5 mg test item/L and the 72-hour $E_rC_{50}$ value was calculated to be 87.1 mg test item/L. The 72-hour $NOE_yC$ was determined to be 1 mg test item/L and the associated 72-hour $LOE_yC$ was 3.2 mg test item/L. The 72-hour $NOE_rC$ was determined to be 1 mg test item/L and the associated 72-hour $LOE_rC$ was 3.2 mg test item/L. The 72-hour $NOE_bC$ was determined to be 1 mg test item/L and the associated 72-hour $LOE_bC$ was 3.2 mg test item/L.

The initial concentrations and the maintenance of the exposure concentrations during the test were determined in the analytical part. All reported results refer to nominal values since the concentrations of the test item were within ± 20% of the nominal concentrations during the test.

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| Comments of zRMS: | The study was performed according to OECD TG 221 and principles of GLP.<br>The validity criteria are met: the doubling time of frond numbers in the control was less than 2.5 days (actually 1.5 days), as required by OECD 221 Guideline.<br><br>There were no deviations to the study plan.<br><br>The study is considered acceptable and suitable for the risk assessment.<br>7d $E_yC_{50\ \text{frond number}}$ = 8.28 mg test item/L,<br>7d $E_yC_{50\ \text{dry weight}}$ = 9.94 mg test item/L,<br>7d $E_rC_{50\ \text{frond number}}$ = 18.3 mg test item/L,<br>7d $E_rC_{50\ \text{dry weight}}$ = 83.6 mg test item/L |

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|---|---|
| Reference: | KCP 10.2.1 |
| Report | GLOB2112dH: Toxicity to the aquatic plant *Lemna gibba* in a static growth inhibition test, Bauer J., 2024b, 177011240 |
| Guideline(s): | Yes, OECD 221 (2006) and SANTE/2020//12830 Rev. 2 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes/No/Supplementary |

**Executive Summary**

The purpose of this study was to determine the inhibitory effect of the test item GLOB2112dH on the growth of the freshwater aquatic plant *Lemna gibba*. For this purpose, cultures of *Lemna gibba* were exposed in a static test to various concentrations under defined conditions. The inhibition of growth in relation to control cultures was determined over a test period of 7 days. The purpose of the analytical part of this study was to verify the concentration of the test item in the test medium.
The 7-day $E_yC_{50}$ value was calculated to be 8.28 and 9.94 µg test item/L for frond number and dry weight, respectively. The 7-day $E_rC_{50}$ value was calculated to be 18.3 and 83.6 µg test item/L for frond number and dry weight. The 7-day $NOE_yC$ and the $LOE_yC$ were determined to be 3.2 and 10 µg test item/L respectively for both frond number and dry weight. The 7-day $NOE_rC$ and the $LOE_rC$ were determined to be 3.2 and 10 µg test item/L respectively for both frond number and dry weight.

**Materials and methods**

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| Test Item: | GLOB2112dH; batch no.: MAM 107683; content of a.i. Mesotrione: 391.9 g/L, content of safener Cyprosulfamide: 117.5 g/L, content of a.i. Thiencarbazone-methyl: 77.47 g/L, according to certificate of analysis. |
| Test Species: | *Lemna gibba* G 3 |
| Test Design: | This study encompassed 6 treatment groups (5 dose rates of the test item and a control) with 3 replicates per test concentration and control.<br>At test start 12 fronds were introduced in each replicate and incubated for 7 days under static conditions. The frond numbers were determined on day 2, 4 and 7. The dry weight of each replicate was deter- |

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| | mined at test termination. |
| Endpoints: | Yield and growth rate based on frond number and dry weight. |
| Test Concentrations: | 100, 32, 10, 3.2 and 1.0 µg test item/L (spacing factor 3.16) and a control |
| Test Conditions: | Water temperature: observed 23.6 to 24.2°C (target: 24°C ± 2°C );<br>pH values of the freshly prepared control medium at test start: 7.5<br>pH values of the aged control medium at test end: 8.9<br>pH values of the treatment media at test start: 7.5<br>pH values of the treatment media at test end: 8.4 to 8.9;<br>(target pH: 7.5 ± 0.1 at test start)<br>continuous illumination:<br>observed mean light intensity: 7328lux (7180 to 7520 lux)<br>(target: 6500-10000 lux) |
| Statistical analysis: | The $E_rC_{50}$ and the $E_yC_{50}$ values, the corresponding $EC_{20}$ and $EC_{10}$ values and where possible their 95%-confidence limits were calculated using Probit analysis.<br>For the determination of the 7-day $LOE_yC$ and $NOE_yC$ values significant differences at the test concentrations compared to the control values were tested using the Williams t-test (frond number and dry weight).<br>The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat® Solutions GmbH. |

**Results**

*Biological results*

Validity criteria:

Doubling Time of fronds in the control:
Achieved: 1.5 days (criteria: < 2.5d). Thus, the validity criterion was met.

The biological results are summarized in the table below.

**Summary of Biological Results**

| Parameter | Yield (frond number) [µg test item/L] | Growth rate (frond number) [µg test item/L] | Yield (dry weight) [µg test item/L] | Growth rate (dry weight) [µg test item/L] |
|---|---|---|---|---|
| $EC_{50}$ (7-day) | 8.28 | 18.3 | 9.94 | 83.6 |
| 95 % conf. limits | 9.36 - 9.30 | 15.4 - 21.8 | 6.52 - 15.2 | 50.5 - > 100 |
| $EC_{20}$ (7-day) | 4.90 | 6.85 | 3.81 | 8.69 |
| 95 % conf. limits | 4.03 - 5.93 | 5.39 - 8.75 | 2.13 - 6.92 | 3.63 - 20.2 |
| $EC_{10}$ (7-day) | 3.73 | 4.09 | 2.30 | 2.66 |
| 95 % conf. limits | 2.85 - 4.85 | 2.96 - 5.62 | 1.05 - 4.96 | < 1.0 - 8.58 |
| 7-day NOEC | 3.2 | 3.2 | 3.2 | 3.2 |
| 7-day LOEC | 10 | 10 | 10 | 10 |

n.d.: not determinable, conf. limits: confidence limits,
Values refer to nominal /geometric mean measured /initial mean measured test concentrations

*Signs of Phytotoxicity and Visual Observations:* The shape of fronds and colonies after the test period of 7 days was not different to those in the control up to and including the nominal test concentration of 3.2 µg test item/L. At the higher test item concentrations the fronds showed deviations from the control replicates after 4 days; *i.e.* shortened roots (10 µg test item/L) and chlorosis (100 µg test item/L).

*Analytical results*

The quantification of the active ingredients Mesotrione and Thiencarbazone-methyl and the safener Cyprosulfamide of the test item GLOB2112dH in the test samples was performed using liquid chromatography with MS/MS detection.

Cyprosulfamide:
At the start of the test 101% of the nominal test concentrations were found (average of all test concentrations). After 7 days test duration, 102% of the nominal value was determined (average of all test concentrations). During the test the *Lemna* were exposed to a mean of 101% of the nominal test concentrations.

Mesotrione:
At the start of the test 101% of the nominal test concentrations were found (average of all test concentrations). After 7 days test duration, 100% of the nominal value was determined (average of all test concentrations). During the test the *Lemna* were exposed to a mean of 100% of the nominal test concentrations.

Thiencarbazone-methyl:
At the start of the test 100% of the nominal test concentrations were found (average of all test concentrations). After 7 days test duration, 99% of the nominal value was determined (average of all test concentrations). During the test the *Lemna* were exposed to a mean of 99% of the nominal test concentrations.

**Conclusion**

The study is valid since all required validity criteria were fulfilled.
The influence of GLOB2112dH on the growth of the freshwater plant *Lemna gibba* was assessed in a static concentration-response test.
The 7-day $E_yC_{50}$ value was calculated to be 8.28 and 9.94 µg test item/L for frond number and dry weight, respectively.
The 7-day $E_rC_{50}$ value was calculated to be 18.3 and 83.6 µg test item/L for frond number and dry weight.
The 7-day $NOE_yC$ and the $LOE_yC$ were determined to be 3.2 and 10 µg test item/L respectively for both frond number and dry weight.
The 7-day $NOE_rC$ and the $LOE_rC$ were determined to be 3.2 and 10 µg test item/L respectively for both frond number and dry weight.
The initial concentrations and the maintenance of the exposure concentrations during the test were determined in the analytical part. All reported results refer to nominal values since the concentrations of the test item were within ± 20% of the nominal concentrations during the test.

| | |
|---|---|
| Comments of zRMS: | The study was performed according to OECD TG 239 and principles of GLP.<br>The validity criteria are met:<br>- Control plants did not show any sign of sublethal effects and were visibly free from contamination by other organisms such as algae and/or bacterial film.<br>- The total shoot length increased by a factor of 5.5 after 14 days of exposure (criteria: factor of ≥ 2).<br>- The fresh weight increased by a factor of 4.8 after 14 days of exposure (criteria: factor of ≥ 2).<br>- The coefficient of variation of yield fresh weight was 21.5 % (criteria: ≤ 35%). as required by OECD 221 Guideline.<br><br>The following deviation from the study plan was noted:<br>According to Study Plan: The obtained pore water and centrifuged sediment from each prepared test vessel will be split into duplicate samples.<br>Deviation to the Study Plan: The obtained pore water and centrifuged sediment from each prepared test vessel was not split into duplicate samples.<br>Reason for the Deviation: Sample handling error. |

| | |
|---|---|
| | Presumed Effect on the Study:<br>None, because the sediment samples were analysed in duplicate nevertheless the centrifuged sediment samples were not split before further storage. Further it has no negative effect on the study that the pore water was only analysed once for each treatment group, since the results are regarded to be sufficient as the pore water being a very small part of the entire test system, is regarded to have no significant effect on the outcome of this study.<br><br>Results refer to geometric mean concentrations based on Thiencarbazone-methyl, since the test item concentrations were not within ± 20% of the nominal concentrations during the test.<br><br>The study is considered acceptable and suitable for the risk assessment.<br><br>$E_yC_{50}$ shoot length = 18.0 µg/L $_{mm}$<br>$E_rC_{50}$ shoot lenght = 27.0 µg/L $_{mm}$<br>$E_yC_{50}$ fresh weight = 7.81 µg/L $_{mm}$<br>$E_rC_{50}$ fresh weight = 14.2 µg/L $_{mm}$<br>$E_yC_{50}$ dry weight = 8.50 µg/L $_{mm}$<br>$E_rC_{50}$ dry weight = 22.0 µg/L $_{mm}$ |

| | |
|---|---|
| Reference: | KCP 10.2.1 |
| Report | GLOB2112dH: Toxicity to the aquatic plant *Myriophyllum spicatum* in a static growth inhibition test with a prior rooting phase, Bauer J., 2024c, 177011215 |
| Guideline(s): | Yes, OECD 239 (2014) and SANTE/2020//12830 Rev. 2 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes/No/Supplementary |

**Executive Summary**

The purpose of this test was to determine the inhibitory effect of the test item GLOB2112dH on the vegetative growth of the freshwater aquatic plant *Myriophyllum spicatum*. Plants of *Myriophyllum spicatum* were exposed in a static test to various concentrations of the test item under defined conditions. The inhibition of growth in relation to control cultures was determined over a test period of 14 days.
The purpose of the analytical part of this study was to verify the concentrations of the test item in the test medium.
The 14-day $NOE_yC$ and the $LOE_yC$ were determined to be 9.21 and 29.7 µg test item/L for total shoot length, 2.40 and 9.21 µg test item/L for fresh weight and 0.589 and 2.40 µg test item/L for dry weight, respectively. The 14-day $NOE_rC$ and the $LOE_rC$ were determined to be 2.40 and 9.21 µg test item/L for total shoot length, 2.40 and 9.21 µg test item/L for fresh weight and 0.589 and 2.40 µg test item/L for dry weight, respectively. The 14-day $E_yC_{50}$ was calculated to be 18.0, 7.81 and 8.50 µg test item/L for total shoot length, fresh weight and dry weight, respectively. The 14-day $E_rC_{50}$ was calculated to be 27.0, 14.2 and 22.0 µg test item/L for shoot length, fresh weight and dry weight, respectively.

**Materials and methods**

| | |
|---|---|
| Test Item: | GLOB2112dH; Batch No.: MAM 107683; Content of Mesotrione: 375 g/L, Thiencarbazone-methyl 75 g/L, Cyprosulfamide 112 g/L (safener) |

| | |
|---|---|
| Test Species: | *Myriophyllum spicatum* |
| Test Design: | This study encompassed 6 treatment groups (5 dose rates of the test item and a control) with five replicates per test concentration and ten replicates for the control.<br>After a pre-rooting phase of 7 days, 1 plant per replicate was incubated for 14 days under static conditions. The shoot length was determined at test start and day 14. Sublethal parameters were assessed at test start, once during the test (e.g. day 6) and at test end. At test end fresh and dry weight of each replicate was determined. The samples collected at start and after 14 days were analysed. |
| Endpoints: | Inhibition of growth expressed in terms of yield and growth rate, based on total shoot length, fresh and dry weight |
| Test Concentrations: | 100, 32, 10, 3.2 and 1.0 µg test item/L (spacing factor 3.2) and a control. Corresponding to geometric mean measured concentrations of 90.7, 29.7, 9.21, 2.40 and 0.589 µg test item/L. |
| Test Conditions: | Water temperature: observed: 20.2 - 22.1°C (target: 20 ± 2 °C);<br><br>light regime: 16 h light : 8 h dark; observed mean light intensity: 9081 lux(8730 - 9360 lux) (target: 8000-10000 lux);<br><br>pH values during pre-rooting phase (Smart & Barko Medium without test item): 8.0 / 7.9,<br>pH values of the control (Smart & Barko Medium without test item):<br>test start: 7.9 - 8.0, on day 6: 9.2 - 9.7, at the end of the test: 9.8 - 10.0,<br>pH values of the test item treatments (Smart & Barko Medium with test item):<br>test start: 7.9, on day 6: 8.5 -9.4, at the end of the test: 7.9 -10.0.<br>(target pH: 7.5 - 8.0 at start of the pre-rooting period and at test start in the control);<br>oxygen concentrations at test start: 8.0 - 8.1 mg/L, on day 6: 10.4 - 14.2 mg/L, at the end of the test: 7.4 - 13.8 mg/L. |
| Statistical analysis: | The $EC_{50/20/10}$ and their 95 %-confidence limits were calculated by 3-param. normal CDF analysis.<br>For the determination of the 14-day LOEC and the 14-day NOEC, the calculated growth rates based on total shoot length, fresh weight and dry weight and yields based on fresh weight and dry weight at each test concentration were tested for significant differences compared to the control values by Williams t-test. The test was chosen as data showed normal distribution and variance homogeneity and the analysis of contrasts revealed a linear trend. The yield based on total shoot length was compared to the control by Welch t-test after Bonferroni-Holm-adjustment as variance homogeneity check failed while normal distribution requirements were fulfilled.<br>The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat® Solutions GmbH. |

**Results**

*Validity criteria*

Control plants did not show any sign of sublethal effects and were visibly free from contamination by other organisms such as algae and/or bacterial film.
The total shoot length increased by a factor of 5.5 after 14 days of exposure (criteria: factor of ≥ 2).
The fresh weight increased by a factor of 4.8 after 14 days of exposure (criteria: factor of ≥ 2).
The coefficient of variation of yield fresh weight was 21.5 % (criteria: ≤ 35%).

Thus, all validity criteria were met.

*Biological results*

Observed effects followed a concentration-response-relationship. Phytotoxic symptoms were observed at 2.40 up to 90.7 µg test item/L and number and extent of the symptoms increased with increasing test concentration. Symptoms observed were shortened shoot tip, absent buoyancy, chlorosis, necrosis, shortened roots and few roots.

**Summary of Biological Results**

| Parameter | Yield (total shoot length) [µg test item/L] | Growth rate (total shoot length) [µg test item/L] | Yield (fresh weight) [µg test item/L] | Growth rate (fresh weight) [µg test item/L] | Yield (dry weight) [µg test item/L] | Growth rate (dry weight) [µg test item/L] |
|---|---|---|---|---|---|---|
| $EC_{50}$ (14-day) | 18.0 | 27.0 | 7.81 | 14.2 | 8.50 | 22.0 |
| 95 % conf. limits | 14.2 - 22.8 | 24.1 - 30.3 | 5.52 - 11.0 | 10.8 - 18.7 | 4.57 - 15.8 | 11.5 - 42.2 |
| $EC_{20}$ (14-day) | 9.50 | 12.6 | 2.83 | 4.23 | 1.04 | 1.58 |
| 95 % conf. limits | 6.84 - 13.3 | 10.7 - 15.0 | 1.72 - 4.71 | 2.83 - 6.39 | < 0.589 - 2.65 | < 0.589 - 4.66 |
| $EC_{10}$ (14-day) | 6.80 | 8.49 | 1.67 | 2.25 | < 0.589 | < 0.589 |
| 95 % conf. limits | 4.39 - 10.4 | 6.75 - 10.6 | 0.868 - 3.20 | 1.30 - 3.84 | < 0.589 - 1.17 | < 0.589 - 1.77 |
| 14-day NOEC | 9.21 | 2.40 | 2.40 | 2.40 | 0.589 | 0.589 |
| 14-day LOEC | 29.7 | 9.21 | 9.21 | 9.21 | 2.40 | 2.40 |

Values refer to geometric mean measured

*Analytical results*

The quantification of the active ingredients Mesotrione and Thiencarbazone-methyl and of the safener Cyprosulfamide of the test item GLOB2112dH in the test samples was performed using liquid chromatography with MS/MS detection.
The concentrations of the active ingredients Mesotrione and Thiencarbazone-methyl and of the safener Cyprosulfamide of the test item GLOB2112dH were determined in the test media (overlying water) of all nominal test concentrations and in the control. The concentrations of Thiencarbazone-methyl were additionally determined in the sediment and pore water of all test concentrations and in the control.
In the controls of overlying water, sediment and pore water all measured concentrations were below the limit of detection for all three analytes.

Cyprosulfamide in overlying water:
At the start of the test 99% of the nominal test concentrations were found (average of all test concentrations). After 14 days test duration, 97 % of the nominal value was determined (average of all test concentrations).

Mesotrione in overlying water:
At the start of the test 104% of the nominal test concentrations were found (average of all test concentrations). After 14 days test duration, 86 % of the nominal value was determined (average of all test concentrations).

Thiencarbazone-methyl in overlying water, sediment and pore water:
At the start of the test 100 % of the nominal test concentrations were found in overlying water (average of all test concentrations). After 14 days test duration, 34 to 80 % of the nominal value was determined in overlying water.

The additional analysis of the concentrations of Thiencarbazone-methyl in sediment and pore water show only small amounts of Thiencarbazone-methyl. 34 to 88% of the nominal was found in the mass balance for total recovery in overlying water, sediment and pore water.

**Conclusion**

The study is valid since all required validity criteria were fulfilled.
The influence of GLOB2112dH on the growth of the dicotyledonous freshwater plant *Myriophyllum spicatum* was assessed in a static concentration-response test.
The 14-day $NOE_yC$ and the $LOE_yC$ were determined to be 9.21 and 29.7 µg test item/L for total shoot length, 2.40 and 9.21 µg test item/L for fresh weight and 0.589 and 2.40 µg test item/L for dry weight, respectively.
The 14-day $NOE_rC$ and the $LOE_rC$ were determined to be 2.40 and 9.21 µg test item/L for total shoot length, 2.40 and 9.21 µg test item/L for fresh weight and 0.589 and 2.40 µg test item/L for dry weight, respectively.
The 14-day $E_yC_{50}$ was calculated to be 18.0, 7.81 and 8.50 µg test item/L for total shoot length, fresh weight and dry weight, respectively.
The 14-day $E_rC_{50}$ was calculated to be 27.0, 14.2 and 22.0 µg test item/L for shoot length, fresh weight and dry weight, respectively.
The correct application of the test item and the maintenance of the exposure concentrations during the test were determined in the analytical part. All reported results refer to geometric mean concentrations based on Thiencarbazone-methyl, since the Thiencarbazone-methyl concentrations were not within ± 20% of the nominal concentrations during the test.

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

No new studies were submitted.

## A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

No new studies were 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

| | |
|---|---|
| Comments of zRMS: | The study is acceptable.<br>The validity criteria were met.<br>The study was conducted according to OECD guidance 213 and 214<br><br>The following endpoint was accepted:<br>Oral LD50 > 547.5 µg product/bee<br>Contact LD50 > 500 µg product/bee |

| | |
|---|---|
| Reference: | KCP 10.3.1.1 |
| Report | GLOB2112dH: effects (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory, Schabio S., 2024, 177011035 |
| Guideline(s): | Yes, OECD 213 and 214, SANTE/2020/12830 Rev.2 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes/No/Supplementary |

**Executive summary**

The purpose of this study was to determine the acute contact and oral toxicity of GLOB2112dH to the honey bee (*A. mellifera* L.). Mortality of the bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed. The purpose of the analytical part of this study was to verify proper dosing of the test item.

The contact $LD_{50}$values (24 and 48h) of GLOB2112dH were estimated to be both > 500.0 µg product/bee. The oral $LD_{50}$values (24 and 48h) of GLOB2112dH were estimated to be both > 547.5 µg product/bee. The contact NOED values (24 and 48 h) were determined to be ≥ 500 µg product/bee. The oral NOED values (24 and 48 h) were determined to be both ≥ 547.5 µg product/bee.

**Materials and methods**

| | |
|---|---|
| Test Item: | GLOB2112dH, Batch No.: MAM 107683, content:<br>Thiencarbazone-methyl: 75 g/L (nominal); 77.47 g/L (pre storage);<br>Mesotrione: 375 g/L (nominal; 391.9 g/L (pre storage);<br>Cyprosulfamide (safener): 112 g/L (nominal); 117.5 g/L (pre storage),<br>according to GLP certificate of analysis. |
| Test Species: | Honey bee (*Apis mellifera* L.); female worker bees; obtained from a healthy and queen-right colony, bred by the test facility, collected in the morning of use. |
| Test Design: | Acute contact and oral dose response test; duration 48 hours; 3 replicates for the contact and oral test, each consisting of 10 bees per cage per treatment; assessment of mortality after 4, 24 and 48 hours; reference item: dimethoate 413 g/L (analysed). |
| Test Dose Levels: | Contact test: 500.0, 250.0, 125.0, 62.5 and 31.3 µg product/bee*<br>Oral test (target): 500.0, 250.0, 125.0, 62.5 and 31.3 µg product/bee*<br>Oral test (actual consumed): 547.5, 281.3, 138.1, 69.8 and 33.9 µg product/bee*<br>* dose levels of the test item were based on the product without taking into account the content of active ingredient. |
| Test Conditions: | Temperature: 25 - 27°C; relative humidity: 60 - 66%; photoperiod: 24 h darkness. |
| Statistical analysis: | Results obtained from the bees treated with the test item were compared to those obtained from the water control group.<br>As no test item treatment group showed mortality above 50.0 %, no statistical evaluation on the contact and oral $LD_{50}$ values have been carried out.<br>The contact $LD_{50}$ values of the reference item were determined according to Probit Analysis (according to Finney 1971).<br>The oral $LD_{50}$ values of the reference item were estimated according to moving average computations (Thompson and Weil, 1952).<br>The contact and oral NOED values of the test item were determined using Fisher's Exact Binomial Test with Bonferroni Correction (one-sided greater, $\alpha$ = 0.05), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis.<br>The software used to perform the statistical analysis was ToxRat Professional, |

Version 3.3.0, ® ToxRat Solutions GmbH.

**Results**

Validity criteria:

| | | |
|---|---|---|
| Control Mortality: | Contact Test<br>untreated control: | 0.0 % |
| | Oral Test<br>untreated control | 0.0 % |
| $LD_{50}$ of Reference Item (24 hrs): | Contact Test: | 0.16 µg a.s./bee |
| | Oral Test: | 0.14 µg a.s./bee |

The contact and oral tests are considered valid as the control mortality in each case was ≤ 10 % and the $LD_{50}$ values obtained with the reference item (dimethoate) were within the required ranges.

Biological results:

Contact Test:

The doses of 500, 250, 125, 62.5 and 31.3 µg product/bee led to no mortality at test termination (48 hours). No mortality occurred in the water control group (tap water containing 0.1 % Triton X-100).

During the first 4 hours each bee in the 250.0, 125.0 and 31.3 µg product/bee dose group was affected (= moving coordination problem).

Oral Test:

The target dose levels of the test item (500.0, 250.0, 125.0, 62.5 and 31.3 µg product/bee) were all achieved. Actual oral consumed doses of 547.5, 281.3, 138.1, 69.8 and 33.9 µg product/bee led to no mortality at test termination (48 hours). No mortality occurred in the water control (50 % w/v sucrose solution = 500 g sucrose/L tap water).

No test item induced behavioural abnormalities were observed in all dose groups during the whole experiment.

**Toxicity of GLOB2112dH to honey bees; laboratory test**

| Test Item | GLOB2112dH | |
|---|---|---|
| Test Species | *Apis mellifera* L. | |
| Exposure | contact<br>(solution in Triton X-100 (0.1 %)/water) | oral<br>(50 % w/v sucrose solution) |
| Application rate [µg product/bee] | 500, 250, 125, 62.5 and 31.3 | Target: 500, 250, 125, 62.5 and 31.3<br>Consumed: 547.5, 281.3, 138.1, 69.8 and 33.9 |
| $LD_{50}$ [µg product/bee] | 24 hours: > 500<br>48 hours: > 500 | 24 hours: > 547.5<br>48 hours: > 547.5 |
| NOED [µg product/bee] | 24 hours: ≥ 500<br>48 hours: ≥ 500 | 24 hours: ≥ 547.5<br>48 hours: ≥ 547.5 |

Contact and $LD_{50}$: estimated, since the mortality did not exceed 50 % in the test.
Contact and oral NOED: were determined using Fisher's Exact Binomial Test with Bonferroni Correction (one-sided greater, α = 0.05).

The contact and oral $LD_{50}$ (24 h) values for the reference item (dimethoate) were calculated to be 0.16 and 0.14 µg a.i./bee, respectively.

Analytical results:

The analytical recovery rates of the active ingredients Thiencarbazone-methyl and Mesotrione and the safener Cyprosulfamide in the test solutions were as follows:

| | Recovery rate [%] | | |
|---|---|---|---|
| Concentration of the Solution | Thiencarbazone-methyl | Mesotrione | Cyprosulfamide |
| **Contact Test** | | | |
| 500 µg product/bee | 110 % | 104 % | 105 % |
| 31.3 µg product/bee | 92 % | 94 % | 93 % |
| **Oral Test** | | | |
| 500 µg product/bee | 82 % | 80 % | 80 % |
| 31.3 µg product/bee | 92 % | 105 % | 88 % |

**Conclusion**

The acute toxicity of GLOB2112dH on adult honey bees (*Apis mellifera* L.) was investigated in an acute contact and an acute oral, dose-response study under laboratory conditions.
The contact $LD_{50}$values (24 and 48h) of GLOB2112dH were estimated to be both > 500.0 µg product/bee.
The oral $LD_{50}$values (24 and 48h) of GLOB2112dH were estimated to be both > 547.5 µg product/bee.
The contact NOED values (24 and 48 h) were determined to be ≥ 500 µg product/bee.
The oral NOED values (24 and 48 h) were determined to be both ≥ 547.5 µg product/bee.

| | |
|---|---|
| Comments of zRMS: | The study is acceptable.<br>The validity criteria were met.<br>The study was conducted according to OECD guidance 246 and 247<br><br>The following endpoint was accepted:<br>Oral LD50 > 564.8 µg product/bee<br>Contact LD50 > 500 µg product/bee |

| | |
|---|---|
| Reference: | KCP 10.3.1.1 |
| Report | GLOB2112dH: acute contact and oral toxicity to bumblebees (*Bombus terrestris* L.) in the laboratory, Chwiesko D., 2024, 177011105 |
| Guideline(s): | Yes, OECD 246 and 247, SANTE/2020/12830 Rev.2 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes/No/Supplementary |

**Executive summary**

The purpose of this study was to determine the acute contact and oral toxicity of GLOB2112dH to the bumblebee (*Bombus terrestris* L.). Mortality of the bumblebees was used as the endpoint related to toxicity. Sub-lethal effects, such as changes in behaviour, were also assessed. The purpose of the analytical part of this study was to verify proper dosing of the test item.
The contact $LD_{50}$ (48 h) value was estimated to be > 500 µg product/bumblebee. The contact NOED (48 h) value was calculated to be ≥ 500 µg product/bumblebee.

The oral $LD_{50}$ (48 h) value was estimated to be > 564.8 µg product/bumblebee. The oral NOED (48 h) value was calculated to be ≥ 564.8 µg product/bumblebee.
The analytical verification confirmed the correct preparation of all the dosing solutions of the test item.

**Materials and methods**

| | |
|---|---|
| Test Item: | GLOB2112dH, Batch No.: MAM 107683, content:<br>Thiencarbazone-methyl: 75 g/L (nominal content),<br>Mesotrione: 375 g/L (nominal content),<br>Cyprosulfamide (safener): 112 g/L (nominal content),<br>according to certificate of analysis.<br>Since the product contains two a.i.'s and a safener, the dose levels of the test item were based on the product without taking into consideration active substance content . |
| Test Species: | Bumblebee (*Bombus terrestris* L.); female worker bumblebees; obtained from a commercial bumblebee breeding company (Koppert Deutschland GmbH, Zeppelinstr. 32, D-47638 Straelen, Germany). |
| Test Design: | Acute Contact Dose Response Test :<br>Duration: 48 h;<br>replicates: 30 per each dose of the test item treatment group (five doses), 30 for the water control treatment group, 30 for the reference item treatment group ,<br>each consisting of 1 bumblebee per cage per treatment;<br>assessment of mortality and behavioural abnormalities:<br>after 4 (± 0.5); 24 (± 2) and 48 (± 2) hours;<br>reference item: dimethoate 413 g/L (analytical).<br>Analytical verification of the concentration of the active ingredients in the contact application solutions of the highest and the lowest concentrations.<br>Acute Oral Dose Response Test:<br>Duration: 48 h;<br>replicates: 35 per each dose of the test item treatment group (five doses), 35 for the water control treatment group, 35 for the reference item treatment group,<br>each consisting of 1 bumblebee per cage per treatment (individual bumblebees which did not take up at least 80 % of the mean food uptake per treatment group were excluded from the evaluation; see section 6.9 Result Evaluation);<br>assessment of mortality and behavioural abnormalities:<br>after 4 (± 0.5); 24 (± 2) and 48 (± 2) hours;<br>reference item: dimethoate 413 g/L (analytical).<br>Analytical verification of the concentration of the active ingredients in the oral feeding solutions of the highest and the lowest concentrations. |
| Test Item Dose Levels: | Contact Dose Response Test (nominal):<br>500, 294.1, 173.0, 101.8 and 59.9 µg product/bumblebee<br>Oral Dose Response Test (target):<br>500, 294.1, 173.0, 101.8 and 59.9 µg product/bumblebee<br>Oral Dose Response Test achieved (mean consumption):<br>564.8. 334.5, 194.8, 117.8 and 69.1 µg product/bumblebee |

Test Conditions:

Recommended temperature: 25 ± 2 °C

Recommended relative humidity: 60 ± 20 %

| | | |
|---|---|---|
| Contact Test: | Acclimatisation: | Temperature: 25 - 26°C<br>Relative Humidity: 60 – 64% |
| | Exposure: | Temperature: 25 – 26°C<br>Relative Humidity: 57 – 64% |

| | | |
|---|---|---|
| Oral Test: | Acclimatisation: | Temperature: 25 - 26°C<br>Relative Humidity: 57 – 64% |
| | Exposure: | Temperature: 25 – 26°C<br>Relative Humidity: 59 – 64% |
| Photoperiod: | Photoperiod: | 24 h darkness (except handling procedures, including treatment and observations). |
| Statistical analysis: | Results obtained from the bumblebees treated with the test item and reference item were compared to those obtained from the water control treatment groups.<br>For the evaluation of the results of the oral test, bumblebees which did not consume at least 80 % of the mean food uptake per treatment group were excluded from the evaluation of mortality and behavioural abnormalities, as well as from the calculation of the final actual doses in the test item treatment groups.<br>As the test item treatment groups in the contact and oral tests did not show any mortality above 50 %, the $LD_{50}$ values could not be calculated by statistical evaluation. The contact and oral $LD_{50}$ values were considered to be higher than the highest dose rates tested.<br>The contact and oral NOED values were determined using Fisher's Exact Binomial Test with Bonferroni Correction (one-sided greater, $\alpha = 0.05$)<br>The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH. | |

**Results**

Validity criteria:

| | | |
|---|---|---|
| Water Control Mortality at test end: | Contact Test: | 6.7 % |
| | Oral Test: | 0.0 %<br>considering bumblebees with food uptake of at least 80 % of the mean food consumption. |
| Reference Item Mortality at test end: | Contact Test: | 96.7 % |
| | Oral Test: | 100.0 %, considering bumblebees with food uptake of at least 80 % of the mean food consumption. |

This study met the OECD 246 (2017) and OECD 247 (2017) validity criteria as the control mortality in both the oral and contact tests was ≤ 10 % and the mortality due to the reference item (dimethoate) was > 50% at test end.

Biological Results:

Contact Test:

In the contact test a droplet of 5 µL* containing the targeted dose levels of 500, 294.1, 173.0, 101.8 and 59.9 µg product/bumblebee was applied on the dorsal thorax of each exposed bumblebee. At the end of the contact toxicity test (48 hours after application) 0.0, 0.0, 0.0, 6.7 and 3.3 % mortality respectively occurred in the test item treatment group. Mortality of 6.7 % occurred in the water control group (tap water containing 0.1 % v/v Triton X-100).

One affected bumblebee was observed in the 500 and 173.0 µg product/bumblebee test item treatment group 24 hours after dosing. No test item induced behavioural effects were observed at any time in the other test item treated groups.

The contact target dose level of the reference item of 10 µg dimethoate/bumblebee was applied on the dorsal thorax of each exposed bumblebee. The mortality in the reference item treatment group was 96.7 % (48 hours after application).

*A 5 µL droplet was chosen in deviation to the guideline recommendation of 2 µL, since a higher volume ensures a more reliable dispersion of the test item; test facility experience has proven that higher volumes are suitable and no adverse effects on the outcome of the study are to be expected; [according to internal test facility experiments 2016 and 2022].

Oral Test:

In the oral test the targeted dose levels of 500, 294.1, 173.0, 101.8 and 59.9 µg product/bumblebee would have been achieved if an exact amount of 40 mg treated feeding solution had been consumed by each exposed bumblebee. This was not the case in all treatment groups and the actual food uptake per bumblebee in the different treatment groups varied between 11 and 48 mg. Therefore, bumblebees which did not consume at least 80 % of the mean food uptake were excluded from the derivation of the endpoints, as well as from the calculation of the actual mean oral doses in the test and reference item treatment groups. This was done to avoid potentially overestimating the final endpoints.

The actual mean consumed oral doses of the test item were 564.8. 334.5, 194.8, 117.8 and 69.1 µg product/bumblebee. There was 0.0, 3.1, 8.8, 6.7 and 5.9 % mortality in the 564.8. 334.5, 194.8, 117.8 and 69.1 µg product/bumblebee test item treatment groups respectively at test end (48 hours after application). For the 564.8. 334.5, 194.8, 117.8 and 69.1 µg product/bumblebee test item treatment groups, 33, 32, 34, 30 and 34 bumblebees respectively were considered for the evaluation (≥ 80 % of the mean food uptake). During the 4, 24 and 48 hours assessment one affected bumblebee was observed in the 194.8 µg product/bumblebee test item treatment group. No test item induced behavioural effects were observed at any time in the other test item treated groups.

For the water control group, 33 bumblebees were considered for the evaluation. No mortality occurred in the water control group (50 % w/v aqueous sucrose solution).

Similarly, the reference item targeted dose level of 4.0 µg dimethoate/bumblebee would have been achieved if exactly 40 mg treated feeding solution had been consumed by each bumblebee. Considering bumblebees consuming a food uptake of at least 80 % of the mean food uptake, the mean consumption corresponded to an actual mean oral dose of 4.3 µg dimethoate/bumblebee. For the reference item treatment group, 30 bumblebees were considered for the evaluation. Under this condition, the mortality in the reference item treatment group was 100.0 % 24 hours after application.

**Toxicity to Bumblebees; Laboratory Tests**

| Test Item | GLOB2112dH | | | |
|---|---|---|---|---|
| Test Species | *Bombus terrestris* L. | | | |
| Exposure | Contact (tap water containing 0.1 % v/v Triton X-100) | | Oral [1] (50 % w/v sucrose solution) | |
| Target dose rates [µg product/bumblebee] [µg a.i./bumblebee] | 500, 294.1, 173.0, 101.8 and 59.9 | | 500, 294.1, 173.0, 101.8 and 59.9 | |
| Actual achieved dose rate [µg product/bumblebee] | n.a. | | 564.8. 334.5, 194.8, 117.8 and 69.1 | |
| Test Duration: | 24 h | 48 h | 24 h | 48 h |
| $LD_{50}$ [µg product/bumblebee] [2,3] | > 500 | > 500 | > 564.8 | > 564.8 |

| | | | | |
|---|---|---|---|---|
| NOED [µg product/bumblebee] [2, 4] | ≥ 500 | ≥ 500 | ≥ 564.8 | ≥ 564.8 |

[1] For the 564.8. 334.5, 194.8, 117.8 and 69.1 µg product/bumblebee, 33, 32, 34, 30 and 34 bumblebees were considered for the evaluation, respectively.
[2] Results obtained from test item treated groups were compared to those obtained from the water control group. 33 bumblebees of the water control group were considered for the evaluation of the oral test.
[3] As the test item treatment groups in the contact and oral test did not show any mortality above 50 %, the $LD_{50}$ values could not be calculated by statistical evaluation. The contact and oral $LD_{50}$ values were considered to be higher than the highest dose rates tested.
[4] The contact and oral NOED values were determined using Fisher's Exact Binomial Test with Bonferroni Correction (one-sided greater, $\alpha = 0.05$)
n.a.= not applicable

Analytical results:

The analytical recovery rates of the active substances Thiencarbazone-methyl, Mesotrione and the safener Cyprosulfamide in the testing solutions were as follows:

| Concentration/bumblebee | Nominal concentration in the solution | Recovery of the nominal value in the solution |
|---|---|---|
| Contact Test: Application solution (500 µg product/bumblebee*, **) | 100 g product/L application solution | Thiencarbazone-methyl: 116 % Mesotrione: 101 % Cyprosulfamide (safener): 111 % |
| Contact Test: Application solution (59.9 µg product/bumblebee*) | 12.0 g product/L application solution | Thiencarbazone-methyl: 94 % Mesotrione: 90 % Cyprosulfamide (safener): 91 % |
| Oral Test: Feeding solution (500 µg product/bumblebee*, **) | 12.5 g product/kg feeding solution | Thiencarbazone-methyl: 87 % Mesotrione: 91 % Cyprosulfamide (safener): 82 % |
| Oral Test: Feeding solution (59.9 µg product/bumblebee*) | 1.50 g product/kg feeding solution | Thiencarbazone-methyl: 91 % Mesotrione: 97 % Cyprosulfamide (safener): 86 % |

* Since the product contains two a.i.'s and a safener, the dose levels of the test item were based on the product without taking into consideration active substance content.
** ≙ stock solution

**Conclusion**

The toxicity of GLOB2112dH to bumblebees was tested in an acute contact and oral toxicity test.
As there was no mortality above 50 % in the test item treatment group in the contact test, the contact $LD_{50}$ (48 h) value was estimated to be > 500 µg product/bumblebee. The contact NOED (48 h) value was calculated to be ≥ 500 µg product/bumblebee.
As there was no mortality above 50 % in the test item treatment groups in the oral test, the oral $LD_{50}$ (48 h) value was estimated to be > 564.8 µg product/bumblebee. The oral NOED (48 h) value was calculated to be ≥ 564.8 µg product/bumblebee.
The analytical verification confirmed the correct preparation of all the dosing solutions of the test item.

## A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

Please refer to A 2.3.1.1.1 above.

## A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

| | |
|---|---|
| Comments of zRMS: | The study was conducted in accordance with OECD 245.<br><br>All validity criteria for the study were met. After 10 days of continuous exposure, |

| | |
|---|---|
| | mortality in the control was 0 % and thus below the threshold of 15 %. Mortality in the reference treatment group was 100 % and thus above the threshold of 50 %.<br><br>Study is acceptable.<br>Following endpoint are accepted:<br><br>LDD50 > 98.92 µg/bee/day<br>NOEC ≥ 4000 mg/kg, the<br>NOEDD ≥ 98.92 µg/bee/day |

| | |
|---|---|
| Reference: | KCP 10.3.1.2 |
| Report | Chronic oral effects of GLOB2112dH to adult woker honeybees (*Apis mellifera* L.) in a 10-day feeding laboratory test, Venturi S., 2023, BT215/23 |
| Guideline(s): | Yes, OECD TG No. 245 (2017) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes/No/Supplementary |

**Executive summary**

The objective of this study was to assess the chronic oral toxicity effects of the test item GLOB2112dH on adult worker honeybees *Apis mellifera* L. under laboratory conditions over a period of 10 days.
The study was carried out as dose-response test with 5 test item concentrations in order to establish the lethal concentrations/dietary doses ($LC_{10,20,50}$/$LDD_{10,20,50}$) and the no observed effect concentration/dietary dose (NOEC/NOEDD) after 10 days of exposure.
The purpose of the analytical part of this study was to verify the content of active substances and the safener in the feeding solutions (diets) at the highest and lowest test item concentrations, as well as in the xanthan control diet.Given that no significant mortality was observed at any of the tested doses, the NOEDD value was estimated as higher than or equal to 98.92 µg/bee/day. The results did not allow to calculate LDDx and LCx values, but they can be estimated to be > 98.92 µg/bee/day.

**Materials and methods**

**Test item**

| | |
|---|---|
| *Name:* | GLOB2112dH |
| *Indication:* | Herbicide |
| *Batch:* | MAM 107683 |
| *Active substances:* | Thiencarbazone-methyl [317815-83-1]; Mesotrione [104206-82-8] |
| *Safener:* | Cyprosulfamide [221667-31-8] |
| *Content:* | Thiencarbazone-methyl 77.47 g/L, Mesotrione 391.9 g/L, Cyprosulfamide 117.5 g/L |
| *Density:* | 1.2153 g/mL |

**Test system**

| | |
|---|---|
| *Species:* | *Apis mellifera* L. |
| *Age:* | Adult worker bees (maximum 2 days old) |
| *Source:* | Healthy colonies (hive no. 2) maintained at BioTecnologie BT S.r.l. |
| *Acclimation:* | 24 hours |
| *Diet:* | 50% (w/v) aqueous sucrose solution |

**Experimental conditions**

| | |
|---|---|
| *Temperature:* | min 31.1°C – max 36.4°C (average measured: 34.0°C) |

*Humidity:* min 39.0% – max 81.1% (average measured: 58.9%)
*Photoperiod:* 24 hours darkness (except during observations)

**Experimental design**
The 10-day chronic oral feeding test in the laboratory was performed as a dose-response test: the test item was diluted in 50% (w/v) aqueous sucrose solution (prepared at least every 4 days), to obtain the feeding solutions indicated in Table 1. The feeding solutions were prepared each day of the test and administered to bees. A negative control with untreated sucrose solution, another control containing xanthan gum at the concentration of 0.1% w/v, and the reference item Dimethoate were tested in parallel.

**Trial layout of the 10-day Chronic Oral Toxicity Test with GLOB2112dH**

| Groups | Expected Doses [µg prod./bee/day] | Concentrations[1] [mg prod. /kg diet] | Number of | | ID Code | |
|---|---|---|---|---|---|---|
| | | | Bees/cage | Cages | From | To |
| **Negative Control** | - | - | 10 | 3 | CTRLa | CTRLc |
| **Xanthan Control** | - | - | 10 | 3 | CXa | CXc |
| **Test item (T1)** | 2.05 | 102.4 | 10 | 3 | T1a | T1c |
| **Test item (T2)** | 5.12 | 256 | 10 | 3 | T2a | T2c |
| **Test item (T3)** | 12.8 | 640 | 10 | 3 | T3a | T3c |
| **Test item (T4)** | 32 | 1600 | 10 | 3 | T4a | T4c |
| **Test item (T5)** | 80 | 4000 | 10 | 3 | T5a | T5c |
| **Reference item** | 0.02 | 1.00 | 10 | 3 | Ra | Rc |
| **Evaporation** | - | - | 0 | 3 | EVAa | EVAc |

[1]Calculated from the reported expected doses, considering a daily mean uptake of food of 20 mg/bee.

**Assessments**
Mortality and sub-lethal effects were recorded every 24 ± 2 h, from Day 1 to Day 10 of the test. The amount of consumed feeding solutions was determined by weighing each of the feeders before and after administration. The daily mean uptake of food per bee was calculated by dividing the daily amount of food consumed per replicate by the number of bees alive at the beginning of each feeding interval.

**Statistics**
The Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm (one-sided greater, $\alpha = 0.05$) was used to determine the NOEDD/NOEC values. The software ToxRatPro 3.3.0 was used for the statistical analysis.

**Results**

All validity criteria were met:
- The average mortality across replicates of the untreated control is ≤ 15% at the end of the test (after 10 days of exposure). Was 0.0% for both the negative control and the xanthan control.
- The average mortality in the reference item group is ≥ 50% at the end of the test (after 10 days of exposure). Was 100%.

The results of the test are displayed in the table below.

**Summarized mean food uptake and cumulative mortality at the end of the test**

| Group | Concentration [mg prod. /kg diet] | Mean daily uptake of[1] | | Mean Mortality | | |
|---|---|---|---|---|---|---|
| | | Feeding solution [mg/bee/day] | Test item [µg/bee/day] | %M | %CM | S[2] |
| **CTRL** | 0 | 26.01 | n/a | 0.0 | n/a | n/a |
| **CX** | 0 | 26.33 | n/a | 0.0 | n/a | n/a |
| **T1** | 102.4 | 23.64 | 2.420 | 6.7 | 6.7 | - |
| **T2** | 256 | 24.11 | 6.171 | 3.3 | 3.3 | - |
| **T3** | 640 | 28.65 | 18.333 | 3.3 | 3.3 | - |
| **T4** | 1600 | 22.49 | 35.982 | 3.3 | 3.3 | - |
| **T5** | 4000 | 24.73 | 98.918 | 10.0 | 10.0 | - |
| **R** | 1.00 | 17.38 | 0.017 | 100 | 100 | n/a |

%M = Mean Mortality; %CM = Corrected Mean Mortality; S = statistical significance; "+" = significant; "-" = not-significant; n/a = not available. [1]Adjusted for evaporation from the feeders. [2]Fisher's Exact Binomial Test with Bonferroni Correction (α = 0.05, one-sided greater).

The content of active substances and safener, analysed in the feeding solutions of the highest and lowest test item concentrations, sampled on D0, was determined to be within 20% of the nominal value. Therefore, the endpoints were calculated using the nominal concentrations and doses. The negative xanthan gum control, sampled on D0, was also analysed resulting in no test item contamination.

**Conclusion**

The effects of GLOB2112dH on adult worker honeybees (*Apis mellifera* L.) were assessed in this 10-day oral chronic test. The average mortality across replicates of any control group was 0.0% (≤ 15%) at the end of the test (10 days after exposure). The reference item Dimethoate tested at 1.00 mg/kg diet (corresponding to 0.017 µg/bee/day) caused 100% mortality by Day 6. The test fulfilled the validity criteria of OECD 245 (2017). Given that no significant mortality was observed at any of the tested doses, the NOEDD value was estimated as higher than or equal to 98.92 µg/bee/day. The results did not allow to calculate LDDx and LCx values, but they can be estimated to be > 98.92 µg/bee/day.

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

| | |
|---|---|
| Comments of zRMS: | The study was conducted in accordance with OECD 239.<br><br>The validity criteria with regards to control larval mortality on D8, control adult emergence on D22 and toxicity of the reference item were met.<br>Study is acceptable.<br><br>There were deviations from guideline, temperature as well as humidity deviated from the range of values specified in the guidelines. The mortality assessment on D7 was not carried out because on D7 there are no treatments or feeding of the larvae and performing a mortality assessment would have involved opening the desiccator with a consequent decrease of temperature and humidity.<br>According to study director deviations did not affect the course of the study and the reliability of the study.<br><br>NOED = 33.33 µg test item/larva<br>NOEC = 216.45 mg test item/kg diet |

| | |
|---|---|
| | Study is acceptable. |

| | |
|---|---|
| Reference: | KCP 10.3.1.3 |
| Report | Effects of GLOB2112dH on honeybees (*Apis mellifera* L.) 22-day larval toxicity test with repeated exposure, Venturi S., 2024, BT131/23 |
| Guideline(s): | Yes, OECD 239, 2021 |
| Deviations: | Yes, The mortality assessment on D7 was not carried out because on D7 there are no treatments or feeding of the larvae and performing a mortality assessment would have involved opening the desiccator with a consequent decrease of temperature and humidity. Therefore, to avoid stress to the larvae (in a very delicate stage such as the transition to pre-pupa) the assessment was performed directly on D8. |
| GLP: | Yes |
| Acceptability: | Yes/No/Supplementary |

**Executive summary**

The objective of this study was to assess the effects on adult emergence of honeybee larvae *Apis mellifera ligustica* L., following a repeated exposure of larvae to the test item GLOB2112dH.
The purpose of the analytical part of this study was to verify the test item content in the water solutions (used to treat the diets administered to the larvae) at the lowest and highest concentrations, as well as in the untreated water which was added to the control diet.
Regarding the effects on larvae on D8 (developmental period) and the effects on adult emergence on D22, the test item GLOB2112dH caused statistically significant mortality from the dose of 100.0 µg test item/larva and above. Therefore, the NOED for larvae on D8 and on D22 was determined to be 33.33 µg test item/larva. The NOEC for larvae on D8 and on D22 was determined to be 216.45 mg test item/kg diet.

**Materials and methods**

**Test item:**

| | |
|---|---|
| **Name:** | GLOB2112dH |
| **Indication:** | Herbicide |
| **Batch:** | MAM 107683 |
| **Active substance:** | Thiencarbazone-methyl [317815-83-1] |
| **Safener:** | Cyprosulfamide [221667-31-8] |
| **Content:** | Thiencarbazone-methyl 77.47 g/L, Mesotrione 391.9 g/L Cyprosulfamide 117.5 g/L |
| **Density:** | 1.2153 g/mL |

**Test system:**

| | |
|---|---|
| **Species:** | *Apis mellifera ligustica* |
| **Age:** | 3 days old bee larvae (D3) |
| **Source:** | Healthy colony maintained at BioTecnologie BT S.r.l. (colonies no. 14, 2, 12) |
| **Diet:** | Dependent on developmental stage: Diet A, Diet B and Diet C |

**Experimental conditions:**

| | | |
|---|---|---|
| **Temperature:** | 31.8 – 35.4°C (average measured during the test: 34.4°C) | |
| **Relative Humidity:** | From D1 to D8 = | 88.3 – 97.2% (average measured during the test: 96.4%) |
| | From D8 to D15 = | 75.9 – 84.4% (average measured during the test: 84.0%) |

From D15 to D22 = 47.7 – 72.1% (average measured during the test: 61.7%)

**Photoperiod:** 24 h darkness (except during observations)

**Experimental period:**
From 29th August to 12th October 2023 (including the analytical phase)

**Study design:**
The 22-day larval toxicity test with repeated exposure in the laboratory was performed as a dose-response test: the test item was dissolved in water and then in the larval food (aqueous sugar solution mixed with royal jelly) and administered daily to the larvae from day 3 (D3) to 6 (D6) of the test. The stock solutions and the treated diet were prepared freshly each day of administration.
The reference item Dimethoate was tested at a cumulative dose of 7.39 µg/larva.
An untreated control was run in parallel with the royal jelly-based diet.
For each test item treatment group, for the control group and the reference item treatment group, 3 replicates of 12 larvae each were set up. The cumulative doses and the concentrations of test item and reference item used for the test are shown in the table below.

**Trial layout**

| Groups | Cumulative doses [µg/larva] | | Concentrations[1] [mg/kg diet] | | ID Code[2] | |
|---|---|---|---|---|---|---|
| | Test item | Active substance/Safener | Test item | Active substance/Safener | From | To |
| **Control (CTRL)** | 0.0 | 0.0 | 0.0 | 0.0 | CTRLa | CTRLc |
| **Test item 1 (T1)** | 11.11 | Meso: 3.62<br>Cypro.: 1.08<br>Thien.: 0.72 | 72.15 | Meso: 23.53<br>Cypro.: 7.03<br>Thien.: 4.71 | T1a | T1c |
| **Test item 2 (T2)** | 33.33 | Meso: 10.87<br>Cypro.: 3.25<br>Thien.: 2.17 | 216.45 | Meso: 70.58<br>Cypro.: 21.08<br>Thien.: 14.12 | T2a | T2c |
| **Test item 3 (T3)** | 100.00 | Meso: 32.61<br>Cypro.: 9.74<br>Thien.: 6.52 | 649.35 | Meso: 211.74<br>Cypro.: 63.24<br>Thien.: 42.35 | T3a | T3c |
| **Test item 4 (T4)** | 300.00 | Meso: 97.83<br>Cypro.: 29.22<br>Thien.: 19.57 | 1948.05 | Meso: 635.23<br>Cypro.: 189.72<br>Thien.: 127.05 | T4a | T4c |
| **Test item 5 (T5)** | 900.00 | Meso: 293.48<br>Cypro.: 87.65<br>Thien.: 58.70 | 5844.16 | Meso: 1905.70<br>Cypro.: 569.17<br>Thien.: 381.14 | T5a | T5c |
| | **Product** | **Active substance** | **Product** | **Active substance** | | |
| **Reference item (R)** | 7.39 | 7.35 | 48.0 | 47.7 | Ra | Rc |

[1]Calculated based on the cumulative amount of 154 mg of treated food administered to each larva during the treatment period, see the calculation example in section 4.5.2.
[2]For each group, 3 replicates and 12 larvae per replicate were used.
Meso. = Mesotrione, Thien. = Thiencarbazone-methyl, Cypro. = Cyprosulfamide.

**Observations:**
Assessments of mortality and any developmental/behavioural abnormality were performed daily from D4 to D8 (except on D7) and on D15 and on D22. Pupal mortality and the emergence rate of adults were also assessed on D22.

**Statistics:**
The Software Tox Rat Pro 3.3.0 was used to perform the statistics.

**Results**

All validity criteria were met:

a) in the control plate the cumulative larval mortality from D3 to D8 was 2.78% (≤ 15%) across all replicates.
b) in the control plate the adult emergence rate on D22 was 97.22% (≥ 70%) across all replicates.
c) in the reference item group (dimethoate) larval mortality was 100% (≥ 50%) across all replicates on D8.

The qualitative observations carried out during the test (*e.g.* larval and pupal behaviour and morphological differences) did not show abnormalities in the surviving treated bees.

The content of active substances/safener was analysed in the lowest and highest test item concentrations of the water stock solutions (prepared on D3) used to treat the diets and was determined to be within 20% of the nominal values of active substance/safener for all samples. Therefore, the endpoints were calculated based on nominal concentrations and doses. No contamination by the test item was detected in the control.

Significant mortality compared to the control was observed at the tested dose of 33.33 µg test item/larva and above. Therefore, the NOED for larvae on D8 was determined to be 11.11 µg test item/larva.
The adult emergence on D22 (compared to the control) was significantly reduced starting from 100.0 µg test item/larva. Therefore, the NOED on D22 was determined to be 33.33 µg test item/larva.
The relevant endpoints from the statistical analysis of data (D22) are summarized in the table below.

**Summary of endpoints on D22 after repeated exposure to the test item**

| | | **for Adult Emergence/Mortality on D22** | |
|---|---|---|---|
| | | **Test item** | **Active substance/Safener** |
| **Critical dose [µg/larva]** | **ED/LD$_{10}$** | 45.63 (5.22-399.00) | Meso.: 14.88 (1.70-130.11)<br>Thien.: 2.98 (0.34-26.02)<br>Cypro.: 4.44 (0.51-38.86) |
| | **ED/LD$_{20}$** | 92.65 (18.77-457.30) | Meso.: 30.21 (6.12-149.12)<br>Thien.: 6.04 (1.22-29.82)<br>Cypro.: 9.02 (1.83-44.54) |
| | **ED/LD$_{50}$** | 270.09 (96.20-758.27) | Meso.: 88.07 (31.37-247.26)<br>Thien.: 17.61 (6.27-49.45)<br>Cypro.: 26.30 (9.37-73.85) |
| | **NOED** | 33.33 | Meso.: 10.75<br>Thien.: 2.12<br>Cypro.: 3.22 |
| **Critical concentration [mg/kg diet]** | **EC/LC$_{10}$** | 296.30 (33.90-2590.91) | Meso.: 96.62 (11.05-844.86)<br>Thien.: 19.32 (2.21-168.97)<br>Cypro.: 28.86 (3.30-252.33) |
| | **EC/LC$_{20}$** | 601.62 (121.88-2969.48) | Meso.: 196.18 (39.74-968.31)<br>Thien.: 39.24 (7.95-193.66)<br>Cypro.: 58.59 (11.87-289.20) |
| | **EC/LC$_{50}$** | 1753.83 (624.68-4923.83) | Meso.: 571.90 (203.70-1605.60)<br>Thien.: 114.38 (40.74-321.12)<br>Cypro.: 170.81 (60.84-479.54) |
| | **NOEC** | 216.45 | Meso.: 69.80<br>Thien.: 13.80<br>Cypro.: 20.93 |

ED/LD/EC/LC$_{10/20/50}$ evaluated by Weibull analysis using linear max. likelihood regression: 95% confidence limits in brackets.
EC/LC$_{10/20/50}$ values derived multiplying ED/LD$_{10/20/50}$ values by 1000/154 mg diet.
Meso. = Mesotrione, Thien. = Thiencarbazone-methyl, Cypro. = Cyprosulfamide.

## Conclusion

Regarding the effects on larvae on D8 (developmental period) and the effects on adult emergence on D22, the test item GLOB2112dH caused statistically significant mortality starting at the dose of 100.0 µg test item/larva and above. Therefore, the NOED for larvae on D8 and on D22 was determined to be 33.33 µg

test item/larva (10.75 µg Mesotrione/larva, 2.12 µg Thiencarbazone-methyl/larva and 3.22 µg Cyprosulfamide/larva). The NOEC for larvae on D8 and on D22 was determined to be 216.45 mg test item/kg diet (69.80 mg Mesotrione/kg diet, 13.80 mg Thiencarbazone-methyl/kg diet and 20.93 mg Cyprosulfamide/kg).

### A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

No new studies were submitted.

### A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

No new studies were submitted.

### A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

No new studies were 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 Using artificial substrates

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| Comments of zRMS: | The study was conducted according to the method IOBC Blümel et al. (2000).<br>Validity criteria were met:<br>- mortality in the control treatment over the initial 7 days did not exceed 20% (actual: 13.3%).<br>- mortality in the toxic reference was 50-100 % (100 %).<br>- the mean cumulative number of eggs produced between 7 and 14 days exceeded 4.0 per female in the control treatment (actual 6.6 eggs/female)<br>The study is acceptable and suitable for the use in the risk assessment.<br><br>LR50> 200.1 ml product/ha<br>ER50> 200.1 ml product/ha<br>NOERmortality ≥ 200.1 mL/ha<br>NOERreproduction > 200.1 mL/ha |

| | |
|---|---|
| Reference: | KCP 10.3.2.1 |
| Report | GLOB2112dH: Effects on the predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae) in the laboratory. A dose response test on glass plates, Leopold J., 2023a, 177011063 |
| Guideline(s): | Yes, Blümel *et al*., 2000 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes/No/Supplementary |

**Executive summary**

The purpose of this study was to determine the effect of GLOB2112dH in the laboratory on mortality of the predatory mite *Typhlodromus pyri via* contact to treated glass surfaces, compared to a water treated control and to a reference item. Additionally, an assessment for sublethal effects was made (reproduction assessment).
The $LR_{50}$ value of GLOB2112dH for mortality is estimated to be higher than 200 mL product/ha. The NOER for mortality is equal to or higher than 200 mL product/ha and the LOER is higher than 200 mL product/ha.
Reproduction of *Typhlodromus pyri* was assessed in the control and in all test item treatments. The $ER_{50}$ value of GLOB2112dH for reproduction is estimated to be higher than 200 mL product/ha since no effects above 50 % were recorded up to the test item application rate of 200 mL product/ha. The NOER for reproduction is equal to or higher than 200 mL product/ha and the LOER is higher than 200 mL product/ha.

**Material and Methods**

| | |
|---|---|
| Test Item: | GLOB2112dH; batch no.: MAM 107683; analysed content of thiencarbazone-methyl: 77.47 g/L, mesotrione: 391.9 g:L, cyprosulfamide (safener): 117.5 g/L. |
| Test Species: | Predatory Mite *(Typhlodromus pyri),* protonymphs not older than 24 hours; source: Katz Biotech AG, Baruth, Germany. |
| Test Design: | This study comprised 7 treatment groups (5 application rates of the test item, control, reference item) with 3 replicates each containing 20 mites. The mites were exposed to dried residues on treated glass plates. Survival of the mites was assessed after 3 and 7 days. For the reproduction assessment surviving mites from the control and from all test item groups where the corrected mortality was ≤ 50 % were sexed and the number of eggs per female was recorded at 3 assessment days within one week. |
| Endpoints: | Mortality after 7 days of exposure; additionally, reproduction capacity for survived mites. |
| Validity Criteria: | Control mortality should not exceed 20 % on day 7 after exposure.<br>Reference item mortality should result in at least 50 % corrected mortality on day 7 after exposure.<br>Control reproductions (number of eggs per female) should be ≥ 4 eggs for the second week. |
| Reference Item: | Danadim Progress (nominal: 400 g dimethoate/L). |
| Test Rates: | Control, 12.5, 25.0, 50.0, 100 and 200 mL product/ha and reference item. The reference item was applied at an application rate of 9.0 mL Danadim Progress/ha. All treatments were applied in 200 L spray volume/ha. The spraying solutions were sprayed onto glass plates via laboratory spraying equipment, which were then air dried. |
| Test Conditions: | Temperature: 24 - 27 °C; relative humidity: 66 - 70 %; photoperiod: 16 h light : 8 h dark; light intensity: 230 - 480 lux. |
| Statistics: | Mortality: $Chi^2$ 2x2 Table Test with Bonferroni Correction; Fisher's Exact Binomial Test (both one-sided greater, $\alpha = 0.05$.<br>Reproduction: Dunnett's t-Test (one-sided smaller, $\alpha = 0.05$). |

**Results**

The mean mortality of *Typhlodromus pyri* was 13.3 % in the control treatment. In the test item treatments, mortality ranged from 5.0 % to 21.7 %, corresponding to corrected mortalities of -9.6 % and 9.6 %. Mortality was not statistically significantly increased compared to the control up to and including the highest application rate of 200 mL product/ha ($Chi^2$ 2x2 Table Test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$).

The reference item applied at a rate of 9.0 mL Danadim Progress/ha produced a statistically significant mortality of 100.0 % (corrected mortality 100.0 %) after 7 days (Fisher's Exact Binomial Test, one-sided greater, α = 0.05).

**Mortality and reproduction of the mites**

| | **Rate** [1] **[mL product/ha]** | **Mortality** [2] **[%]** | **Mortality corr.** [3] **[%]** | **Reproduction** [4] **[eggs/female]** | **Effect on reproduction [%]** [5] |
|---|---|---|---|---|---|
| Control | 0 | 13.3 | -- | 6.6 | -- |
| GLOB2112dH | 12.5 | 20.0 n.s. | 7.7 | 7.5 n.s. | -13.0 |
| | 25.0 | 5.0 n.s. | -9.6 | 8.8 n.s. | -32.3 |
| | 50.0 | 8.3 n.s. | -5.8 | 7.3 n.s. | -10.3 |
| | 100 | 13.3 n.s. | 0.0 | 6.1 n.s. | 8.7 |
| | 200 | 21.7 n.s. | 9.6 | 6.8 n.s. | -1.9 |
| reference item Danadim Progress | 9.0 mL product/ha | 100.0 * | 100.0 | -- | -- |

| **Endpoints** [6] | |
|---|---|
| | [mL product/ha] |
| Mortality: $LR_{50}$ Value | > 200 |
| NOER for Mortality | ≥ 200 |
| LOER for Mortality | > 200 |
| Reproduction: $ER_{50}$ Value | > 200 |
| NOER for Reproduction | ≥ 200 |
| LOER for Reproduction | > 200 |

1) Application rate in 200 L spray volume/ha
2) Mortality: after 7 days of exposure to spray residues on glass plates
(Chi² 2x2 Table Test with Bonferroni Correction; one-sided greater; α = 0.05; n.s. = not significant)
3) Corrected mortality according to Abbott and improvements by Schneider-Orelli; negative values indicate better survivorship compared to the control
4) Reproduction: mean number of eggs/female,
(Dunnett's t-Test; one-sided smaller; α = 0.05; n.s. = not significant)
5) Calculated on the exact raw data; negative values indicate better reproductive performance compared to the control
6) The $LR_{50}$ and $ER_{50}$ value could not be calculated as no mortality or effects on reproduction above 50% were noted.

Reproduction of *T. pyri* was assessed in the control and in all test item treatments. The mean reproduction was 6.6 eggs per female in the control treatment. In the test item treatments, reproduction was between 6.1 and 8.8 eggs per female, corresponding to reductions of the reproductive performance of 8.7 % and -32.3 %. Reproduction was not statistically significantly reduced compared to the control up to and including the highest application rate of 200 mL product/ha (Dunnett's t-Test, one-sided smaller, α = 0.05).

Validity criteria:

The reference item applied at a rate of 9.0 mL Danadim Progress/ha produced a statistically significant corrected mortality of 100.0 % after 7 days (should be ≥ 50 % corrected mortality). The control mortality was 13.3 % after 7 days (should not exceed 20 %). The mean control reproduction rate was 6.6 eggs per female after 14 days (should be ≥ 4 eggs per female in the second week). All validity criteria were met.

**Conclusion**

Under worst case laboratory conditions, the $LR_{50}$ value of GLOB2112dH for mortality is estimated to be higher than 200 mL product/ha. The NOER (no observed effect rate) for mortality is equal to or higher than 200 mL product/ha and the LOER (lowest observed effect rate) is higher than 200 mL product/ha.

Reproduction of *Typhlodromus pyri* was assessed in the control and in all test item treatments. Reproduction was not statistically significantly affected up to and including the highest application rate of 200 mL product/ha. The $ER_{50}$ value of GLOB2112dH for reproduction is estimated to be higher than 200 mL product/ha since no effects above 50 % were recorded up to the test item application rate of 200 mL product/ha. The NOER for reproduction is equal to or higher than 200 mL product/ha and the LOER is higher than 200 mL product/ha.
All validity criteria were met. The study is considered valid.

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| Comments of zRMS: | The study was conducted according to the method Mead-Briggs et al., 2000 and Mead-Briggs et al., 2010<br>Validity criteria were met:<br>-mortality in the control treatment at 48 h did not exceed 13 % (actual: 7.50%).<br>-mortality in the toxic reference treatment ≥50 % at 48 h (actual: 100 %).<br>- all wasps survived the 24-hour oviposition period,<br>-the mean number of mummies in the control treatment was >5.0 per per female (actual: 31.4) and no more than two females should fail to produce mummies (actual: no zero values).<br>There were no deviations.<br>The study is acceptable and suitable for the use in the risk assessment.<br><br>48h LR50> 200.2 ml product/ha<br>ER50 > 200.2 ml product/ha<br>NOERmortality > 200.2 ml/ha<br>NOERreproduction > 200.2 ml/ha |

| | |
|---|---|
| Reference: | KCP 10.3.2.1 |
| Report | GLOB2112dH: Effects on the parasitoid *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) in the laboratory. A dose response test on glass plates, Leopold J., 2023b, 177011001 |
| Guideline(s): | Yes, Mead-Briggs *et al.* 2000 and Mead-Briggs *et al.* 2010 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes/No/Supplementary |

**Executive summary**

The purpose of this study was to determine the effect of GLOB2112dH in the laboratory on mortality of the parasitoid *A. rhopalosiphi via* contact to treated glass surfaces, compared to a water treated control and to a reference item. Additionally, an assessment for sublethal effects was made (reproduction assessment).
The $LR_{50}$ value of GLOB2112dH was estimated to be higher than 200 mL product/ha. The NOER for mortality was equal to or higher than 200 mL product/ha and the LOER was higher than 200 mL product/ha.
Reproduction of *Aphidius rhopalosiphi* was assessed in the control and in all test item treatments. The $ER_{50}$ value of GLOB2112dH for reproduction is estimated to be higher than 200 mL product/ha. The NOER for effects on reproduction was equal to or higher than 200 mL product/ha and the LOER was higher than 200 mL product/ha.

**Material and methods**

Test Item: GLOB2112dH; batch no.: MAM 107683; analysed content of

| | |
|---|---|
| | thiencarbazone-methyl: 77.47 g/L, mesotrione: 391.9 g:L, cyprosulfamide (safener): 117.5 g/L. |
| Test Species: | Parasitoid *(Aphidius rhopalosiphi),* adults not older than 48 hours; source: Katz Biotech AG, Baruth, Germany. |
| Test Design: | This study encompassed 7 treatment groups (5 application rates of the test item, control, reference item) with 4 replicates each containing 10 adult parasitoids. The parasitoids were exposed to dried residues on treated glass plates. Survival of the parasitoids was assessed after 2, 24 and 48 hours. After 48 hours, for treatment groups where the corrected mortality was ≤ 50 % the reproductive capacity was assessed by confining females individually over untreated barley plants infested with the host cereal aphids, *Rhopalosiphum padi*. The females were removed after 24 hours and the aphid-infested plants left for a further 11 -12 days before the numbers of aphid mummies that had developed were assessed. |
| Endpoints: | Mortality of exposed parasitoids; additionally, reproductive capacity for female survivors. |
| Validity Criteria: | Control mortality should be ≤ 13 %.<br>Reference item mortality should be ≥ 50 % corrected mortality.<br>Mean reproduction rate of control treatment should be ≥ 5 mummies per female.<br>No more than 2 female parasitoids should produce zero values. |
| Reference Item: | Danadim Progress (nominal: 400 g dimethoate/L). |
| Test Rates: | Control, 12.5, 25.0, 50.0, 100 and 200 mL product/ha and reference item. The reference item was applied at an application rate of 0.3 mL Danadim Progress/ha. All treatments were applied in 200 L spray volume/ha. The spraying solutions were sprayed onto glass plates *via* laboratory spraying equipment, which were then air dried. |
| Test Conditions: | Temperature: 20 - 21 °C; relative humidity: 74 - 83 % (acclimatisation and exposure period), 76 - 79 % (post-exposure period, within the test units); photoperiod: 16 h light : 8 h dark; light intensity: 820 - 1710 lux (acclimatisation, exposure and parasitisation period), 14320 - 17210 lux (post-parasitisation period). |
| Statistics: | Mortality: $Chi^2$ 2x2 Table Test with Bonferroni Correction, Fisher's Exact Binomial Test (both one-sided greater, $\alpha = 0.05$).<br>Reproduction: Williams t-Test (one-sided smaller, $\alpha = 0.05$). |

**Results:**

The mean mortality of *Aphidius rhopalosiphi* was 7.5 % in the control treatment. In the test item treatments, mortality was between 0.0 % and 15.0 %, corresponding to corrected mortalities of -8.1 % and 8.1 %. Mortality was not statistically significantly increased compared to the control up to and including the highest application rate of 200 mL product/ha ($Chi^2$ 2x2 Table Test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$).

The reference item applied at a rate of 0.3 mL Danadim Progress/ha produced a statistically significant corrected mortality of 100.0 % after 48 hours (Fisher's Exact Binomial Test, one-sided greater, $\alpha = 0.05$).

**Mortality and parasitisation efficiency of the parasitoid wasp *Aphidius rhopalosiphi***

| | **Rate [1)]** **[mL product/ha]** | **Mortality [2)]** **[%]** | **Mortality corr. [3)]** **[%]** | **Reproduction [4)]** **[mummies/female]** | **Effect on reproduction [5)] [%]** |
|---|---|---|---|---|---|
| Control | 0 | 7.5 | -- | 31.4 | -- |
| GLOB2112dH | 12.5 | 5.0 n.s. | -2.7 | 39.4 n.s. | -25.6 |
| | 25.0 | 0.0 n.s. | -8.1 | 38.0 n.s. | -21.0 |
| | 50.0 | 2.5 n.s. | -5.4 | 42.3 n.s. | -34.7 |
| | 100 | 0.0 n.s. | -8.1 | 23.3 n.s. | 25.8 |

| | Rate [1)] [mL product/ha] | Mortality [2)] [%] | Mortality corr. [3)] [%] | Reproduction [4)] [mummies/female] | Effect on reproduction [5)] [%] |
|---|---|---|---|---|---|
| | 200 | 15.0 n.s. | 8.1 | 25.6 n.s. | 18.3 |
| **Endpoints [6)]** | | | | | |
| | | | [mL product/ha] | | |
| Mortality: $LR_{50}$ Value | | | > 200 | | |
| NOER for Mortality | | | ≥ 200 | | |
| LOER for Mortality | | | > 200 | | |
| Reproduction: $ER_{50}$ Value | | | > 200 | | |
| NOER for Reproduction | | | ≥ 200 | | |
| LOER for Reproduction | | | > 200 | | |

1) Application rate in 200 L spray volume/ha
2) Mortality: after 48 hours of exposure to spray residues on glass plates,
($Chi^2$ 2x2 Table Test with Bonferroni Correction, one-sided greater, α = 0.05; n.s. = not significant)
3) Corrected mortality according to Abbott and improvements by Schneider-Orelli; negative values indicate better survivorship compared to control
4) Reproduction: mean number of parasitised aphids/female,
(Williams t-Test, one-sided smaller, α = 0.05; n.s. = not significant)
5) Calculated on the exact raw data; negative values indicate better performance compared to the control
6) The $LR_{50}$ and $ER_{50}$ value could not be calculated as no mortality or effects on reproduction above 50% were noted.

No behavioural abnormalities (affected and/or moribund parasitoids) were observed at any test item application rate after 2, 24 and 48 hours with exception of the lowest test item rate of 12.5 mL product/ha where one moribund parasitoid was observed after 48 hours.
Reproduction of *A. rhopalosiphi* was assessed in the control and in all test item application rates. The mean reproduction rate was 31.4 mummies per female in the control treatment. In the test item treatments, reproduction ranged from 23.3 to 42.3 mummies per female, corresponding to a reduction of 25.8 % and -34.7 %. Reproduction was not statistically significantly reduced compared to the control up to and including the highest application rate of 200 mL product/ha (Williams t-Test, one-sided smaller, α = 0.05).

Validity criteria:

The reference item applied at a rate of 0.3 mL Danadim Progress/ha produced a statistically significant corrected mean mortality of 100.0 % after 48 hours (should be ≥ 50 % corrected mortality). The mean control mortality was 7.5 % after 48 hours of exposure (should not exceed 13 %). The mean control reproduction rate was 31.4 mummies per female (should be ≥ 5.0 mummies per female). No female parasitoid produced zero values in the control treatment (no more than 2 female parasitoids producing zero values). All validity criteria were met.

**Conclusion**

Under worst case laboratory conditions, the $LR_{50}$ value of GLOB2112dH was estimated to be higher than 200 mL product/ha. The NOER (no observed effect rate) for mortality was equal to or higher than 200 mL product/ha and the LOER (lowest observed effect rate) was higher than 200 mL product/ha.
Reproduction of *Aphidius rhopalosiphi* was assessed in the control and in all test item treatments. Reproduction was not statistically significantly reduced up to and including the highest test item application rate of 200 mL product/ha. The $ER_{50}$ value of GLOB2112dH for reproduction is estimated to be higher than 200 mL product/ha. The NOER for effects on reproduction was equal to or higher than 200 mL product/ha and the LOER was higher than 200 mL product/ha.
All validity criteria were met. The study is considered valid.

### A 2.3.2.2 KCP 10.3.2.2 Extended laboratory tests

No new studies were 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

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|---|---|
| Comments of zRMS: | The study was performed according to OECD TG 222 and principles of GLP.<br>The validity criteria are met:<br>For the control group:<br>- Adult mortality: ≤ 10 % (being 0.0 %)<br>- Number of juveniles per replicate: ≥ 30 (being 37 to 101)<br>- Coefficient of variation of reproduction: ≤ 30 % (being 25.6 %).<br><br>There were no deviations to the study plan.<br><br>The study is considered acceptable and suitable for the risk assessment.<br><br>$NOEC_{mortality}$ ≥ 25 mg/kg dw<br>$NOEC_{body\ weight}$ = 4.29 mg/kg dw<br>$NOEC_{reproduction}$ = 1.32 mg/kg dw<br>$EC_{10}$ = 1.49 mg/kg dw<br>$EC_{20}$ = 1.99 mg/kg dw<br>$EC_{50}$ = 3.48 mg/kg dw |

| | |
|---|---|
| Reference: | KCP 10.4.1.1 |
| Report | GLOB2112dH: Effects on reproduction and growth of earthworms *Eisenia andrei* in artificial soil, Hübner S., 2024, 177011022 |
| Guideline(s): | Yes, OECD 222 (2016) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes/No/Supplementary |

**Executive summary**

The purpose of this study was to investigate the effects of GLOB2112dH on the mortality, body weight, feeding activity and reproduction of the adult earthworm *Eisenia andrei*.
The NOEC for mortality of the earthworm *Eisenia andrei* was determined to be ≥25.0 mg test item/kg soil dry weight, *i.e.* the highest concentration tested. The LOEC for mortality was estimated to be >25.0 mg test item/kg soil dry weight. The $LC_{50}$ was estimated to be >25.0 mg test item/kg soil dry weight.
The NOEC for body weight changes was determined to be 4.29 mg test item/kg soil dry weight. The LOEC for body weight changes was determined to be 7.72 mg test item/kg soil dry weight.
The NOEC for reproduction was determined to be 1.32 mg test item/kg soil dry weight. The LOEC for reproduction was determined to be 2.38 mg test item/kg soil dry weight.

The $EC_{10}$ was determined to be 1.49 mg test item /kg soil dry weight. The $EC_{20}$ was determined to be 1.99 mg test item/kg soil dry weight and the $EC_{50}$ was determined to be 3.48 mg test item/kg soil dry weight.

**Materials and methods**

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|---|---|
| Test Item: | GLOB2112dH; batch no.: MAM 107683; authenticated content (pre-storage): Mesotrione 391.9 g/L, Cyprosulfamide (safener) 117.5 g/L, Thiencarbazone-methyl 77.47 g/L |
| Test Species: | Earthworm (*Eisenia andrei,* Bouché, 1972), adult earthworms (with clitellum and weight range 305 to 600 mg), approximately 8 to 9 months old, source: from an in-house culture. |
| Test Design: | 56-day test in treated artificial soil prepared according to OECD; different concentrations of the test item were incorporated into the soil; 9 treatment groups (8 test item concentrations, control); 4 replicates for the test item treatments and 8 replicates for the control with 10 earthworms each. Assessment of adult earthworm mortality, behavioural effects and biomass development was carried out after 28 days exposure of adult earthworms in treated artificial soil. Reproduction rate (number of offspring) was assessed after additional 28 days (assessed 56 days after application). |
| Endpoints: | Mortality, weight change, feeding activity and reproduction rate were determined. |
| Reference Item: | Carbendazim (600 g/L nominal). The effects of the reference item were investigated in a separate GLP study. |
| Test Concentrations: | Control, 0.408, 0.735, 1.32, 2.38, 4.29, 7.72, 13.9 and 25.0 mg GLOB2112dH/kg soil dry weight. |
| Test Conditions: | Artificial soil according to OECD 222; initial pH 5.7 to 5.9, pH at experimental end 6.0 to 6.3; water content 24.7% to 25.7% (53.7% to 56.0% of maximum water holding capacity, WHC) at experimental start and 23.0% to 24.8% (49.9% to 53.9% of the maximum WHC) at experimental end; temperature: within the range of 18 °C to 22 °C; photoperiod: 16 h light : 8 h dark, light intensity: within the range of 400 lux to 800 lux. |
| Statistics: | Standard procedures, Fisher's Exact Test (mortality), Williams t-test (body weight changes and reproduction), 4-param. normal CDF (EC values). |

**Results**

All study validity criteria were met:

| | |
|---|---|
| Mean Control Mortality: | Should not exceed 10% over initial 4-week test period. Was 0%. |
| Reproduction of Control: | Should be ≥ 30 earthworms per replicate container. Was 37 to 101. |
| Coefficient of Variation of Reproduction in Control: | Should not exceed 30%. Was 25.6%. |

A mortality of up to 5% was found in the test item treated groups, which was not statistically significantly different compared to the control, where 0% of the earthworms died (Fisher's Exact Test, $\alpha = 0.05$, one-sided greater).
The body weight changes of the earthworms after 28 days exposure to GLOB2112dH were not statistically significantly different compared to the control up to and including the test concentration of 4.29 mg test item/kg soil dry weight (Williams t-test, $\alpha = 0.05$, one-sided smaller). At the test concentration of 7.72 mg test item/kg soil dry weight and above, body weights were statistically significantly reduced compared to the control.

No statistically significant effects on reproduction were observed up to and including the test concentration of 1.32 mg test item/kg soil dry weight (Williams t-test, $\alpha = 0.05$, one-sided smaller). At the test concentration of 2.38 mg test item/kg soil dry weight and above, reproduction was statistically significantly reduced compared to the control.
No behavioural abnormalities were observed in any of the treatment groups. The feeding activity up to and including the concentration of 2.38 mg test item/kg soil dry weight was comparable to the control whereas the food intake at the test concentration of 4.29 mg test item/kg soil dry weight and above appeared to be reduced.

**Effect of GLOB2112dH on earthworms (*Eisenia andrei*) in a 56-day reproduction study**

| **GLOB2112dH [mg test item/kg soil dry weight]** | **Control** | **0.408** | **0.735** | **1.32** | **2.38** | **4.29** | **7.72** | **13.9** | **25.0** |
|---|---|---|---|---|---|---|---|---|---|
| Mortality (day 28) [%] | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 3 | 5 |
| Statistical Significance [1)] | - | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| Body weight change (day 28) [%] | 26.6 | 35.7 | 29.5 | 29.1 | 28.6 | 27.7 | 18.1 | 11.2 | 8.1 |
| Statistical Significance [2)] | - | n.s. | n.s. | n.s. | n.s. | n.s. | * | * | * |
| Mean No. of juveniles (day 56) | 70 | 71 | 69 | 83 | 45 | 45 | 27 | 19 | 23 |
| Statistical Significance [2)] | - | n.s. | n.s. | n.s. | * | * | * | * | * |
| Reproduction in [%] of control (day 56) | - | 101 | 99 | 118 | 64 | 65 | 39 | 28 | 32 |
| Food consumption [g] | 25.0 | 25.0 | 25.0 | 24.8 | 24.3 | 21.3 | 21.0 | 19.8 | 19.3 |
| **Endpoints [mg test item/kg soil dry weight]** | | | | | | | | | |
| NOEC (day 28 mortality) | ≥25.0 | | | | | | | | |
| LOEC (day 28 mortality) | >25.0 | | | | | | | | |
| $LC_{50}$ [3)] | >25.0 | | | | | | | | |
| NOEC (day 28 weight) | 4.29 | | | | | | | | |
| LOEC (day 28 weight) | 7.72 | | | | | | | | |
| NOEC (day 56 reproduction) | 1.32 | | | | | | | | |
| LOEC (day 56 reproduction) | 2.38 | | | | | | | | |
| EC Values (reproduction) [4)] | $EC_{10}$ = 1.49 | | | $EC_{20}$ = 1.99 | | | $EC_{50}$ = 3.48 | | |
| 95% confidence limits | 1.13 – 1.97 | | | 1.61 – 2.47 | | | 2.68 – 4.51 | | |

The results represent rounded values calculated from the exact raw data.
- = not applicable
n.s. = not significantly different compared to the control
* = significantly different compared to the control
[1)] Fisher's Exact Test, $\alpha = 0.05$, one-sided greater
[2)] Williams t-test, $\alpha = 0.05$, one-sided smaller
[3)] estimated value
[4)] 4-param. normal CDF

Reference Item Test:

In the most recent test with the reference item Carbendazim (performed under the test facility Study No. 105689022 from January to March 2023), there were statistically significant effects on reproduction at a concentration of 1.00 mg a.i./kg soil dry weight and above, which is in line with the guideline OECD 222 (effects should be observed between 1 and 5 mg a.i./kg soil dry weight). The $EC_{50}$ for reproduction was calculated as 1.10 mg a.i./kg soil dry weight.

**Conclusion**

In an earthworm reproduction and growth study with GLOB2112dH the No Observed Effect Concentration (NOEC) for mortality of the earthworm *Eisenia andrei* was determined to be ≥25.0 mg test item/kg soil dry weight, *i.e.* the highest concentration tested. The Lowest Observed Effect Concentration (LOEC) for mortality was estimated to be >25.0 mg test item/kg soil dry weight. The $LC_{50}$ was estimated to be >25.0 mg test item/kg soil dry weight.
The NOEC for body weight changes was determined to be 4.29 mg test item/kg soil dry weight. The LOEC for body weight changes was determined to be 7.72 mg test item/kg soil dry weight.

The NOEC for reproduction was determined to be 1.32 mg test item/kg soil dry weight. The LOEC for reproduction was determined to be 2.38 mg test item/kg soil dry weight.
The $EC_{10}$ was determined to be 1.49 mg test item /kg soil dry weight. The $EC_{20}$ was determined to be 1.99 mg test item/kg soil dry weight and the $EC_{50}$ was determined to be 3.48 mg test item/kg soil dry weight.

### A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

No new studies were 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

| | |
|---|---|
| Comments of zRMS: | The study was performed according to OECD TG 232 and principles of GLP.<br>The validity criteria are met:<br>For the control group:<br>Mean adult mortality: ≤ 20 % (observed: 8%)<br>Mean number of juveniles per test vessel: ≥ 100 (observed:830 to 1207)<br>Coefficient of variation for the mean number of juveniles: < 30 % (observed: 13.4%).<br><br>The following deviation from the study plan was noted:<br>On day 9 of exposure, the light period was shortened for approximately 5.5 hours due to a technical problem. Therefore, the light regime was 10.25 h light : 13.75 h dark at that day instead of 16 h light : 8 h dark. Based on the long-term exposure of the soil animals, a short interruption of the light period was no impact on the study.<br><br>The study is considered acceptable and suitable for the risk assessment.<br><br>$NOEC_{mortality}$ ≥ 1000 mg/kg dw<br>$NOEC_{reproduction}$ = 309 mg/kg dw<br>$EC_{10}$ = 306.7 mg/kg dw<br>$EC_{20}$ = 454.3 mg/kg dw<br>$EC_{50}$ = 963.6 mg/kg dw |

| | |
|---|---|
| Reference: | KCP 10.4.2.1 |
| Report | GLOB2112dH: Effects on reproduction of Collembola (*Folsomia candida*) in artificial soil, Hübner S., 2023a, 177011016 |
| Guideline(s): | Yes, OECD 232 (2016) |
| Deviations: | On day 9 of exposure, the light period was shortened for approximately 5.5 hours due to a technical problem. Therefore, the light regime was 10.25 h light : 13.75 h dark at that day instead of 16 h light : 8 h dark. Based on the long-term exposure of the soil animals, a short interruption of the light period will not affect the Collembola. There was no impact on the study. |
| GLP: | Yes |
| Acceptability: | Yes/No/Supplementary |

**Executive summary**

The purpose of the study was to determine the effects of GLOB2112dH on mortality and reproduction of Collembola (*Folsomia candida*).
The NOEC of GLOB2112dH for mortality of *Folsomia candida* was determined to be ≥1000 mg test item/kg soil dry weight. The LOEC for mortality was estimated to be >1000 mg test item/kg soil dry weight. The $LC_{50}$ was estimated to be >1000 mg test item/kg soil dry weight.
The NOEC of GLOB2112dH for reproduction of *Folsomia candida* was determined to be 309 mg test item/kg soil dry weight. The LOEC for reproduction was determined to be 556 mg test item/kg soil dry weight. The $EC_{10}$ for *Folsomia candida* in artificial soil was determined to be 306.7 mg test item/kg soil dry weight, $EC_{20}$ was determined to be 454.3 mg test item/kg soil dry weight and the $EC_{50}$ was determined to be 963.6 mg test item/kg soil dry weight.

**Materials and methods**

| | |
|---|---|
| Test Item: | GLOB2112dH; batch no.: MAM 107683; authenticated content (pre-storage): Mesotrione 391.9 g/L, Cyprosulfamide (safener) 117.5 g/L, Thiencarbazone-methyl 77.47 g/L |
| Test Species: | Collembola *Folsomia candida*, 10 - 12 days old, from cultures held at the laboratory. |
| Test Design: | 28-day exposure in treated artificial soil. Different concentrations of the test item were mixed homogeneously into the soil which was placed into glass vessels before the Collembola were introduced on top of the soil; 8 test item concentrations and one control were tested; 4 replicates/concentration with 10 Collembola each (8 replicates for the control). Feeding of Collembola with approximately 2 mg dry yeast for each test vessel at the beginning of the test and on day 14. Assessment of adult mortality, behavioural effects and reproduction was performed after 28 days. |
| Endpoints: | Mortality of adult Collembola, behavioural effects, number of juveniles. |
| Reference Item: | Boric acid (the effects of the reference item were investigated in a separate GLP study). |
| Test Concentrations: | Control, 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg GLOB2112dH/kg soil dry weight. |
| Test Conditions: | Artificial soil according to OECD 232; pH at experimental start 5.6 to 5.8, pH at experimental end 5.8 to 5.9; water content at experimental start 16.2% to 16.4% (50.7% to 51.4% of the maximum water holding capacity); water content at experimental end 14.6% to 16.1% (45.7% to 50.3% of the maximum water holding capacity); temperature: within the range of 18°C to 22°C; illumination: 16 h light : 8 h dark, light intensity within the range of 400 to 800 lux. |
| Statistics: | Standard procedures, Fisher's Exact Test (mortality), Williams t-test (reproduction), Probit analysis (EC values). |

**Results**

All validity criteria for the study were met:

| | |
|---|---|
| Control Mortality: | Should be ≤20%. Was 8%. |
| Control Reproduction: | Should reach ≥100 juveniles per container. Was 830 to 1207. |
| Coefficient of Variation of the Control Reproduction: | Should be less than 30%. Was 13.4%. |

A mortality of up to 10% was observed in the test item treated groups, which was not statistically significantly different compared to the control, where 8% of the Collembola died (Fisher's Exact test, $\alpha = 0.05$, one-sided greater).

Reproduction of the Collembolan exposed to GLOB2112dH was not statistically significantly different compared to the control up to and including the test concentration of 309 mg test item/kg soil dry weight (Williams t-test, $\alpha = 0.05$, one-sided smaller). At the test concentration of 556 mg test item/kg soil dry weight and above, reproduction was statistically significantly decreased compared to the control.
No behavioural abnormalities were observed in any of the treatment groups. The results are shown in the table below.

**Summary of the Effects of GLOB2112dH on Collembola (*Folsomia candida*) in a 28-day Reproduction Study**

| **GLOB2112dH [mg/kg soil dry weight]** | **Control** | **16.3** | **29.4** | **52.9** | **95.3** | **171** | **309** | **556** | **1000** |
|---|---|---|---|---|---|---|---|---|---|
| Mean mortality (day 28) [%] | 8 | 8 | 0 | 3 | 10 | 5 | 3 | 8 | 8 |
| Significance [1)] | - | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| Mean no. of juveniles (day 28) | 1024 | 959 | 963 | 978 | 1015 | 977 | 1088 | 690 | 515 |
| Reproduction in [%] of control (day 28) | - | 94 | 94 | 95 | 99 | 95 | 106 | 67 | 50 |
| Statistical significance [2)] | - | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | * | * |

| **Endpoints [mg/kg soil dry weight]** | | | |
|---|---|---|---|
| NOEC (mortality) | ≥1000 | | |
| LOEC (mortality) | >1000 | | |
| $LC_{50}$ (mortality) [3)] | >1000 | | |
| NOEC (reproduction) | 309 | | |
| LOEC (reproduction) | 556 | | |
| EC Values (reproduction) [4)] | $EC_{10}$: 306.7 | $EC_{20}$: 454.3 | $EC_{50}$: 963.6 |
| 95% confidence limits | 200.3 – 387.6 | 349.2 – 534.6 | 830.7 – 1202.8 |

n.s. = not significantly different compared to the control
* = significantly different compared to the control
[1)] Fisher's Exact Test, $\alpha = 0.05$, one-sided greater
[2)] Williams t-test, $\alpha = 0.05$, one-sided smaller
[3)] estimated value
[4)] Probit analysis
- not applicable

Reference Item Test:
In a separate study, the reference item Boric acid showed statistically significant effects on reproduction at concentrations of 30.5 mg/kg soil dry weight and above. The $EC_{50}$ for reproduction was calculated to be 79.3 mg/kg soil dry weight.

**Conclusion**

The No Observed Effect Concentration (NOEC) of GLOB2112dH for mortality of *Folsomia candida* was determined to be ≥1000 mg test item/kg soil dry weight. The Lowest Observed Effect Concentration (LOEC) for mortality was estimated to be >1000 mg test item/kg soil dry weight. The $LC_{50}$ was estimated to be >1000 mg test item/kg soil dry weight.
The NOEC of GLOB2112dH for reproduction of *Folsomia candida* was determined to be 309 mg test item/kg soil dry weight. The LOEC for reproduction was determined to be 556 mg test item/kg soil dry weight.
The $EC_{10}$ for *Folsomia candida* in artificial soil was determined to be 306.7 mg test item/kg soil dry weight, $EC_{20}$ was determined to be 454.3 mg test item/kg soil dry weight and the $EC_{50}$ was determined to be 963.6 mg test item/kg soil dry weight.

| | |
|---|---|
| Comments of zRMS: | The study was performed according to OECD TG 226 and principles of GLP.<br>The validity criteria are met:<br>For the control group:<br>- Mean mortality of adult females: ≤ 20 % (observed: 9%)<br>- Mean number of juveniles per replicate: ≥ 50 (observed: 179 to 296)<br>- Coefficient of variation (mean number of juveniles per replicate): ≤ 30 % (observed: 15.9 %). |

| | |
|---|---|
| | There were no deviations to the study plan.<br><br>The study is considered acceptable and suitable for the risk assessment.<br><br>$NOEC_{mortality}$ = 95.4 mg/kg dw<br>$NOEC_{reproduction}$ = 95.4 mg/kg dw<br>$EC_{10}$ = 105.6 mg/kg dw<br>$EC_{20}$ = 152.2 mg/kg dw<br>$EC_{50}$ = 306.0 mg/kg dw |

| | |
|---|---|
| Reference: | KCP 10.4.2.1 |
| Report | GLOB2112dH: Effects on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil, Hübner S., 2023b, 177011089 |
| Guideline(s): | Yes, OECD 226 (2016) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes/No/Supplementary |

**Executive summary**

The purpose of the study was to determine the effects of GLOB2112dH on mortality and reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil.
The overall NOEC was determined to be 95.4 mg test item/kg soil dry weight. The overall LOEC was determined to be 172 mg test item/kg soil dry weight. The $LC_{50}$ was determined to be 128.0 mg test item/kg soil dry weight. The $EC_{10}$ was determined to be 105.6 mg test item/kg soil dry weight, $EC_{20}$ was determined to be 152.2 mg test item/kg soil dry weight and the $EC_{50}$ was determined to be 306.0 mg test item/kg soil dry weight.

**Materials and methods**

| | |
|---|---|
| Test Item: | GLOB2112dH; batch no.: MAM 107683; authenticated content (pre-storage): Mesotrione 391.90 g/L, Cyprosulfamide (safener) 117.5 g/L, Thiencarbazone-methyl 77.47 g/L |
| Test Species: | Predatory mite *Hypoaspis aculeifer*, adult females, approximately 12 days after reaching the adult stage (33 days after placing adult females in clean rearing vessels and the start of the egg laying period in the synchronisation), cultured by the test facility. |
| Test Design: | 14 days exposure in treated artificial soil. Different concentrations of the test item were mixed homogeneously into the soil, which was filled into glass vessels before the predatory mites were introduced on top of the soil; 8 test item concentrations and one control were tested; 4 replicates per test item concentration and 8 replicates for the control, with 10 female predatory mites in each replicate. Feeding of the mites with cheese mites (*Tyrophagus putrescentiae) ad libitum* at test start and on day 2, 5, 7, 9 and 12. Assessment of adult mortality and reproduction performed after 14 days. |
| Endpoints: | Adult mortality, number of juveniles. |
| Reference Item: | Dimethoate (the effects of the reference item were investigated in a separate GLP study). |
| Test Concentrations: | Control, 5.05, 9.08, 16.4, 29.4, 53.0, 95.4, 172 and 309 mg GLOB2112dH/kg soil dry weight. |
| Test Conditions: | Artificial soil based on OECD 226; initial pH 5.7 to 5.9, pH at experimental end 5.7 to 5.8; water content at experimental start 16.8% to 17.1% (52.6% to |

| | |
|---|---|
| | 53.5% of the maximum water holding capacity); at experimental end 15.5% to 16.8% (48.4% to 52.5% of the maximum water holding capacity); temperature: within the range of 18°C to 22°C; illumination: 16 h light : 8 h dark (within the range of 400 to 800 lux). |
| Statistics: | Standard procedures, Step-down Cochran-Armitage Test (mortality), Williams t-test (reproduction), Moving Averages Computation ($LC_{50}$), 3-param. normal CDF Analysis (EC values). |

**Results**

All validity criteria for the study were met:

| | |
|---|---|
| Control Mortality: | ≤ 20% of the introduced adult female animals. Mean mortality was 9%. |
| Control Reproduction: | ≥ 50 juveniles per test unit. The number of juvenile mites per replicate was 179 to 296 |
| Coefficient of Variation (CV) of the Control Reproduction: | Should be ≤30%. Was 15.9% |

A mortality of 5% to 15% was observed up to and including the test concentration of 95.4 mg test item/kg soil dry weight, which was not statistically significantly different compared to the control, where 9% of the adult mites died (Step-down Cochran-Armitage Test, $\alpha = 0.05$, one-sided greater). At the test concentration of 172 mg test item/kg soil dry weight and above, mortality was statistically significantly increased compared to the control.
Reproduction of the predatory mites exposed to GLOB2112dH was not statistically significantly different compared to the control up to and including the test concentration of 95.4 mg/kg soil dry weight (Williams t-test, $\alpha = 0.05$, one-sided smaller). At the test concentration of 172 mg test item/kg soil dry weight and above, reproduction was statistically significantly decreased compared to the control.
No behavioural abnormalities were observed in any of the treatment groups. The results are shown in the table below.

**Summary of the Effects of GLOB2112dH on the Predatory Mite *Hypoaspis aculeifer* in a 14-day Reproduction Study**

| **GLOB2112dH [mg/kg soil dry weight]** | **Control** | **5.05** | **9.08** | **16.4** | **29.4** | **53.0** | **95.4** | **172** | **309** |
|---|---|---|---|---|---|---|---|---|---|
| Mortality (day 14) [%] | 9 | 5 | 8 | 15 | 15 | 10 | 5 | 90 | 100 |
| Statistical significance[1)] | - | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | * | * |
| No. of juveniles (day 14) | 246 | 246 | 217 | 216 | 230 | 234 | 224 | 165 | 120 |
| Reproduction in [%] of control (day 14) | - | 100 | 88 | 88 | 94 | 95 | 91 | 67 | 49 |
| Statistical significance [2)] | - | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | * | * |

| **Endpoints [mg/kg soil dry weight]** | | | |
|---|---|---|---|
| NOEC (mortality) | 95.4 | | |
| LOEC (mortality) | 172 | | |
| $LC_{50}$ (mortality) [3)] | 128.0 (95% confidence limits 115.9 – 141.3) | | |
| NOEC (reproduction) | 95.4 | | |
| LOEC (reproduction) | 172 | | |
| EC Values (reproduction) [4)] | $EC_{10}$ = 105.6 | $EC_{20}$ = 152.2 | $EC_{50}$ = 306.0 |
| 95% confidence limits | 83.6 – 130.1 | 129.9 – 175.7 | 290.3 – 323.8 |

n.s. = not significantly different compared to the control * = significantly different compared to the control
[1)] Step-Down Cochran-Armitage Test, $\alpha = 0.05$, one-sided greater
[2)] Williams t-test, $\alpha = 0.05$, one-sided smaller
[3)] Moving Averages Computation
[4)] 3-param. normal CDF
- not applicable

The reference item Dimethoate showed statistically significant treatment related effects on reproduction at a concentration of 2.23 mg a.i./kg soil dry weight and above. The $EC_{50}$ for reproduction was 3.25 mg a.i./kg soil dry weight.

**Conclusion**

GLOB2112dH caused no statistically significant effects on mortality and reproduction of *Hypoaspis aculeifer* up to and including the test concentration of 95.4 mg test item/kg soil dry weight. At 172 mg test item/kg soil dry weight and above, mortality was statistically significantly increased and reproduction was statistically significantly decreased compared to the control. Therefore, the overall No Observed Effect Concentration (NOEC) was determined to be 95.4 mg test item/kg soil dry weight. The overall Lowest Observed Effect Concentration (LOEC) was determined to be 172 mg test item/kg soil dry weight.
The $LC_{50}$ for *Hypoaspis aculeifer* in artificial soil was determined to be 128.0 mg test item/kg soil dry weight. The $EC_{10}$ was determined to be 105.6 mg test item/kg soil dry weight, $EC_{20}$ was determined to be 152.2 mg test item/kg soil dry weight and the $EC_{50}$ was determined to be 306.0 mg test item/kg soil dry weight.

| | |
|---|---|
| Comments of zRMS: | The study has been assessed and accepted in dRR Callisto, 2020 (Finalization date).<br><br>$EC_{10}$ = 134 mg pm/kg dw soil |

| | |
|---|---|
| Reference: | KCP 10.4.2.1/01 |
| Report | Dickinson RA, (2015) R169649 - Collembola (*Folsomia candida*) Reproduction Test in Soil, Report Number ENV-14-015. Agrochemex Ltd., Aldhams research station, Manningtree, Essex, CO11 2NF, United Kingdom. (Syngenta File No. CA3511_10011) |
| Guideline(s): | OECD Guideline for Testing of Chemicals, Method 232 (adopted 7 September 2009): Collembolan reproduction test in soil. |
| Deviations: | No |

| | |
|---|---|
| GLP: | Yes |
| Acceptability: | Acceptable with minor deviations |
| Duplication (if vertebrate study) | No |

## Materials and methods

| | |
|---|---|
| **Test Material** | R169649 (MNBA; CA3511) |
| **Lot/Batch #:** | 454319 |
| **Actual content of active ingredients:** | 99.9% |
| **Description:** | Off-white powder |
| **Stability of test compound:** | Stable under standard conditions |
| **Reanalysis/Expiry date:** | 30 June 2017 |
| **Treatments** | |
| **Test rates:** | 17.2, 30.9, 55.6, 100, 180, 324, 583 and 1050 mg R169649/kg soil dry weight |
| **Control:** | Oven dried sand |
| **Toxic standard:** | Boric acid (Separate study – No.: ENV-13-051, date: February 2014) |
| **Application method:** | R169649 mixed in oven dried sand was mixed into artificial soil prior to introduction of collembolans |
| **Test organisms** | |
| **Species:** | Collembolans *Folsomia candida* (Willem) |
| **Age:** | 9 to 12 days old |
| **Source:** | Bias Labs Ltd., UK |
| **Feeding:** | Approximately 10 mg ground baker's yeast at the start of the test and after 7, 14 and 21 days |
| **Test design** | |
| **Arenas:** | Glass test vessels (60 mL capacity) with lids |
| **Substrate** | Artificial soil comprising 5 % sphagnum peat, 20 % kaolinite clay, 69.77 % quartz sand (> 50 % of the particles between 0.05 mm and 0.2 mm) and 0.23 % calcium carbonate. 30 g wet weight of artificial soil was added to each test vessel. |
| **Replication:** | Treated groups 4, control group 8, plus an additional vessel per treatment for measurement purposes |
| **No./arena:** | 10* |
| **Duration of test:** | 28 days |
| **Environmental test conditions** | |
| **Temperature:** | 16.4 to 21.6 °C |
| **pH of soil:** | Test start: 5.96 to 6.50<br>Test end: 5.63 to 6.21 |
| **Water content of soil:** | Test start: 15.86 to 16.37 % soil moisture content<br>Test end: 13.86 to 15.05 % soil moisture content |
| **Photoperiod:** | 16 hours light and 8 hours dark at 715 to 720 Lux |

**During the extraction process it was noted that some of the test vessels contained more than 10 organisms. This was considered most likely due to an addition error on Day 0, and was not considered significant or to have affected the integrity of the study.*

### Study Design and Methods

Experimental dates: 24 November 2014 to 22 December 2014

The highest test concentration was prepared by weighing 630.2 mg of the test item and making up to 30.0597 g with oven dried sand. This was mixed thoroughly and serially diluted with oven dried sand to prepare the lower test concentrations. Aliquots of the respective treated sand were thoroughly mixed with artificial soil at 25 % of the WHC, and distilled water was added to achieve a final nominal water content of 50 % of WHC. The control was treated with oven dried sand only.

Nominally ten juvenile collembolans were transferred after the application to the substrate surface of each test vessel using a pooter. Four and eight replicates were used for each test item treatment and control group, respectively (+ one replicate per treatment not loaded with collembolans for measurement purposes). The test organisms were fed four times during the experiment (at the start of the test and after 7, 14 and 21 days) with approximately 10 mg of ground baker's yeast per test vessel. Four weeks after introducing the test organisms, the surviving parental collembolans and offspring (juveniles) were counted.

All values presented throughout this report were calculated using the original raw data and were not based on rounded values.
The percentage mortality of the springtails was calculated for each treatment, both before and after correction for any control treatment losses using Abbott's formula (1925), modified by Schneider-Orelli (1947). The 28-day mortality data for the individual test-item treatments were compared to those for the control using Fisher Exact /Bonferroni-Holm Test ($\alpha = 0.05$). The $LC_{50}$ was determined by nonlinear regression analysis. The percentage reduction in reproductive performance in the test item treatment groups, compared to the control group, was calculated.

For the fecundity assessment, the data from the test-item treatments were compared to the control data using Wilcoxon/Bonferroni Adjustment Test ($\alpha = 0.05$). The results were used to determine the NOEC with respect to reproduction. The median effect concentration ($EC_{50}$) and also values for the $EC_{20}$ and $EC_{10}$ were determined by nonlinear regression analysis.

## Results and Discussion

Mortality and fecundity are summarised in the table below.

**Table A 23: Effects of residues of R169649 on mortality and reproduction of *Folsomia candida***

| Endpoint | Treatment group (mg R169649/kg soil d.w.) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Control** | **17.2** | **30.9** | **55.6** | **100** | **180** | **324** | **583** | **1050** |
| **% Mortality of parental collembolans after 4 weeks[a]** | 11 | 3 | 3 | 0 | 0 | 18 | 23 | 33* | 40* |
| **% corrected mortality[b]** | - | -10 | -10 | -13 | -13 | 7 | 13 | 24 | 32 |
| **Mean number of juveniles after 4 weeks[c]** | 323 | 267 | 400 | 505 | 466 | 264 | 86* | 88* | 59* |
| **Standard deviation** | 96.8 | 54.3 | 189.1 | 61.9 | 117.7 | 145.9 | 9.1 | 24.8 | 18.5 |
| **CV (%)** | 30.0 | 20.4 | 47.3 | 12.3 | 25.2 | 55.2 | 10.7 | 28.1 | 31.2 |
| **% reduction compared to control[d]** | - | 17 | -24 | -56 | -44 | 18 | 74 | 73 | 82 |
| **NOEC (mortality)** | 324 | | | | | | | | |
| **NOEC (reproduction)** | 180 | | | | | | | | |
| **LOEC (mortality)** | 583 | | | | | | | | |
| **LOEC (reproduction)** | 324 | | | | | | | | |
| **$LC_{50}$** | > 1050 | | | | | | | | |
| **$EC_{10}$** | 134 (95 % confidence limits: n.d. and 225) | | | | | | | | |
| **$EC_{20}$** | 163 (95 % confidence limits: n.d. and 274) | | | | | | | | |
| **$EC_{50}$** | 237 (95 % confidence limits: 133 and 423) | | | | | | | | |

[a] Mortality amongst springtails originally introduced. Individual treatments compared to the control data using Fisher Exact/Bonferroni-Holm Test ($\alpha = 0.05$), and an asterisk indicates where there was a significant difference.
[b] Derived using Abbott's formula (Abbott, 1925), modified by Schneider-Orelli (Schneider-Orelli, 1947)
[c] Fecundity data were compared to the control data using Wilcoxon/Bonferroni Adjustment Test ($\alpha = 0.05$). Treatments marked with an asterisk (*) differed significantly from the control.
[d] A negative value indicates an increase in reproduction relative to the control and a positive value indicates a decrease
d.w.: dry weight

n.d.: could not be determined

**Validity Criteria**

The validity criteria for the control group were met:

- Control treatment mortality was 11 % (must be < 20 %)
- The mean number of juveniles recorded in the control treatment was 323 (must be > 100 per replicate)
- The coefficient of variation of reproduction in the control was 30 % (must not be > 30 %)

**Conclusion**

The toxicity of R169649 to the reproduction and the parental mortality of collembola species *Folsomia candida* were determined. The NOECs for survival and reproduction were determined to be 324 and 180 mg R169649/kg soil dry weight, respectively. The $EC_{50}$ for number of juvenile collembolans was determined to be 237 mg R169649/kg soil dry weight.

| | |
|---|---|
| Comments of zRMS: | The study has been assessed and accepted in dRR Callisto, 2020 (Finalization date).<br><br>$NOEC_{mortality\ and\ reproduction}$ = 1050 mg pm/kg dw soil. |

| | |
|---|---|
| Reference: | KCP 10.4.2.1/02 |
| Report | Ramsden C, (2015), R169649 – Predatory Mite (*Hypoaspis (Geolaelaps) aculeifer*) Reproduction Test in Soil, Report Number ENV-14-012. AgroChemex Environmental Ltd., Aldhams Farm Research Station, Dead Lane, Manningtree, Essex, CO11 2NF, United Kingdom. (Syngenta file No. CA3511_10010) |
| Guideline(s): | OECD (2008). OECD Guideline for Testing of Chemicals, Section 2 – Effects on Biotic Systems, Method 226 (adopted 3 October 2008): Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil. |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Acceptable with minor deviations |
| Duplication (if vertebrate study) | No |

**Materials and methods**

| | |
|---|---|
| **Test Material** | R169649<br>NMSBA ( = MNBA) |
| **Lot/Batch #:** | 454319 |
| **Purity:** | 99.9 % ± 0.5 % w/w |
| **Description:** | Off white powder |
| **Stability of test compound:** | Stable under test conditions |
| **Reanalysis/Expiry date:** | 30 June 2017 |
| **Density:** | Not applicable |
| **Treatments** | |
| **Test rates:** | 17.2, 30.9, 55.6, 100, 180, 324, 583 and 1050 mg R169649/kg soil dry weight |
| **Control:** | Oven dried sand |
| **Toxic standard:** | Boric acid (separate study ENV-14-017; November 2014) |
| **Test organisms** | |
| **Species** | *Hypoaspis aculeifer* |
| **Source:** | Obtained from Bias Labs Ltd., UK |

| | |
|---|---|
| **Food:** | Cheese mites, *Tyrophagus putrescentiae* , three times per week, *ad libitum* |
| **Age at test start:** | 28 – 35 days |
| **Test design** | |
| **Vessels:** | Glass test vessels (volume: 60 mL; inner diameter: 38 mm) fitted with a 53 µm plastic mesh, and screw tops with a hole approximately 10 mm in diameter. Each vessel was filled with approximately 20 g soil d.w. |
| **Substrate:** | Artificial soil comprising 5% sphagnum peat, 20 % kaolinite clay, 74.77% quartz sand (with > 50% of particles between 50 – 200 µm) and 0.23% calcium carbonate |
| **Replication:** | Control group: 8 (+3 temperature surrogates)<br>Treated group: 4 (+1 surrogate per concentration) |
| **No. of mites/arena :** | 10 |
| **Duration of test:** | 14 days |
| **Environmental test conditions** | |
| **Temperature:** | 17 to 21 °C (mean: 19 °C)* |
| **pH:** | Test start: 5.20 to 5.94<br>Test end: 5.40 to 6.03 |
| **Water content of soil:** | Test start: 16.11 to 16.59 % wet weight of soil<br>Test end: 13.51 to 15.54 % wet weight of soil |
| **Photoperiod:** | 16 h light : 8 h dark, 480 lux |

** The temperature briefly dropped below the intended minimum (i.e. the guideline range of 18 – 22 °C) but there was no apparent effect on control mites and no impact was identified on the outcome or validity of the study.*

**Study Design and Methods**

Experimental dates: 12 November 2014 to 29 November 2014

Adult females of the soil mite *Hypoaspis aculeifer* were exposed to concentrations of R169649 incorporated into the test soil. A 30 g aliquot of the highest test concentration was prepared using exactly weighed amounts of the test item and oven-dried sand in a 60-mL glass jar, which was shaken and inverted repeatedly until well mixed. The lower test item concentrations were prepared by serial dilution with sand, starting with an appropriate volume from the aliquot of the highest concentration. Appropriate amounts of the test concentrations were then mixed with pre-moistened soil, and distilled water added such that a final moisture content value of 50 % WHC was achieved. Adult females were transferred to the test vessels which contained untreated (control) or test item treated artificial soil. Ten adult females were introduced to each test vessel. As a source of food, cheese mites (*Tyrophagus putrescentiae*) were added throughout the test. The test was carried out under controlled light-dark cycle. Fourteen days after introducing the test organisms, the surviving mites and the juveniles of *Hypoaspis aculeifer* were extracted by heat/light extraction. From these data the mortality of the adult females and the reproductive output were calculated.

The mean number of dead adult female mites for each treatment, the mean number of juvenile mites for each treatment, the NOEC, the LOEC, and the $EC_{50}$ at day 14 were determined.
Mortality data were corrected for control mortality according to Abbott (1925) modified by Schneider-Orelli (1947), and statistically analysed using Fisher's Exact/Bonferroni-Holm test ($p = 0.05$). Reproduction data was statistically analysed using a Dunnett Multiple Comparison Test ($p = 0.05$).
The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The software used to perform the statistical analysis was CETIS™, Version 1.8.7.14. The $LC_{50}$ and $EC_{50}$ were not able to be determined by statistical analysis due to the outcome of the study.

**Results and Discussion**

Mortality and fecundity are summarised in the table below.

**Table A 24: Effects of residues of R169649 on mortality and reproduction of *Hypoaspis aculeifer***

| Endpoint | Treatment group (mg R169649/kg soil d.w.) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Control** | **17.2** | **30.9** | **55.6** | **100** | **180** | **324** | **583** | **1050** |
| | **Mortality of adult mites after 14 days** | | | | | | | | |
| **% mortality[a]** | 5.0 | 10.0 | 17.5 | 17.5 | 2.5 | 15.0 | 5.0 | 7.5 | 15.0 |
| **% corrected mortality[b]** | 0.0 | 5.3 | 13.2 | 13.2 | -2.6 | 10.5 | 0.0 | 2.6 | 10.5 |
| | **Number of juveniles after 14 days** | | | | | | | | |
| **Mean no. progeny per replicate[c]** | 115 | 93 | 102 | 103 | 117 | 91 | 109 | 95 | 108 |
| **standard deviation** | 16.7 | 15.4 | 12.2 | 14.5 | 5.7 | 21.5 | 20.4 | 6.4 | 32.8 |
| **% reduction compared to control[d]** | n.a. | 19.2 | 11.2 | 10.3 | -1.8 | 21.4 | 5.8 | 17.7 | 6.0 |

The results represent rounded values calculated from the exact raw data
[a] There were no statistically significant differences compared to the control for mortality (Fisher's Exact/Bonferroni Holm test)
[b]According to Abbott (1925) modified by Schneider-Orelli (1947)
[c] There were no statistically significant differences compared to the control for reproduction (Dunnett Multiple Comparison Test)
[d] A positive value indicates a decrease and a negative an increase in reproduction, relative to the control
d.w.: dry weight

***Validity Criteria***

The validity criteria for the control group were met:

- Mean mortality of adult females: ≤ 20 % (observed: 5.0 %)
- Mean number of juveniles per replicate: ≥ 50 (calculated: 115)
- Coefficient of variation (mean number of juveniles per replicate): ≤ 30 % (calculated: 14.5 %)

**Conclusion**

The effects of R169649 on the mortality and reproductive output of the soil mite species *Hypoaspis aculeifer* were determined during a 14-day test.

The NOEC for mortality and reproduction was determined to be 1050 mg R169649/kg soil dry weight, and the 14-day $EC_{50}$ and $LC_{50}$ could not be determined but were considered to be > 1050 mg R169649/kg soil dry weight, the highest concentration tested.

## A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

No new studies were submitted.

## A 2.5 KCP 10.5 Effects on soil nitrogen transformation

| | |
|---|---|
| Comments of zRMS: | The study was conducted according to OECD guideline 216 and principles of GLP. All the validity criterion are met: the variation between the replicate control samples did not exceed the validity criterion of 15% throughout the study.<br><br>There were no deviations to the study plan.<br><br>The study is considered to be reliable and suitable for the risk assessment. |

| | |
|---|---|
| | The test item had no impact on nitrogen transformation of soil microorganisms when applied at 0.32 mg and 1.62 mg test item/kg soil dry weight treatment. |

| | |
|---|---|
| Reference: | KCP 10.5 |
| Report | GLOB2112dH: Effects on the Activity of the Soil Microflora in the Laboratory (Nitrogen Transformation), Hammesfahr U., 2023, 177011080 |
| Guideline(s): | Yes, OECD 216 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes/No/Supplementary |

**Executive summary**

The purpose of this study was to assess the effects of the test item on the activity (nitrogen transformation) of soil microflora in the laboratory.
The test item had no impact on nitrogen transformation of soil microorganisms when applied at 0.32 mg and 1.62 mg test item/kg soil dry weight treatment.

**Material and Methods**

| | |
|---|---|
| **Test Item:** | GLOB2112dH, Batch No. MAM 107683 |
| **Test System:** | Biologically active agricultural soil: Loamy sand |
| **Test Design:** | Determination of nitrogen-transformation in soil enriched with lucerne meal. Comparison of test item treated soil with a non-treated soil.<br>Three replicates per treatment. $NH_4$-, $NO_2$- and $NO_3$-nitrogen formed in the nitrification process was determined by continuous flow analysis.<br>Sampling scheme: 0, 7, 14 and 28 days after treatment |
| **Test Rates:** | Control<br>0.32 mg GLOB2112dH/kg soil dry weight<br>1.62 mg GLOB2112dH/kg soil dry weight |
| **Endpoints:** | Effects on $NO_3$-nitrogen production after 28 days exposure (soil nitrogen transformation). |
| **Reference Item:** | Effects of sodium chloride were determined at a rate of 16 g/kg dry soil in a separate study (test facility study code: 116527080) once a year. |
| **Test Conditions:** | Moisture: 47% to 52% of maximum water holding capacity ($WHC_{max}$).<br>Temperature: 20°C ± 2°C, in the dark. |
| **Statistics:** | Calculation of mean values per treatment, standard deviation and coefficient of variation. Normality and homogeneity of variances were tested using the R/S-Test ($\alpha = 0.01$) and Levene´s test ($\alpha = 0.01$), respectively and pair-wise comparisons of treated and control values according to Student's t-test ($\alpha = 0.05$) were conducted. |

**Findings**

| | |
|---|---|
| **Nitrogen Transformation - Nitrate Content:** | No adverse effects of the test item on nitrate content in soil were observed on day 28. On day 28 differences to the control were 7.97% and -9.90% in the 0.32 mg and 1.62 mg test item/kg soil dry weight treatment, respectively. |
| **Nitrogen Transformation - Mineral Nitrogen Content:** | The mineral nitrogen contents in soil were within the trigger range of ± 25% set by EPPO and SETAC guidelines on day 28. On day 28 differ- |

ences to the control were 7.80% and -9.81% in the 0.32 mg and 1.62 mg test item/kg soil dry weight treatment, respectively.

**Nitrogen Transformation - Nitrate Formation Rates:** The incremental soil nitrate formation rates did not exceed the trigger range of ± 25% set by OECD guideline No. 216 on the 14 - 28 day determination. Differences to the control were 4.00% and -3.02% in the 0.32 mg and 1.62 mg test item/kg soil dry weight treatment, respectively.

**Validity Criteria:** The variation between the replicate control samples did not exceed the validity criterion of 15% throughout the study.

**Conclusion:** The test item had no impact on nitrogen transformation (nitrate content, mineral nitrogen content and nitrate formation rates) of soil microorganisms when applied at 0.32 mg and 1.62 mg test item/kg soil dry weight treatment.

**Effects of the test item on Nitrogen Transformation in a Loamy Sand Soil**

| **Nitrogen Transformation - $NO_3$ – Nitrogen (mg / kg soil dry weight) Mean Values** | | | | | | |
|---|---|---|---|---|---|---|
| | Control | | 0.32 mg GLOB2112dH/kg soil dw | | 1.62 mg GLOB2112dH/kg soil dw | |
| Sampling | Nitrate-N Content | Replicate Variation[1] | Nitrate-N Content | Deviation[2] | Nitrate-N Content | Deviation[2] |
| Day 0 | 28.897 | 0.53 | 28.551 | -1.20 | 28.180* | -2.48 |
| Day 7 | 19.332 | 5.28 | 20.558 | 6.34 | 15.716* | -18.70 |
| Day 14 | 30.899 | 1.54 | 33.998* | 10.03 | 26.758* | -13.40 |
| Day 28 | 46.666 | 1.54 | 50.385* | 7.97 | 42.046* | -9.90 |
| **Nitrogen Transformation - Mineral Nitrogen[3] (mg / kg soil dry weight) Mean Values** | | | | | | |
| | Control | | 0.32 mg GLOB2112dH/kg soil dw | | 1.62 mg GLOB2112dH/kg soil dw | |
| Sampling | Mineral-N Content | Replicate Variation[1] | Mineral -N Content | Deviation[2] | Mineral -N Content | Deviation[2] |
| Day 0 | 35.782 | 0.59 | 35.501 | -0.79 | 35.290 | -1.37 |
| Day 7 | 20.514 | 5.04 | 21.696 | 5.76 | 16.805* | -18.08 |
| Day 14 | 31.768 | 1.51 | 34.863* | 9.74 | 27.641* | -12.99 |
| Day 28 | 47.295 | 1.54 | 50.983* | 7.80 | 42.653* | -9.81 |
| **Nitrogen Transformation - $NO_3$ – Nitrogen Formation Rate (mg / kg soil dry weight per day)[4]** | | | | | | |
| | Control | | 0.32 mg GLOB2112dH/kg soil dw | | 1.62 mg GLOB2112dH/kg soil dw | |
| Interval[4] | Nitrate-N Formation | | Nitrate-N Formation | Deviation[2] | Nitrate-N Formation | Deviation[2] |
| Day 0 - 7 | -1.366 | | -1.142 | 16.40 | -1.780* | -30.31 |
| Day 7 - 14 | 1.652 | | 1.920* | 16.22 | 1.577 | -4.54 |
| Day 14 - 28 | 1.126 | | 1.171 | 4.00 | 1.092 | -3.02 |

[1] = % variation within control replicates (coefficient of variation. calculated as standard deviation / mean value x 100)
[2] = % difference to control
[3] = mineral nitrogen = sum of nitrite- nitrate- and ammonium-nitrogen
[4] = related to successive intervals between samplings
positive values = stimulatory effect; negative values = inhibitory effect
dw = dry weight
* statistically significantly different from control (Student's t-test; α = 0.05)

## A 2.6 KCP 10.6 Effects on terrestrial non-target higher plants

### A 2.6.1 KCP 10.6.1 Summary of screening data

No new studies were submitted.

### A 2.6.2 KCP 10.6.2 Testing on non-target plants

| | |
|---|---|
| Comments of zRMS: | The study was conducted according to OECD guideline 208 and principles of GLP. All the validity criterion are met:<br>- Seedling emergence in the control: ≥ 70 % (actual 85% - 100%),<br>- mean survival of emerged control seedlings: ≥90 % (actual 100 %),<br>- no visible phytotoxic effects were seen in the control and the plants exhibit only normal variation in growth and morphology for the particular species,<br>- environmental conditions for a particular species were identical and growing media contained the same amount of soil matrix, support media, or substrate from the same source.<br><br>There was no deviation to the study plan.<br><br>The study is considered to be reliable and suitable for the risk assessment.<br><br>$ER_{50}$ > 28 mL/ha |

| | |
|---|---|
| Reference: | KCP 10.6 |
| Report | GLOB2112dH: Effects on terrestrial (non-target) plants : seedling emergence and seedling growth test, Dommes A.B., 2024a, 177011086 |
| Guideline(s): | Yes, OECD 208, 2006 and SANTE/2020/12830 Rev.2 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes/No/Supplementary |

**Executive summary**

The purpose of this study was to determine a dose-dependent effect of the test item on seedling emergence and seedling growth of six non-target plant species representing six plant families. Parameters measured were plant fresh weight, plant height, phytotoxicity, emergence and mortality.
The most sensitive species in terms of fresh weight was *Beta vulgaris* with an $ER_{50}$ value of 36.4 mL GLOB2112dH/ha. The most sensitive species in terms of plant height was *Lolium perenne* with an $ER_{50}$ value of 39.6 mL GLOB2112dH/ha. The emergence rate showed no statistically significant reduction for any species tested. Mortality was observed for *Lactuca sativa* at 28.0 and 84.0 mL GLOB2112dH/ha (15 and 5 %), for *Lolium perenne* and *Allium cepa* at 84.0 and 9.33 mL GLOB2112dH/ha, respectively (each 6 %), and for *Beta vulgaris* at 28.0 and 84.0 mL GLOB2112dH/ha (20 and 71 %). The mortality observed for *Beta vulgaris* was statistically significant. The most sensitive species in terms of phytotoxicity was *Lolium perenne* with an $ER_{50}$ value of 66.8 mL GLOB2112dH/ha.

**Materials and methods**

Test Item: GLOB2112dH; Batch-No.: MAM 107683; authenticated content of a.s.:

391.9 g/L Mesotrione, 77.47 g/L Thiencarbazone-methyl, and 117.5 g/L Cyprosulfamide (safener) (nominal: 375 g/L Mesotrione, 75 g/L Thiencarbazone-methyl, and 112 g/L Cyprosulfamide (safener)).

Test Species and Rates: Six species, four dicotyledonous and two monocotyledonous species, representing six plant families, were tested with the following concentrations beside a control with deionised water:

**Plant Species and tested Rates**

| Species | Rate [mL test item/ha] | | | | | |
|---|---|---|---|---|---|---|
| | 0.346 | 1.04 | 3.11 | 9.33 | 28.0 | 84.0 |
| *Brassica napus* | x | x | x | x | x | |
| *Lactuca sativa* | | x | x | x | x | x |
| *Cucumis sativus* | | x | x | x | x | x |
| *Beta vulgaris* | | x | x | x | x | x |
| *Lolium perenne* | | x | x | x | x | x |
| *Allium cepa* | | x | x | x | x | x |

Test Design: On the day after sowing different rates of the test item were sprayed in 100 L/ha of deionised water onto the soil. At least 20 seeds were tested per rate and species. The exposure time was 14 or 21 days after 50% emergence in the respective control (DAE) depending on the growth of the seedlings. The concentration of the active ingredient in the stock solution was verified analytically.

Endpoints: $ER_{10}$, $ER_{20}$, $ER_{50}$, NOER and LOER based on fresh weight and plant height.
$ER_{10}$, $ER_{20}$, $ER_{50}$ based on phytotoxicity.
Observation of emergence and mortality.

Test Conditions: The study was performed in a growth chamber. Exposure conditions were as follows:
Mean temperature was 19.7 °C (16.4 °C to 22.3 °C). Mean humidity was 62 % (51 % to 78 %). Photoperiod: 16 hours light / 8 hours dark. Mean light intensity during the day was 331 µE/m²/s (300 to 400 µE/m²/s).

Statistics: Fresh weight and plant height data were tested for normal distribution, homogeneity of variance and linear or quadratic trends using the Shapiro-Wilk's test ($\alpha = 0.01$), the Levene's test ($\alpha = 0.01$) and a trend analysis of contrasts ($\alpha = 0.05$). If the data were normally distributed and homogeneous the Dunnett's Multiple t-test Procedure (one-sided smaller, $\alpha = 0.05$) or if the data showed a monotonic dose response the Williams Multiple Sequential t-test Procedure (one-sided smaller, $\alpha = 0.05$) was used for comparing treatment groups and control. If the data were normally distributed but not homogeneous the Multiple Sequentially-rejective Welch-t-test after Bonferroni-Holm (one-sided smaller, $\alpha = 0.05$) was used. If the data were neither normally distributed nor homogeneous, the Multiple Sequentially-rejective Median (2x2-Table) Test after Bonferroni-Holm (one-sided smaller, $\alpha = 0.05$) was used.
In order to determine the $ER_{10}$, $ER_{20}$ and $ER_{50}$ values for fresh weight and plant height a regression analysis was performed (Probit-, Logit- or Weibull-Analysis). In the case that no significant dose response relation on the mean values for each treatment group was found ($p(F) > 0.05$) the regression analysis was performed using all replicates for fitting.
For the mortality and emergence data Fisher's Exact Binomial Test with Bonferroni Correction (one-sided smaller, $\alpha = 0.05$), Chi² 2x2 Table Test with Bonferroni Correction (one-sided greater, $\alpha = 0.05$), or Step-down Cochran-Armitage Test Procedure (one-sided greater, $\alpha = 0.05$) was used.
The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH.
In order to determine the $ER_{10}$, $ER_{20}$ and $ER_{50}$ values on phytotoxicity data, a regression analysis was performed (Probit- or Weibull-Analysis). The soft-

ware used to perform this statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

**Results**

All validity criteria were met:

| | |
|---|---|
| Emergence Rate of the Control Seeds: | Should be at least 70%. Was 85-100%. |
| Mean Survival of Emerged Control Seedlings: | Should be at least 90%. Was 100%.. |
| Growth and Morphology of the Control Plants: | The control seedlings exhibited no visible phytotoxic effects and the plants exhibited only normal variation in growth and morphology for that particular species. |
| Environmental Conditions: | Environmental conditions for a particular species were identical and growing media contained the same amount of soil matrix, support media, or substrate from the same source. |

The analytical recovery rate of the active ingredient Mesotrione in the stock solution was 91% of the nominal value (mean, n = 2). The analytical recovery rate of the active ingredient Thiencarbazone-methyl in the stock solution was 93% of the nominal value (mean, n = 2). The analytical recovery rate of the safener Cyprosulfamide in the stock solution was 94% of the nominal value (mean, n = 2).

The most sensitive species in terms of fresh weight was *Beta vulgaris* with an $ER_{50}$ value of 36.4 mL GLOB2112dH/ha ($ER_{20}$ value of 5.32 mL GLOB2112dH/ha). It was followed by *Allium cepa, Lolium perenne*, and *Lactuca sativa* with $ER_{50}$ values of 43.7, 43.9 and 48.9 mL GLOB2112dH/ha, respectively ($ER_{20}$ values of 18.7, 24.0 and 17.2 mL GLOB2112dH/ha, respectively). The next sensitive species was *Cucumis sativus*, for which an $ER_{50}$ value of 139 mL GLOB2112dH/ha was extrapolated ($ER_{20}$ value of 66.5 mL GLOB2112dH/ha). The least sensitive species was *Brassica napus*, which showed no statistically significant fresh weight reduction up to and including the highest tested rate of 28.0 mL GLOB2112dH/ha.

**Summary of effect rates (based on fresh weight)**

| | NOER | LOER | Statistical Analysis | | $ER_{10}$ | $ER_{20}$ | $ER_{50}$ | Statistical Analysis |
|---|---|---|---|---|---|---|---|---|
| | [mL test item/ha] | | | | [mL test item/ha] | | | |
| *Brassica napus* | ≥ 28.0 | > 28.0 | [1] | | >28.0§ | >28.0§ | >28.0§ | |
| *Lactuca sativa* | 28.0 | 84.0 | [1] | | 10.0 | 17.2 | 48.9 | [2] |
| | | | | lower 95%-cl | 2.88 | 7.10 | 27.4 | |
| | | | | upper 95%-cl | 34.5 | 41.7 | 87.5 | |
| | | | | $r^2$ = 0.848 | | | | |
| *Cucumis sativus* | 28.0 | 84.0 | [3] | | 43.2 | 66.5 | 139* | [4] |
| | | | | lower 95%-cl | 39.8 | 64.0 | 129 | |
| | | | | upper 95%-cl | 47.0 | 69.1 | 150 | |
| | | | | $r^2$ = 0.995 | | | | |
| *Beta vulgaris* # | 1.04 | 3.11 | [5] | | 1.49 | 5.32 | 36.4 | [6] |
| | | | | lower 95%-cl | 0.359 | 2.19 | 20.3 | |
| | | | | upper 95%-cl | 6.17 | 12.9 | 65.5 | |
| | | | | $r^2$ = 0.301 | | | | |
| *Lolium perenne* | 28.0 | 84.0 | [5] | | 17.6 | 24.0 | 43.9 | [2] |
| | | | | lower 95%-cl | 16.0 | 22.4 | 41.8 | |
| | | | | upper 95%-cl | 19.3 | 25.8 | 46.1 | |
| | | | | $r^2$ = 0.998 | | | | |
| *Allium cepa* | 28.0 | 84.0 | [5] | | 12.0 | 18.7 | 43.7 | [2] |
| | | | | lower 95%-cl | 7.58 | 13.3 | 35.2 | |
| | | | | upper 95%-cl | 19.1 | 26.3 | 54.3 | |
| | | | | $r^2$ = 0.969 | | | | |

results represent rounded values based on exact data
n.d. not determined due to mathematical reasons
* the $ER_x$-value is extrapolated
§ estimated value
# the $ER_x$-values are calculated on each replicate per rate
[1] Multiple Sequentially-rejective Welsh-t-test after Bonferroni-Holm, α = 0.05, one-sided smaller
[2] Probit Analysis, cl = confidence limits
[3] Multiple Sequentially-rejective Median (2x2-Table) Test after Bonferroni-Holm, α = 0.05, one-sided smaller
[4] Logit Analysis, cl = confidence limits
[5] Williams Multiple Sequential t-test Procedure, α = 0.05, one-sided smaller
[6] Weibull Analysis, cl = confidence limits

The most sensitive species in terms of plant height was *Lolium perenne* with an $ER_{50}$ value of 39.6 mL GLOB2112dH/ha ($ER_{20}$ value of 16.9 mL GLOB2112dH/ha). It was followed by *Allium cepa, Beta vulgaris*, and *Lactuca sativa* with $ER_{50}$ values of 78.8, 108 (extrapolated), and 113 (extrapolated) mL GLOB2112dH/ha, respectively ($ER_{20}$ values of 25.8, 13.0, and 33.0 mL GLOB2112dH/ha, respectively). For *Cucumis sativus* no $ER_{50}$ value could be determined ($ER_{10}$ value of 12.0 mL GLOB21112dH/ha). The least sensitive species was *Brassica napus*, which showed no statistically significant plant height reduction up to and including the highest tested rate of 28.0 mL GLOB2112dH/ha.

**Summary of effect rates (based on plant height)**

| | NOER [mL test item/ha] | LOER [mL test item/ha] | Statistical Analysis | | $ER_{10}$ [mL test item/ha] | $ER_{20}$ [mL test item/ha] | $ER_{50}$ [mL test item/ha] | Statistical Analysis |
|---|---|---|---|---|---|---|---|---|
| *Brassica napus* | ≥28.0 | >28.0 | [1] | | >28.0§ | >28.0§ | >28.0§ | |
| *Lactuca sativa* | 28.0 | 84.0 | [2] | | 7.00 | 21.2 | 113* | [3] |
| | | | | lower 95%-cl | 1.82 | 10.0 | 52.1 | |
| | | | | upper 95%-cl | 13.9 | 44.2 | 638 | |
| | | | | $r^2$ = 0.937 | | | | |
| *Cucumis sativus* | 3.11 | 9.33 | [4] | | 12.0 | >84.0§ | >84.0§ | [3] |
| | | | | lower 95%-cl | 4.64 | n.d. | n.d. | |
| | | | | upper 95%-cl | 31.1 | n.d. | n.d. | |
| | | | | $r^2$ = 0.899 | | | | |
| *Beta vulgaris* # | <1.04 | 1.04 | [2] | | 3.21 | 13.0 | 108* | [3] |
| | | | | lower 95%-cl | 0.968 | 6.69 | 46.7 | |
| | | | | upper 95%-cl | 10.7 | 25.4 | 250 | |
| | | | | $r^2$ = 0.296 | | | | |
| *Lolium perenne* | 9.33 | 28.0 | [4] | | 10.9 | 16.9 | 39.6 | [5] |
| | | | | lower 95%-cl | 4.91 | 9.43 | 27.3 | |
| | | | | upper 95%-cl | 24.1 | 30.5 | 57.6 | |
| | | | | $r^2$ = 0.915 | | | | |
| *Allium cepa* | 9.33 | 28.0 | [4] | | 14.4 | 25.8 | 78.8 | [5] |
| | | | | lower 95%-cl | 6.70 | 15.5 | 52.4 | |
| | | | | upper 95%-cl | 31.0 | 42.9 | 118 | |
| | | | | $r^2$ = 0.923 | | | | |

results represent rounded values based on exact data
n.d. not determined due to mathematical reasons
* the $ER_x$-value is extrapolated
§ estimated value
# the $ER_x$-values are calculated on each replicate per rate
[1] Dunnett's Multiple t-test Procedure, α = 0.05, one-sided smaller
[2] Multiple Sequentially-rejective Welsh-t-test after Bonferroni-Holm, α = 0.05, one-sided smaller
[3] Weibull Analysis, cl = confidence limits
[4] Williams Multiple Sequential t-test Procedure, α = 0.05, one-sided smaller
[5] Probit Analysis, cl = confidence limits

The emergence rate showed no statistically significant reduction for any species tested.
Mortality was observed for *Lactuca sativa* at 28.0 and 84.0 mL GLOB2112dH/ha (15 and 5 %), for *Lolium perenne* and *Allium cepa* at 84.0 and 9.33 mL GLOB2112dH/ha, respectively (each 6 %), and for *Beta vulgaris* at 28.0 and 84.0 mL GLOB2112dH/ha (20 and 71 %). The mortality observed for *Beta vulgaris* was statistically significant.
The values for the phytotoxicity data (visual injury) used for the $ER_x$ calculation are a rating system and are not measured. The most sensitive species in terms of phytotoxicity was *Lolium perenne* with an $ER_{50}$ value of 66.8 mL GLOB2112dH/ha ($ER_{20}$ value of 37.3 mL GLOB2112dH/ha). It was followed by *Lactuca sativa* with an $ER_{50}$ value of 84.6 mL GLOB2112dH/ha (extrapolated, $ER_{20}$ value of 38.5 mL GLOB2112dH/ha). For the other tested species, no $ER_x$ values could be calculated. Phytotoxic effects observed were chlorosis (all species), deformation (all species except *Lactuca sativa*), necrosis (*Cucumis sativus*, *Beta vulgaris*, and *Lolium perenne*), and discoloration in *Lolium perenne*.

**Summary of effect rates (based on phytotoxicity (visual injury))**

| | | $ER_{10}$ | $ER_{20}$ | $ER_{50}$ | Statistical Analysis |
|---|---|---|---|---|---|
| | | [mL test item/ha] | | | |
| *Brassica napus* | | >28.0§ | >28.0§ | >28.0§ | |
| *Lactuca sativa* | | 22.8 | 38.5 | 84.6* | [1] |
| | lower 95%-cl | 0.00 | 0.045 | 41.1 | |
| | upper 95%-cl | 45.3 | 76.6 | 34844 | |
| *Cucumis sativus* | | n.d. | n.d. | n.d. | |
| *Beta vulgaris* | | n.d. | n.d. | n.d. | |
| *Lolium perenne* | | 27.5 | 37.3 | 66.8 | [2] |
| | lower 95%-cl | n.d. | n.d. | n.d. | |
| | upper 95%-cl | n.d. | n.d. | n.d. | |
| *Allium cepa* | | n.d. | n.d. | >84.0§ | |

results represent rounded values based on exact data
n.d. not determined due to mathematical reasons
§ estimated value
* the $ER_x$- value is extrapolated
[1] Weibull Analysis, cl = confidence limits
[2] Probit Analysis, cl = confidence limits

## Conclusion

GLOB2112dH was tested for effects on seedling emergence and seedling growth of six plant species out of six different plant families.
The most sensitive species in terms of fresh weight was *Beta vulgaris* with an $ER_{50}$ value of 36.4 mL GLOB2112dH/ha ($ER_{20}$ value of 5.32 mL GLOB2112dH/ha).
The most sensitive species in terms of plant height was *Lolium perenne* with an $ER_{50}$ value of 39.6 mL GLOB2112dH/ha ($ER_{20}$ value of 16.9 mL GLOB2112dH/ha).
The emergence rate showed no statistically significant reduction for any species tested.
Mortality was observed for *Lactuca sativa* at 28.0 and 84.0 mL GLOB2112dH/ha (15 and 5 %), for *Lolium perenne* and *Allium cepa* at 84.0 and 9.33 mL GLOB2112dH/ha, respectively (each 6 %), and for *Beta vulgaris* at 28.0 and 84.0 mL GLOB2112dH/ha (20 and 71 %). The mortality observed for *Beta vulgaris* was statistically significant.
The most sensitive species in terms of phytotoxicity was *Lolium perenne* with an $ER_{50}$ value of 66.8 mL GLOB2112dH/ha ($ER_{20}$ value of 37.3 mL GLOB2112dH/ha). Phytotoxic effects observed were chlorosis (all species), deformation (all species except *Lactuca sativa*), necrosis (*Cucumis sativus*, *Beta vulgaris*, and *Lolium perenne*), and discoloration in *Lolium perenne*.

| | |
|---|---|
| Comments of zRMS: | The study was conducted according to OECD guideline 227 and principles of GLP.<br>All the validity criterion are met:<br>- Seedling emergence: ≥ 70 % (actual 81 - 100 %).<br>- For control group: mean plant survival for the duration of the study: ≥ 90 % (actual 100 %),<br>- The control plants exhibited no visible phytotoxic effects and the plants exhibited only normal variation in growth and morphology for that particular species<br>- Environmental conditions for a particular species were identical and the growing media contained the same amount of soil matrix, support media, or substrate from the same source,.<br><br>The following deviation from the study plan was noted:<br>According to Study Plan: *Allium cepa*: 4 plants per pot at application<br>Deviation to the Study Plan: 5 plants were found at 7 DAA assessment in pot 1 of treatment rate 66.7 mL test item/ha. |

| | |
|---|---|
| | Reason for the Deviation:<br>Miscounted while thinning before application or emerged later.<br>Presumed Effect on the Study:<br>None. The plants were small, so no shading in the spray application or overcrowding within the first week after application is probable. At day 7, one plant from the respective pot was randomly chosen and removed.<br><br>The study is considered to be reliable and suitable for the risk assessment.<br><br>$ER_{50}$ = 17.1 mL/ha |

| | |
|---|---|
| Reference: | KCP 10.6 |
| Report | GLOB2112dH: Effects on terrestrial (non-target) plants : vegetative vigour test, Dommes A.B., 2024b, 177011087 |
| Guideline(s): | Yes, OECD 227, 2006 and SANTE/2020/12830 Rev.2 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes/No/Supplementary |

**Executive summary**

The purpose of this study was to determine a dose-dependent effect of the test item on the vegetative vigour of six non-target plant species representing six plant families. Parameters measured were plant fresh weight, plant height, phytotoxicity and mortality.
The most sensitive species in terms of fresh weight were *Helianthus annuus* and *Solanum lycopersicum* with ER50 values of 17.1 and 29.2 mL GLOB2112dH/ha, respectively. The most sensitive species in terms of plant height was *Solanum lycopersicum* with an $ER_{50}$ value of 24.6 mL GLOB2112dH/ha. No statistically significant mortality was observed for any species tested. Phytotoxic effects observed were chlorosis (all species), necrosis (all species except *Allium cepa*), and deformation (all species except *Brassica oleracea* and *Allium cepa*). Additionally, discoloration was observed in *Brassica oleracea*, *Cucumis sativus*, and *Lolium perenne*. The most sensitive species in terms of phytotoxicity was *Solanum lycopersicum* with an $ER_{50}$ value of 21.8 mL GLOB2112dH/ha.

**Materials and methods**

| | |
|---|---|
| Test Item: | GLOB2112dH; batch no.: MAM 107683;<br>content of a.s.: 391.9 g/L Mesotrione, 77.47 g/L Thiencarbazone-methyl, and 117.5 g/L Cyprosulfamide (safener) (nominal: 375 g/L Mesotrione and 75 g/L g/L Thiencarbazone-methyl, and 112 g/L Cyprosulfamide (safener)). |
| Test Species and Rates: | Six plant species from six different plant families were tested. Based on a non GLP range finding test following rates beside a control with deionised water were tested: |

**Plant species and tested rates**

| Species | Rate [mL test item/ha] | | | | | |
|---|---|---|---|---|---|---|
| | 0.823 | 2.47 | 7.41 | 22.2 | 66.7 | 200 |
| *Brassica oleracea* | | x | x | x | x | x |
| *Cucumis sativus* | | x | x | x | x | x |
| *Solanum lycopersicum* | | x | x | x | x | x |
| *Helianthus annuus* | x | x | x | x | x | x |
| *Lolium perenne* | | x | x | x | x | x |

| | | | | | |
|---|---|---|---|---|---|
| *Allium cepa* | **x** | **x** | **x** | **x** | **x** |

| | |
|---|---|
| Test Design: | The plants were grown until they had reached the 2 to 4 true leaf stage prior to dosing. Test rates were calculated for a water amount 100 L/ha and were administered onto the plants using laboratory spraying equipment. At least 20 plants were tested per rate and species. The concentration of the active ingredients and the safener in the stock solution was verified analytically. The exposure time was 21 days. |
| Endpoints: | $ER_{10}$, $ER_{20}$, $ER_{50}$ and NOER and LOER based on plant fresh weight and plant height; $ER_{10}$, $ER_{20}$, $ER_{50}$ based on phytotoxicity; Observation of mortality. |
| Test Conditions: | The study was performed in growth chambers.<br>Pre-application conditions were as follows:<br>Mean temperature was 20.4 °C (17.4 °C to 23.8 °C). Mean humidity was 60 % (45 % to 75 %). Photoperiod: 16 hours light / 8 hours dark. Mean light intensity during the day was 317 µE/m²/s (300 to 360 µE/m²/s).<br>Exposure conditions were as follows:<br>Mean temperature was 21.4 °C (19.9 °C to 23.0 °C). Mean humidity was 60 % (53 % to 75 %). Photoperiod: 16 hours light / 8 hours dark. Mean light intensity during the day was 336 µE/m²/s (300 to 400 µE/m²/s). |
| Statistics: | Plant fresh weight data and plant height data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk's test ($\alpha = 0.01$) and the Levene's test ($\alpha = 0.01$). If the data were normally distributed, homogeneous, and showed a monotonic dose response the Williams Multiple Sequential t-test Procedure (one-sided smaller, $\alpha = 0.05$) was used for comparing treatment groups and control. If the data were normally distributed and not homogeneous the Multiple Sequentially-rejective Welch-t-test after Bonferroni-Holm (one-sided smaller, $\alpha = 0.05$) was used. If the data were not normally distributed and not homogeneous the Multiple Sequentially-rejective Median (2x2-Table) Test after Bonferroni-Holm (one-sided smaller, $\alpha = 0.05$) was used for comparing treatment groups and control.<br>In order to determine the $ER_{10}$, $ER_{20}$ and $ER_{50}$ values, a regression analysis was performed (Probit, Logit, or Weibull Analysis).<br>For the mortality data the Fisher's Exact Binomial Test with Bonferroni Correction (one-sided greater, $\alpha = 0.05$) was used.<br>The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH.<br>In order to determine the $ER_{10}$, $ER_{20}$ and $ER_{50}$ values on phytotoxicity data, a regression analysis was performed (Probit-, Logit-, and Weibull Analysis). The software used to perform this statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH. |

**Results**

All validity criteria of the study were met:

| | |
|---|---|
| Emergence Rate of the Seeds: | Should be at least 70% for each plant species. Was 81-100%. |
| Mean Survival of Control Plants: | Should be at least 90%. Was 100%. |
| Growth and Morphology of the Control Plants: | The control plants exhibited no visible phytotoxic effects and the plants exhibited only normal variation in growth and morphology for that particular species. |
| Environmental Conditions: | Environmental conditions for a particular species were identical and the growing media contained the same amount of soil matrix, support media, or substrate from the same source. |

The analytical recovery rate of the active ingredient Mesotrione in the stock solution was 96% of the nominal value. The analytical recovery rate of the active ingredient Thiencarbazone-methyl in the stock solution was 88% of the nominal value. The analytical recovery rate of the safener Cyprosulfamide in the stock solution was 89% of the nominal value. In the control solution no test item ingredients were detected.

The most sensitive species in terms of fresh weight were *Helianthus annuus* and *Solanum lycopersicum* with $ER_{50}$ values of 17.1 and 29.2 mL GLOB2112dH/ha, respectively ($ER_{20}$ values of 5.89 and 16.5 mL GLOB2112dH/ha, respectively). They were followed by *Lolium perenne* and *Brassica oleracea* with $ER_{50}$ values of 69.3 and 102 mL GLOB2112dH/ha, respectively ($ER_{20}$ values of 29.7 and 24.5 mL GLOB2112dH/ha, respectively). The least sensitive species were *Allium cepa* and *Cucumis sativus* with $ER_{50}$ values of 131 and 132 mL GLOB2112dH/ha, respectively ($ER_{20}$ values of 60.5 and 51.8 mL GLOB2112dH/ha, respectively).

**Summary of effect rates (based on plant fresh weight)**

| | NOER | LOER | Statistical Analysis | | $ER_{10}$ | $ER_{20}$ | $ER_{50}$ | Statistical Analysis |
|---|---|---|---|---|---|---|---|---|
| | [mL test item/ha] | | | | [mL test item/ha] | | | |
| *Brassica oleracea* | < 2.47 | 2.47 | [1] | | 11.6 | 24.5 | 102 | [2] |
| | | | | lower 95%-cl | 3.84 | 11.7 | 72.8 | |
| | | | | upper 95%-cl | 20.5 | 37.2 | 158 | |
| | | | | $r^2$ = 0.963 | | | | |
| *Cucumis sativus* | 22.2 | 66.7 | [3] | | 27.9 | 51.8 | 132 | [4] |
| | | | | lower 95%-cl | 4.24 | 15.4 | 88.8 | |
| | | | | upper 95%-cl | 51.6 | 79.9 | 189 | |
| | | | | $r^2$ = 0.923 | | | | |
| *Solanum lycopersicum* | 7.41 | 22.2 | [5] | | 11.4 | 16.5 | 29.2 | [4] |
| | | | | lower 95%-cl | 3.78 | 8.76 | 23.2 | |
| | | | | upper 95%-cl | 15.9 | 21.1 | 43.6 | |
| | | | | $r^2$ = 0.911 | | | | |
| *Helianthus annuus* | 0.823 | 2.47 | [5] | | 2.91 | 5.89 | 17.1 | [4] |
| | | | | lower 95%-cl | 1.84 | 4.33 | 14.7 | |
| | | | | upper 95%-cl | 4.02 | 7.37 | 19.7 | |
| | | | | $r^2$ = 0.982 | | | | |
| *Lolium perenne* | 66.7 | 200 | [1] | | 16.9 | 29.7 | 69.3 | [4] |
| | | | | lower 95%-cl | 6.23 | 15.5 | 53.1 | |
| | | | | upper 95%-cl | 26.7 | 41.0 | 90.0 | |
| | | | | $r^2$ = 0.955 | | | | |
| *Allium cepa* | 22.2 | 66.7 | [3] | | 40.5 | 60.5 | 131 | [2] |
| | | | | lower 95%-cl | 5.73 | 16.1 | 84.8 | |
| | | | | upper 95%-cl | 68.0 | 91.1 | 218 | |
| | | | | $r^2$ = 0.896 | | | | |

results represent rounded values based on exact data
[1] Multiple Sequentially-rejective Welsh-t-test after Bonferroni-Holm, α = 0.05, one-sided smaller
[2] Probit Analysis, cl = confidence limits
[3] Williams Multiple Sequential t-test Procedure, α = 0.05, one-sided smaller
[4] Weibull Analysis, cl = confidence limits
[5] Multiple Sequentially-rejective Median (2x2-Table) Test after Bonferroni-Holm, α = 0.05, one-sided smaller

The most sensitive species in terms of plant height was *Solanum lycopersicum* with an $ER_{50}$ value of 24.6 mL GLOB2112dH/ha ($ER_{20}$ value of 10.9 mL GLOB2112dH/ha). This was followed by *Helianthus annuus*, *Cucumis sativus* and *Lolium perenne* with $ER_{50}$ values of 38.3, 85.5, and 142 mL GLOB2112dH/ha, respectively (ER20 values of 8.29, 30.9, and 15.0 mL GLOB2112dH/ha, respectively). They were followed by *Allium cepa* for the $ER_{50}$ was estimated to be > 200 mL/ha ($ER_{20}$ value of 126 mL GLOB2112dH/ha). The least sensitive species was *Brassica oleracea* for which no reduction of plant height was observed.

**Summary of effect rates (based on plant height)**

| | NOER [mL test item/ha] | LOER [mL test item/ha] | Statistical Analysis | | $ER_{10}$ [mL test item/ha] | $ER_{20}$ [mL test item/ha] | $ER_{50}$ [mL test item/ha] | Statistical Analysis |
|---|---|---|---|---|---|---|---|---|
| *Brassica oleracea* | ≥ 200 | > 200 | [1] | | > 200[§] | > 200[§] | > 200[§] | |
| *Cucumis sativus* | 22.2 | 66.7 | [1] | | 15.8 | 30.9 | 85.5 | [2] |
| | | | | lower 95%-cl | 2.26 | 8.54 | 52.6 | |
| | | | | upper 95%-cl | 31.3 | 50.8 | 128 | |
| | | | | $r^2$ = 0.929 | | | | |
| *Solanum lycopersi-cum* | 2.47 | 7.41 | [1] | | 6.80 | 10.9 | 24.6 | [3] |
| | | | | lower 95%-cl | 3.71 | 7.17 | 20.0 | |
| | | | | upper 95%-cl | 9.60 | 14.1 | 30.3 | |
| | | | | $r^2$ = 0.973 | | | | |
| *Helianthus annuus* | 2.47 | 7.41 | [1] | | 3.73 | 8.29 | 38.3 | [4] |
| | | | | lower 95%-cl | 0.591 | 2.30 | 22.3 | |
| | | | | upper 95%-cl | 8.29 | 15.4 | 69.6 | |
| | | | | $r^2$ = 0.910 | | | | |
| *Lolium perenne* | 7.41 | 22.2 | [5] | | 4.62 | 15.0 | 142 | [4] |
| | | | | lower 95%-cl | 1.70 | 8.08 | 98.6 | |
| | | | | upper 95%-cl | 8.46 | 22.6 | 240 | |
| | | | | $r^2$ = 0.979 | | | | |
| *Allium cepa* | 22.2 | 66.7 | [5] | | 43.4 | 126 | > 200[§] | [4] |
| | | | | lower 95%-cl | 18.5 | 87.6 | n.d. | |
| | | | | upper 95%-cl | 66.0 | 189 | n.d. | |
| | | | | $r^2$ = 0.949 | | | | |

results represent rounded values based on exact data
n.d. not determined due to mathematical reasons
[§] estimated value
[1] Multiple Sequentially-rejective Welsh-t-test after Bonferroni-Holm, α = 0.05, one-sided smaller
[2] Weibull Analysis, cl = confidence limits
[3] Logit Analysis, cl = confidence limits
[4] Probit Analysis, cl = confidence limits
[5] Williams Multiple Sequential t-test Procedure, α = 0.05, one-sided smaller

No statistically significant mortality was observed for any species tested.
Phytotoxic effects observed were chlorosis (all species), necrosis (all species except *Allium cepa*), and deformation (all species except *Brassica oleracea* and *Allium cepa*). Additionally, discoloration was observed in *Brassica oleracea*, *Cucumis sativus*, and *Lolium perenne*.
The values for the phytotoxicity data used for the ERx calculation are a rating system and are not measured. The most sensitive species in terms of phytotoxicity was *Solanum lycopersicum* with an $ER_{50}$ value of 21.8 mL GLOB2112dH/ha ($ER_{20}$ value of 12.9 mL GLOB2112dH/ha). This was followed by *Helianthus annuus* and *Cucumis sativus* with $ER_{50}$ values of 32.1 and 36.8 mL GLOB2112dH/ha, respectively ($ER_{20}$ values of 18.0 and 16.2 mL GLOB2112dH/ha, respectively). They were followed by *Lolium perenne* and *Brassica oleracea* with $ER_{50}$ values of 64.1 and 77.8 mL GLOB2112dH/ha, respectively ($ER_{20}$ values of 30.5 and 35.4 mL GLOB2112dH/ha, respectively). The least sensitive species in terms of phytotoxicity was *Allium cepa* for which a calculation of ERx values was not possible on account of no or only very small effects.

**Summary of effect rates (based on phytotoxicity)**

| | | $ER_{10}$ | $ER_{20}$ | $ER_{50}$ | Statistical Analysis |
|---|---|---|---|---|---|
| | | [mL test item/ha] | | | |
| *Brassica oleracea* | | 23.4 | 35.4 | 77.8 | [1] |
| | lower 95%-cl | 2.84 | 8.07 | 39.9 | |
| | upper 95%-cl | 44. 3 | 63.2 | 186 | |
| *Cucumis sativus* | | 10.0 | 16.2 | 36.8 | [2] |
| | lower 95%-cl | 2.12 | 5.36 | 20.6 | |
| | upper 95%-cl | 18.4 | 27.3 | 67.8 | |
| *Solanum lycopersi-cum* | | 9.12 | 12.6 | 21.8 | [2] |
| | lower 95%-cl | 2.17 | 4.53 | 13.1 | |
| | upper 95%-cl | 14.6 | 19.0 | 36.0 | |
| *Helianthus annuus* | | 12.8 | 18.0 | 32.1 | [2] |
| | lower 95%-cl | 3.47 | 7.02 | 19.5 | |
| | upper 95%-cl | 20.7 | 27.6 | 54.2 | |
| *Lolium perenne* | | 18.7 | 30.5 | 64.1 | [3] |
| | lower 95%-cl | 0.657 | 3.03 | 23.6 | |
| | upper 95%-cl | 39.2 | 56.7 | 127 | |
| *Allium cepa* | | n.d. | > 200[§] | > 200[§] | |

results represent rounded values based on exact data
n.d. not determined due to mathematical reasons
[§] estimated value
[1] Probit Analysis, cl = confidence limits
[2] Logit Analysis, cl = confidence limits
[3] Weibull Analysis, cl = confidence limits

**Conclusion**

GLOB2112dH was tested for effects on the vegetative vigour using six plant species out of six different plant families.
The most sensitive species in terms of fresh weight were *Helianthus annuus* and *Solanum lycopersicum* with $ER_{50}$ values of 17.1 and 29.2 mL GLOB2112dH/ha, respectively ($ER_{20}$ values of 5.89 and 16.5 mL GLOB2112dH/ha, respectively).
The most sensitive species in terms of plant height was *Solanum lycopersicum* with an $ER_{50}$ value of 24.6 mL GLOB2112dH/ha ($ER_{20}$ value of 10.9 mL GLOB2112dH/ha).
No statistically significant mortality was observed for any species tested.
Phytotoxic effects observed were chlorosis (all species), necrosis (all species except *Allium cepa*), and deformation (all species except *Brassica oleracea* and *Allium cepa*). Additionally, discoloration was observed in *Brassica oleracea*, *Cucumis sativus*, and *Lolium perenne*.
The most sensitive species in terms of phytotoxicity was *Solanum lycopersicum* with an $ER_{50}$ value of 21.8 mL GLOB2112dH/ha ($ER_{20}$ value of 12.9 mL GLOB2112dH/ha).

## A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants

No new studies were submitted.

## A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

No new studies were submitted.

## A 2.8 KCP 10.8 Monitoring data

No new studies were submitted.