REGISTRATION REPORT

**Part B**

**Section 9**

**Ecotoxicology**

Detailed summary of the risk assessment

Product code: Cymoxanil 33% + Zoxamide 33% WG

Product name(s): **Lieto 66 WG**

Chemical active substances:

Zoxamide, 330 g/kg

Cymoxanil, 330 g/kg

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(product re-registration)

Applicant: Sipcam Oxon S.p.A.

Submission date: 30/12/2020

MS Finalisation date: September 2021

Revision date: December 2021

## DATA PROTECTION CLAIM

Under Article 59 of Regulation 1107/2009/EC, the applicant claims data protection for these studies. The data protection status and corresponding justification as valid for the respective country will be confirmed in the respective PART A.

## STATEMENT FOR OWNERSHIP

The summaries and evaluations contained in this review report may be based on unpublished proprietary data submitted for the purpose of the assessment undertaken by the regulatory authority that prepared it. Other registration authorities should not grant, amend, or renew a registration on the basis of the summaries and evaluation of unpublished proprietary data contained in this review report unless they have received the data on which the summaries and evaluation are based, either –

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# Version history

| When | What |
|---|---|
| 30th December 2020 | Submission of initial Version 0 by the applicant |
| September 2021 | zRMS finalized evaluation |
| December 2021 | Finalization of the RA according to comments received |
| | |

# Table of Contents

# 9 Ecotoxicology (KCP 10)

This document reviews the ecotoxicity on the plant protection product 'Cymoxanil 33% + Zoxamide 33% WG'. The product contains the two active, substances cymoxanil and zoxamide, which are both in the Annex of Regulation (EU) 540/2011 (former Annex I of Directive 91/414/EEC). 'Cymoxanil 33% + Zoxamide 33% WG' is an authorised plant protection product in European countries, for which re-registration has been requested under art. 43 of Reg. (EU) 1107/2009 on behalf of the sponsor Gowan Crop Protection Ltd., UK. The dossier follows the data requirements of

- Regulation (EC) No. 544/2011 for the active substance cymoxanil,
- Regulation (EC) No. 283/2013 for the active substance zoxamide and
- Regulation (EC) No. 284/2013 for the plant protection product 'Cymoxanil 33% + Zoxamide 33% WG'.

This document is for the renewal of the authorisation of the product according to Article 43 of Regulation (EC) No 1107/2009, following the renewal of approval of the active substance zoxamide according to Regulation (EU) 2018/1981 of 13 December 2018.
The aim of this step of the art. 43 process is to update the existing dossier information with regard to and limited to the information on the active substance zoxamide as follows:

- To comply with data requirements or criteria which were not in force when the authorisation of the plant protection product was granted and
- to demonstrate that the product meets the requirements set out in the Regulation on the renewal of the approval of the active substance zoxamide to comply with provisions of article 29 of Regulation (EU) No 1107/2009.

This dossier contains the consolidated version of the previous assessment for the parts which do not require a re-evaluation, including all assessments and data on cymoxanil. The consolidated text has been shaded in grey in the present dRR section. Please note that for product authorization the core document in the central zone also included Romania with a tomato use, since Romania at that time belonged to the S-EU zone.

A full risk assessment according to Uniform Principles is provided which demonstrates that the product is safe for the environment.

Review Comments:

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

As it is re-registration of product 'Cymoxanil 33% + Zoxamide 33% WG' under art. 43 of Reg. (EU) 1107/2009, according SANCO/2010/13170 rev. 14; 7 October 2016; art. 3.11: "*For products containing two or more active substances -and when the 1st substance is renewed- there is no need to evaluate data related to the 2nd substance*." Thus, zRMS evaluate only data for Zoxamide and for formulation. Information for Cymoxanil are taken directly from fRR for 'Cymoxanil 33% + Zoxamide 33% WG' dated January 2012 evaluated by Great Britain, as it was highlighted by Applicant above.

## 9.1 Critical GAP and overall conclusions

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

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Use-No. * | Member state(s) | Crop and/or situation (crop destination / purpose of crop) | F, Fn, Fpn G, Gn, Gpn or I ** | Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group) | **Application** | | | | **Application rate** | | | PHI (days) | Remarks: e.g. g safener/ synergist per ha | **Conclusion** | | | | | | |
| | | | | | Method / Kind | Timing / Growth stage of crop & season | Max. number a) per use b) per crop/ season | Min. interval between applications (days) | kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season | g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season | Water L/ha min/max | | | Birds | Mammals | Aquatic organisms | Bees | Non-target arthro- | Soil organisms | Non-target plants |
| **Zonal uses (field or outdoor uses, certain types of protected crops)** | | | | | | | | | | | | | | | | | | | | |
| 1-12 | PL, NL, HU, DE, AT, BE, RO, SI, CZ, IE, UK, SK | Potatoes | F | late blight | Foliar spraying | BBCH 21-89 April to September | a) 3 b) 3 | 7-10 | a) 0.45 kg/ha b) 1.35 kg/ha | a) 0.1485 kg/ha b) 0.4455 kg/ha | 200-1000 | 7 | | | | | | | | |
| 13-16 | RO, AT, HU, SI | Wine and table grapes | F | downy mildew | Foliar spraying | BBCH 14 till PHI of 28 days March to October | a) 3 b) 3 | 7-10 | a) 0.40-0.45 kg/ha b) 1.35 kg/ha | a) 0.132-0.1485 kg/ha b) 0.4455 kg/ha | 200-1000 | 28 | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | |
| **Interzonal uses (use as seed treatment, in greenhouses (or other closed places of plant production), as post-harvest treatment or for treatment of empty storage rooms)** | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | |
| **Minor uses according to Article 51 (field uses)** | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | |
| **Minor uses according to Article 51 (interzonal uses)** | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | |

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

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

## Explanation for column 15 – 21 "Conclusion"

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

**Remarks table:**

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

Effects of 'Cymoxanil 33% + Zoxamide 33% WG' on birds and mammals were not evaluated as part of the EU reviews of the active substances. Therefore, a risk assessment for 'Cymoxanil 33% + Zoxamide 33% WG' with the proposed use pattern has been provided and is considered adequate.

The assessment of effects on birds and mammals was carried out according to EFSA Guidance on Risk Assessment for Birds and Mammals (EFSA Journal 2009, 7(12):1438) based on EU concluded data for the active substances and data on the formulated product. As a result, an acceptable acute and chronic risk to birds was demonstrated for both active substances as well as for the potentially relevant metabolites of zoxamide and the formulated product 'Cymoxanil 33% + Zoxamide 33% WG'. Secondary poisoning for earthworm and fish-eating birds and via drinking water is not likely.

Information on unacceptable effects to reptiles and amphibians are not available.

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

The aquatic risk assessment was carried out according to the EFSA (2013) aquatic guidance document (EFSA Journal 2013;11(7):3290).

New studies are available for zoxamide and its metabolites, which were either requested by EFSA (2017) during AIR zoxamide or are required according to Regulation (EC) No. 283/2013, respectively. The studies on 'CYMOXANIL 33% + ZOXAMIDE 33% WG' are all available from the product authorisation. A comparison of the results for 'CYMOXANIL 33% + ZOXAMIDE 33% WG' with the results for the active substances revealed no additive or synergistic effects. Instead, the assessment confirms that zoxamide drives the aquatoxicity of the mixture product.

The metabolites of zoxamide are of lower toxicity than the parent active substance.

For zoxamide metabolites, PEC/RAC ratios are below 1 for all aquatic organism when FOCUS SW Step 1+2 PECs are considered, indicating an acceptable risk at a lower tier. However, for the active substance zoxamide FOCUS SW Step 4 PECs are required.
The aquatic risk assessment was carried out according to the EFSA (2013) aquatic guidance document (EFSA Journal 2013;11(7):3290).

New studies are available for zoxamide and its metabolites, which were either requested by EFSA **(2017) during AIR zoxamide or are** required according to Regulation (EC) No. 283/2013, respectively. The studies on 'CYMOXANIL 33% + ZOXAMIDE 33% WG' are all available from the product authorisation. A comparison of the results for 'CYMOXANIL 33% + ZOXAMIDE 33% WG' with the results for the active substances revealed no additive or synergistic effects. Instead, the assessment confirms that zoxamide drives the aquatoxicity of the mixture product.

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- For the intended worst-case GAP uses of zoxamide on potato fields and in vineyards (with **3 x 0.45 kg prod./ha**) the calculated PEC/RAC ratios (lower than 1.0) indicate an acceptable risk for aquatic organisms. However, several scenarios needed refinement. Therefore, further PEC/RAC ratios were calculated based on FOCUS SW Step 4 PECs considering a more realistic half-life of zoxamide on/in plants as well as run-off reducing vegetated buffer zones and drift reducing measures (drift reducing buffer zones and equipment/nozzles), leading to a reduction of the exposure of surface water bodies. As a result, the implementation of the following measures is necessary:
- For potatoes a 10 m non-sprayed, vegetated buffer zone.

For vineyards 20 m non-sprayed, vegetated buffer zone.

FOCUS SW Step 4 calculations with the reduced max. dose in grapes authorised in certain EU zone countries were included for completeness. Also for worst-case GAP uses with 3x 0.4 kg prod./ha in vines a vegetated buffer zone of 20 m to receive a PEC/RAC ratio lower than 1.0 in case that the R4 scenario is applicable.

### 9.1.1.3

### 9.1.1.4 Review Comments:

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

The standard RAs demonstrate that applications of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' according to good agricultural practice are of low risk to the aquatic ecosystem with appropriate mitigation measures.

### 9.1.1.5 Effects on bees (KCP 10.3.1)

The bee risk assessment is based on the EPPO (2010) bee guidance (EPPO Standard PP3/10 (3) Environmental risk assessment scheme for plant protection products. Chapter 10: Honeybees), as updated from the EPPO 2001 guidace, which is referred to in the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002).

For zoxamide, cymoxanil and 'CYMOXANIL 33% + ZOXAMIDE 33% WG' the HQ values for acute oral and contact toxicity to adult honeybees are below the relevant trigger of 50, indicating that no unacceptable risk is expected following the application of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' according to the intended use pattern in the field.

Two honey bee field studies from 2018 and 2019 with additional assessments on colony and brood development are available, which have been performed under southern EU (Spanish) and central EU (German) conditions. As a result, two consecutive foliar applications of 'Cymoxanil 33% + Zoxamide 33% WG' at a rate of each 1.35 kg product/ha (the 3-fold max. single application rate of 0.45 kg product/ha) with an interval of 7 days under field conditions on full-flowering *Phacelia tanacetifolia* B. caused no adverse effects on honeybee behaviour, mortality, foraging activity, colony weight development, colony strength, conditions of the colonies, overall bee brood development as well as detailed brood development over two consecutive investigated brood cycles.

An additional study on the toxicity to honey bee larvae has been carried out with zoxamide.

Additional studies with the formulated product on the chronic adult honey bee and honey bee larvae tox-

icity are available or are submitted with this dossier, but without risk assessment. The EFSA bee guidance document (2014) is not yet voted and therefore not taken into account.

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

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 (Candolfi et al., 2000)[1]. It is based on the hazard quotient (HQ) approach, assessing the risk to non-target arthropods in-field and off-field and considering either the $LR_{50}$ (lethal rate) or the $ER_{50}$ (effect rate) on reproduction

Based on the results of extended laboratory studies with *T. pyri, Aphidius, Chrysoperla* and *Orius* the $PER_{in\text{-}field}$ was below the rate with ≤ 50 % effect for all representative uses. Therefore, also off-field no risk to non-target arthropods is expected from the intended uses of 'CYMOXANIL 33% + ZOXAMIDE 33% WG'. The risk assessment for the off-field area is therefore covered by the risk assessment for the in-field area.

In addition, the aged residue test carried out with the product on *Typhlodromus pyri* (Colli M., 2006; report no. BT031/06) showed no adverse effects at the rate of 2.25 kg product/ha after 7 days, indicating an acceptable potential of re-colonisation/recovery within an ecologically relevant period.

It is therefore possible to conclude that the GAP uses of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' do not pose any risk to non-target arthropods.

The product 'CYMOXANIL 33% + ZOXAMIDE 33% WG' shows in general a low toxicity to non-target arthropods.

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

The risk assessment was conducted according to the 'Guidance document on terrestrial ecotoxicology under Council Directive 91/414/EEC' (Anonymous 2002)[2] and the requirements of Regulation (EC) No. 284/2013 for the plant protection product 'Cymoxanil 33% + Zoxamide 33% WG'.
The risk assessment was conducted according to the Guidance Document on Terrestrial Ecotoxicology (2002).

New data on zoxamide and its metabolites are provided within this submission in order to fulfill requests of EFSA (2017). Moreover, new studies with 'CYMOXANIL 33% + ZOXAMIDE 33% WG' have been performed in order to fulfil the data requirements for plant protection products in accordance with Regulation (EC) No 1107/2009.

All acute and chronic TER values for earthworms and other non-target soil organisms (meso- and macrofauna) exposed to zoxamide and its relevant soil metabolites are greater than the Commission Regulation (EU) No. 546/2011 triggers of 10 and 5 – even if considering worst-case assumptions.

For 'CYMOXANIL 33% + ZOXAMIDE 33% WG', the TER values for the chronic earthworm, *Folsomia* and *Hypoaspis aculeifer* risk assessment are above the trigger of 5 for the grape and potato uses. For these

[1] Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods: from the ESCORT 2 Workshop (European standard characteristics of non-target arthropod regulatory testing): a joint BART, EPPO/CoE, OECD, and IOBC Workshop organised in conjunction with SETAC Europe and EC: held at Wageningen International Conference Centre, Wageningen, the Netherlands : 21-23 March 2000.

[2] Guidance document on terrestrial ecotoxicology under Council Directive 91/414/EEC, SANCO/10329/2002 rev. 2 dated 17th October 2002

calculations, $PEC_{soil,accu}$ values considering single applications were taken into account; multiple applications and long-term PECs are better described by active substance data.

In addition, the overall toxicity of zoxamide and its metabolite and of zoxamide, cymoxanil and their metabolites on earthworms has been assessed in field earthworm studies with ~~Zoxium 240 SC and~~ 'CYMOXANIL 33% + ZOXAMIDE 33% WG'~~, respectively. In these studies,~~ potential effects of field populations of earthworms after spray application of ~~Zoxium 240 SC and~~ 'CYMOXANIL 33% + ZOXAMIDE 33% WG' to bare soils at exaggerated application pattern did not show any statistically significant effects on single species, ecological groups and total earthworm abundance or biomass one year after application (~~Schulz L. 2020, Report No. 18 48 FEW 0001 and~~ Schulz L. 2020, Report No. 19 48 FEW 0003).

Overall, the risk to earthworms and other non-target soil organisms (meso- and macrofauna) is acceptable following use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' according to the proposed use pattern.

The risk of 'Cymoxanil 33% + Zoxamide 33% WG', zoxamide, cymoxanil and their relevant metabolites to soil micro-organisms was evaluated by comparison of no-effect concentrations, derived from laboratory tests, with $PEC_{soil}$ values.

Since the formulated product at a soil concentration higher than expected after the use of 'Cymoxanil 33% + Zoxamide 33% WG' had no effects ≥ 25% on the N transformation processes in soil at the end of a 28-day incubation period in the laboratory, the intended GAP uses of 'Cymoxanil 33% + Zoxamide 33% WG' can be regarded as safe for soil micro-organisms and their function in the field. The effects were below the trigger of 25% given in Commission Regulation (EU) no. 546/2011.

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

The risk assessment was conducted according to the 'Guidance document on terrestrial ecotoxicology The risk assessment was conducted according to the Guidance Document on Terrestrial Ecotoxicology (2002).

During AIR of zoxamide, only screening data on terrestrial vascular plants were considered relevant and sufficient (see RAR zoxamide, 2017). No adverse effects were seen at dose rates up to 500 g a.s./ha. Therefore, no studies on seedling emergence and vegetative vigour were regarded to be required. Moreover, as the active substance is not an herbicide and/or plant growth regulator, Tier I studies examining the effects on seedling emergence and vegetative vigour were not requested.

To assess the effects of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' on terrestrial plants, a vegetative vigour test on different species of non-target-plants was carried out. This study showed that application of 1.35 kg formulation/ha (equivalent to 0.4455 kg a.s./ha) had no significant phytotoxic effects on two mono- and four dicotyledon species of plants. The only significant effect observed was a 16% decrease in dry biomass with *P. sativum*. The $EC_{50}$ was therefore assumed to be higher than 0.4455 kg a.s./ha. Thus, no effects over 50% were observed at an application rate three times higher than the single rate proposed for 'CYMOXANIL 33% + ZOXAMIDE 33% WG'. It is therefore possible to conclude that 'CYMOXANIL 33% + ZOXAMIDE 33% WG' poses no unacceptable risk to terrestrial non-target plants in off-crop areas following the proposed uses.

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

No additional data is available or necessary.

### 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).

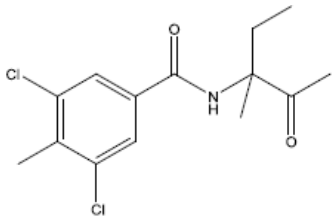
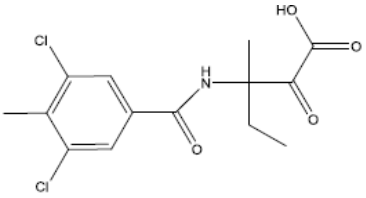
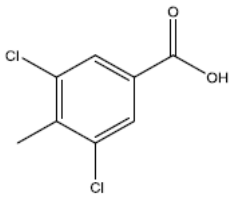
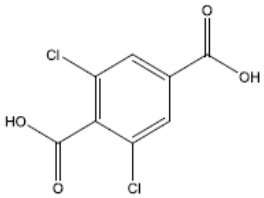
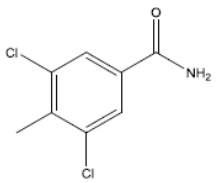
**Table 9.1-2: Critical use pattern of 'Cymoxanil 33% + Zoxamide 33% WG' grouped according to crop group and application pattern**

| Grouping according to crop group and application pattern | | | |
|---|---|---|---|
| **Group** | **Intended uses** | **Relevant use parameters for grouping** | **Crop group and application pattern** |
| Birds and mammals (chapter 9.2 and 9.3) | Wine grapes | Worst-case use pattern per crop group according to EFSA/2009/1438 | Vines;<br>3 × 148.5 g/ha zoxamide + cymoxanil (7-days interval, BBCH 14 till PHI of 28 days) |
| | Potatoes | | Root and tuber crops;<br>3 × 148.5 g/ha zoxamide + cymoxanil (7-days interval, BBCH 21 till PHI of 7 days) |
| Aquatic organisms (chapter 9.5) | Wine grapes | Worst-case use pattern per crop group | Vines;<br>3 × 148.5 g/ha zoxamide + cymoxanil (7-days interval, BBCH 14 till PHI of 28 days) |
| | Potatoes | | Root and tuber crops;<br>3 × 148.5 g/ha zoxamide + cymoxanil (7-days interval, BBCH 21 till PHI of 7 days) |
| Bees (chapter 9.6) | Wine grapes | Worst-case use pattern per crop group | Vines;<br>3 × 148.5 g/ha zoxamide + cymoxanil (7-days interval, BBCH 14 till PHI of 28 days) |
| | Potatoes | | Root and tuber crops;<br>3 × 148.5 g/ha zoxamide + cymoxanil (7-days interval, BBCH 21 till PHI of 7 days) |
| Soil meso- and macrofauna (chapter 9.8) | Wine grapes | Worst-case use pattern per crop group | Vines;<br>3 × 148.5 g/ha zoxamide + cymoxanil (7-days interval, BBCH 14 till PHI of 28 days) |
| Microbial activity (chapter 9.9) | Potatoes | | Root and tuber crops;<br>3 × 148.5 g/ha zoxamide + cymoxanil (7-days interval, BBCH 21 till PHI of 7 days) |
| Arthropods other than bees (chapter 9.7) | Wine grapes | Worst-case use pattern per crop group | Vines;<br>3 × 148.5 g/ha zoxamide + cymoxanil (7-days interval, BBCH 14 till PHI of 28 days) |
| Non-target terrestrial plants (chapter 9.10) | Potatoes | | Root and tuber crops;<br>3 × 148.5 g/ha zoxamide + cymoxanil (7-days interval, BBCH 21 till PHI of 7 days) |

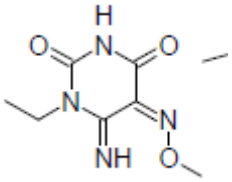
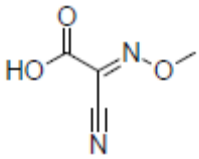
### 9.1.3 Consideration of metabolites

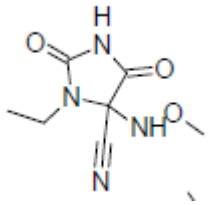
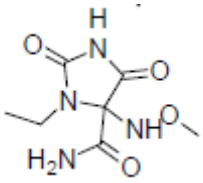
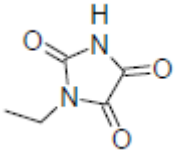
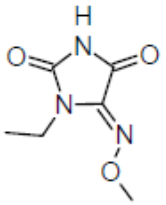
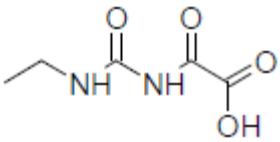
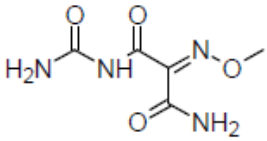
A list of metabolites found in environmental compartments is provided below. The need for conducting a metabolite-specific risk assessment in the context of the evaluation of 'Cymoxanil 33% + Zoxamide 33% WG' is indicated in the table(s).

## Table 9.1-3: Metabolites of zoxamide

| Metabolite | Molar mass | Chemical structure | Maximum observed occurrence in compartments | Risk assessment required? |
|---|---|---|---|---|
| RH-127450 | 302.15 |  | Soil:<br>Max. 15.1% AR after 7 days<br>Water/sediment system:<br>Max. 17.1% AR in surface water (day 28), max. 23.1% AR in sediment, max. 39.3% AR in total system (after incubation at 10°C) | Yes<br>(soil, water/sediment system) |
| RH-163353 | 332.15 |  | Soil:<br>Max. 15% AR after 3 days<br>Water/sediment:<br>Max. 15.8% AR at day 28 in the water phase, max. 7.4% AR at day 106 in the sediment, max. 20.6% AR (day 56) in the total system | Yes<br>(soil, water/sediment system) |
| RH-24549 | 205.0 |  | Soil:<br>Max. 33.8% AR after 7 days<br>Water/sediment:<br>Max. 5% AR (whole system) | Yes<br>(soil, water/sediment system) |
| RH-141455 | 235.02 |  | Soil:<br>Max. 8.4% AR after 14 days<br>Water/sediment:<br>Max. 2.1% AR (whole system) | Yes<br>(soil, water/sediment system) |
| RH-139432 | 204.06 |  | Soil:<br>Max. 4.9% AR after 14 days<br>Surface water:<br>Max. of 21.4% AR (day 28) in surface water of OECD 309 study (max. of 42.4% AR on day 30 in an aquatic photolysis study at pH 4 is regarded as environmentally not relevant). | Yes<br>(water/sediment system) |

## Table 9.1-4: Metabolites of cymoxanil

| Metabolite | Molar mass | Chemical structure | Maximum observed occurrence in compartments | Exposure assessment required due to |
|---|---|---|---|---|
| IN-U3204 | 198.2 |  | Soil: max 24.7% AR by 0.33 day<br>Water/sediment: max in water 24.7% AR after 0.13 d, max in sediment 0.5% AR after 3 d | $PEC_{gw}$: leaching potential to groundwater<br>$PEC_{soil}$: occurrence in soil<br>$PEC_{sw/sed}$: occurrence in surface water |
| IN-W3595 | 128.1 |  | Soil: max 10.1% AR by 1 day<br>Water/sediment: max in water 26.1% AR after 0.25 d, max in sediment 2.3% AR after 1 d | $PEC_{gw}$: leaching potential to groundwater<br>$PEC_{soil}$: occurrence in soil<br>$PEC_{sw/sed}$: occurrence in surface water |

| Metabolite | Molar mass | Chemical structure | Maximum observed occurrence in compartments | Exposure assessment required due to |
|---|---|---|---|---|
| IN-JX915 | 198.2 |  | Soil: 10.9% AR (n=1)<br>Water/sediment: max in water 7.2% AR after 1 d, max in sediment 1.2% AR after 1 d | $PEC_{gw}$: leaching potential to groundwater<br>$PEC_{soil}$: occurrence in soil<br>$PEC_{sw/sed}$: occurrence in surface water |
| IN-KQ960 | 216.9 |  | Groundwater: max 6.3% AR by 3 days<br>Water/sediment: max in water 13.0% AR after 1 d, max in sediment 5.5% AR after 30 d | $PEC_{gw}$: leaching potential to groundwater<br>$PEC_{sw/sed}$: occurrence in surface water |
| IN-TA226 | 142.1 |  | Water/sediment: max in water 11.1% AR after 3 d, max in sediment 1.0% AR after 8 d | $PEC_{sw/sed}$: occurrence in surface water |
| IN-R3273 | 171.2 |  | Water/sediment: max in water 5.0% AR after 3 d, max in sediment 0.5% AR after 3 d | $PEC_{sw/sed}$: occurrence in surface water |
| IN-KP533 | 160.1 |  | Water/sediment: max in water 20.5% AR after 10 d, max in sediment 6.5% AR after 1 d | $PEC_{sw/sed}$: occurrence in surface water |
| M5 | 198.2 |  | Water/sediment: max in water 22.9% AR after 1 d, max in sediment 0.0% AR | $PEC_{sw/sed}$: occurrence in surface water |

## 9.2 Effects on birds (KCP 10.1.1)

The risk assessment for birds was performed in accordance with the current "Guidance document on risk assessment for birds & mammals on request from EFSA" (EFSA Journal 2009; 7(12): 1438).

### 9.2.1 Toxicity data

Effects of ‘Cymoxanil 33% + Zoxamide 33% WG’ on birds were not evaluated as part of the EU assessment of active substances.

Avian toxicity studies have been carried out with zoxamide and cymoxanil. Full details of these studies are provided in the EU review dossiers, for zoxamide in the RAR (2017) and the EFSA Peer Review

Conclusion (2017) and for cymoxanil in the DAR (2005) and its addenda (2007, 2013) as well as the EFSA Scientific Report of Cymoxanil (2008) Peer Review Conclusion (2013).

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

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

| Species | Substance | Exposure system | Results | Reference |
|---|---|---|---|---|
| **Zoxamide** | | | | |
| Bobwhite quail<br>*Colinus virginianus* | zoxamide | Oral<br>1 d<br>Acute | **$LD_{50}$ > 2000 mg a.s./kg bw/d** | EFSA (2017) |
| Bobwhite quail<br>*Colinus virginianus* | zoxamide | Dietary<br>8 d<br>Short-term | $LDD_{50}$ = 1889.3 mg a.s./kg bw/d | EFSA (2017) |
| Mallard duck<br>*Anas plathyrhynchos* | zoxamide | Dietary<br>8 d<br>Short-term | $LDD_{50}$ = 1597.7 mg a.s./kg bw/d | EFSA (2017) |
| Mallard duck<br>*Anas plathyrhynchos* | zoxamide | Dietary<br>22 weeks<br>Reproduction | **NOEL = 122.8 mg a.s./kg bw/d** | EFSA (2017) |
| Bobwhite quail<br>*Colinus virginianus* | zoxamide | Dietary<br>22 weeks<br>Reproduction | NOEL = 170.9 mg a.s./kg bw/d | EFSA (2017) |

**Bold letters:** endpoints used for the risk assessment

The EU review concluded the data reported in the following table for the a.s. cymoxanil should be considered as relevant for Member States when applying the Uniform Principles.

**Table 9.2-2: EU Endpoints: Toxicity of Cymoxanil to birds**

| Study | EU agreed endpoints (EFSA Scientific Report of Cymoxanil (2008)) |
|---|---|
| Acute toxicity | $LD_{50}$ > 2000 mg a.s./kg bw |
| Long-term toxicity | NOAEL = 14.9 mg a.s./kg bw /day |

### 9.2.1.1 Justification for new endpoints

All endpoints are in agreement with EU review data.

## 9.2.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the EFSA (2009) birds and mammals guidance document (EFSA Journal 2009; 7(12): 1438). The major route of exposure is by feeding on e.g. contaminated vegetation, small insects or earthworms.

According to the EFSA (2009) guidance document a short-term risk assessment is not necessary for spray applications due to the poor distinction between short and long-term exposure. The risk assessment for short-term periods is covered by the long-term risk assessment.

**Toxicity of active substances *vs.* formulation**

Data on the toxicity to avian species are only available for the active substances. Accordingly, the risk assessment was based on these data, which were considered sufficient.

*Acute Toxicity*

*Acute Toxicity*

It was considered appropriate to perform the risk assessment separately for Cymoxanil and Zoxamide using the endpoints given above. Nevertheless, according to the conservative approach indicated by EFSA Guidance Document, the acute risk of a formulated products containing more than one active ingredient should be evaluated assuming additive properties of the actives. The acute risk to birds for the formulated product 'CYMOXANIL 33% + ZOXAMIDE 33% WG' was assessed considering the intended uses summarized in Table 9.2-1 and 9.2-2, default values from the guidance document and a "surrogate" $LD_{50}$ for the formulated product.
As result, a $TER_A$ above the trigger of 10 was already derived at screening level, demonstrating an acceptable acute risk for birds after application of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' according to the intended GAP uses. The $TER_A$ calculation is summarised in Table 9.2-3.

**Table 9.2-3: Acute risk to birds for 'CYMOXANIL 33% + ZOXAMIDE 33% WG'**

| Crop * | Indicator species | "Surrogate" $LD_{50}$ (mg prod./kg bw) | Daily Dietary Dose (mg prod./kg bw) | TER | Trigger |
|---|---|---|---|---|---|
| Potato (Central EU); | Small omnivorous bird | 2000 | 103.76 | 19.3 | *10* |
| Grape | Small omnivorous bird | 2000 | 53.7 | 37.2 | *10* |

* Worst-case scenarios

Moreover, based on acute testing of the formulated product in mammals (please refer to Ferguson et al., 1999, report no. 99R-102 with a $LD_{50}$ oral, rat of 1469 mg/kg bw) it can be concluded that the formulated product is of low toxicity. Thus, it is not expected that the mixture of the active substances in 'CYMOXANIL 33% + ZOXAMIDE 33% WG' results in a higher toxicity compared to the toxicity of the single compounds. Therefore, a surrogate acute $LD_{50(mix)}$ was calculated assuming dose additivity. The calculation was performed in line with the recommendations of the EFSA (2009) guidance document [3]. A "surrogate" $LD_{50}$ for the total load of actives was calculated using the formula:

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

where:

X (a.s.$_i$) = fraction of the active substance [$i$] in the mixture (the sum of fraction (a.s.$_i$) must be 1)
$LD_{50}$ (a.s.$_i$) = acute toxicity for the active substance [$i$]

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

Since the formulated product contains the same amount of the two active ingredients, the fraction X (a.s.$_i$) for Cymoxanil and Zoxamide is 0.5 each. Thus, a total load is calculated. Using the above ecotoxicological endpoints in the formula, the "surrogate" $LD_{50}$ results:

$$LD_{50\,(mix)} = \left(\frac{0.5}{2000} + \frac{0.5}{2000}\right)^{-1} = \left(0.00025 + 0.00025\right)^{-1} = 2000 \text{ mg a.s./kg b.w.}$$

$LD_{50(mix)}$ was calculated at >2 000 mg a.s./kg b.w. for acute toxicity, which is in the same range as the $LD_{50}$ values for the single substances.

As a result, the risk assessment has been performed based on the active substance toxicity data.

*Reproductive Toxicity*

The combined risk of the two active ingredients was assessed assuming additive toxicity of cymoxanil and zoxamide. The formula ∑ trigger/TER

was used; if the sum is < 1, acceptable risk can be concluded.

The results of the screening assessment of zoxamide and of Tier 1 assessment for cymoxanil, reported below at point 9.2.2.1, were used for this calculation. Following a risk-envelope approach, the worst-case scenario for each crop was considered.

| | Potato<br>Omnivorous bird "lark" | | Grape<br>Frugivorous bird "starling" | |
|---|---|---|---|---|
| | **TER** | **trigger/TER** | **TER** | **trigger/TER** |
| Zoxamide | 12.04 | 0.415 | 20.7 | 0.242 |
| Cymoxanil | 5.8 | 0.862 | 5.5 | 0.909 |
| **COMBINED RISK** | | **1.277** | | **1.151** |

Since the sum of trigger/TER values is >1, further consideration is necessary. The Tier-1 risk assessment reported below for cymoxanil comes from the Registration Report of UK, where a worst-case use pattern was assessed (potato: 6 applications with 5-day interval; grape: 5 applications with 7-day interval). As a consequence, the MAF values used for Tier-1 reproductive assessment were 2.99 and 2.4 for potato and grape respectively. However, this dRR supports an intended use of 3 applications with 7-day interval, which gives a default MAF of 2.0. If this MAF is used in the Tier-1 assessment of cymoxanil, the following TER values are obtained:

| **Scenario** | **Appl. rate** | **Shortcut value** | **$F_{twa}$** | **MAF** | **DDD** | **NOEL** | **TER** |
|---|---|---|---|---|---|---|---|
| Potato<br>Omnivorous bird "lark" | 0.1485 | 10.9 | 0.53 | **2** | 1.7 | 14.9 | **8.7** |
| Grape<br>Frugivorous bird "starling" | 0.1485 | 14.4 | 0.53 | **2** | 2.3 | 14.9 | **6.6** |

Using these TER values in the combined risk assessment, the sum of trigger/ER values is lower than 1 for both scenarios, indicated acceptable risk for birds.

| | Potato<br>Omnivorous bird "lark" | | Grape<br>Frugivorous bird "starling" | |
|---|---|---|---|---|
| | **TER** | **trigger/TER** | **TER** | **trigger/TER** |

| | | | | |
|---|---|---|---|---|
| Zoxamide | 12.04 | 0.415 | 20.7 | 0.242 |
| Cymoxanil | 8.7 | 0.575 | 6.6 | 0.758 |
| **COMBINED RISK** | | **0.990** | | **0.999** |

It is to be noted that this combined assessment is quite conservative, because for the a.s. zoxamide the results of the screening assessment were used. If a Tier-1 assessment was performed for zoxamide, the results of the combined risk assessment would be even more favourable.

**Relevance of metabolites**

Metabolites of zoxamide

Metabolites further considered in the birds and mammals risk assessment were identified based on the results of the plant metabolism studies and for the metabolites regarded potentially relevant for the ecotox risk assessment (see table 9.1-3).

In the metabolism studies (see DAR 2017 Volume 3-CA B.7.2) conducted on grapes, tomatoes, cucumbers and peas all metabolites found generally occurred at levels <10% TRR. The major residue observed in all studies was always the parent compound.
Only one exception was found for tomatoes: In tomato green and red fruits RH-141452 occurred at 15% and 11.2 % AR, respectively.
In the potato metabolism study (Volume 3-CA B.7.2.1.2) no parent zoxamide was found in potato tubers. Instead, the main residues in potato tubers comprised RH-141452 and RH-141455.

Both RH-141452 and RH-141455 are rat metabolites found at low levels in urine (Swenson, R.E., Frederick, C.B., Graves, D.D. 1998a, Report No: 94R-235, ER Ref No: 24.1). RH-141452 (designated M-17 in the rat metabolism study) was isolated from rat urine by acid/base extraction, and identified by HPLC and TLC by comparison with an authentic reference standard. Analysis by LC-MS gave a molecular weight consistent with RH-141452. The identity was confirmed by derivatisation (methylation with diazomethane) followed by GC/MSD analysis. RH-141452 (M-17) was estimated to account for 0.37% of the administered dose in the low dose female group. RH-141455 was not detected by this method.

Both RH-141452 and RH-141455 were, however, found in rat urine using a non-radiolabelled residue method. Rat urine was diluted with water, acidified and extracted with ethyl acetate to separate the acidic components. After concentration and derivatization (methylation) with diazomethane, the metabolites were identified by GC/MSD and quantified using GC-ECD. Using this method, RH-141455 was found at 0.006% of dose in males and 0.004% of dose in females. RH-141452 was found at higher levels of 0.037% of dose in males and 0.034% of dose in females.
Metabolism of zoxamide to RH-141452 and RH-141455 has the effect of increasing the polarity of the residue and thereby increasing water solubility, which facilitates excretion. From the structures of RH-141452 and RH-141455, which are small molecules containing aromatic carboxylic acids, it would be predicted that these compounds would be readily excreted largely unchanged. Rat metabolism studies have been performed with both RH-141452 and RH-141455, which confirm this expectation (Wu, D., Gu, Z., 1998a, Report No: 97RC-154, ER Ref No: 27.1 and Wu, D., Gu, Z. 1998b, Report No: 98RC-017, ER Ref No: 27.2).

Following oral administration of RH-141452 to rats, the majority of the RH-141452 was eliminated unchanged through urine, accounting for >94% of the administered dose. Three minor conjugates, M-2, M-3 (glucuronide conjugates), and M-4 (glycine conjugate) were also found in the urine samples, accounting for ~3% of the administered dose. An additional 1.6% of the administered radioactivity was excreted in the faeces as the parent chemical.

Following oral administration of RH-141455 to rats, greater than 96% of radioactivity excreted in faeces (73%) and urine (11%) was identified to be unchanged parent. Some very minor metabolites were also observed in urine samples but were not identified due to their extremely low percentage of dose.

The hydrolysis of zoxamide and the subsequent oxidation steps to form RH-141452 and RH-141455 are regarded as detoxification reactions and therefore, both metabolites would be expected to be less toxic than parent zoxamide. In acute oral toxicity studies in male and female mice, the acute oral $LD_{50}$ of RH-141452 and RH-141455 in male and female mice were both > 5000 mg/kg bw.

Genotoxicity testing of both metabolites revealed no harmful effects.

In addition, a comparison of the toxicological profile of zoxamide and two metabolites, RH-141452 and RH-141455 has been made using OECD QSAR Toolbox version 3.4.0.17 (Pellizzaro, M. and Da Silva, M., 2017; see RAR (2017)). This analysis also indicates that both metabolites are expected to have a lower toxicity than the parent zoxamide.

Taking into account that RH-141452

- is not genotoxic,
- showed an acute oral toxicity of $LC_{50}$ > 5000 mg/kg bw/d (see EFSA, 2017), which is above 2000 mg/kg bw/d, the dose for classification and
- is predicted to have a similar toxicity as RH-141455 (which is regarded as toxicologically non relevant)

this metabolite can also be regarded as toxicologically not relevant without a further proof (i.e. without further repeated dose toxicity studies).

During AIR, the metabolites RH-127450 and RH-24549 were both identified as being relevant for the soil and aquatic risk assessments. RH-127450 and RH-24549 together with the parent compound have log Pow values > 3 and have the potential to bioaccumulate. Therefore, an assessment of secondary poisoning is required for these metabolites and zoxamide.

Metabolites of cymoxanil

The risk of plant, soil and water metabolites of the active substances is covered by the risk assessments for the parent compounds and the product 'CYMOXANIL 33% + ZOXAMIDE 33% WG'.

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

The results of the acute and reproductive first-tier (screening) risk assessment are summarised in the following tables.

Zoxamide

**Table 9.2-2: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in wine grapes**

| **Intended use** | | **Wine grapes** | | | |
|---|---|---|---|---|---|
| **Active substance/product** | | **zoxamide** | | | |
| **Application rate (g/ha)** | | 3 × 148.5 | | | |
| **Acute toxicity (mg/kg bw)** | | > 2000 | | | |
| **TER criterion** | | 10 | | | |
| **Crop scenario** | **Indicator/generic focal species** | **$SV_{90}$** | **$MAF_{90}$** | **$DDD_{90}$** | **$TER_a$** |

| | | | | | |
|---|---|---|---|---|---|
| **Growth stage** | | | | **(mg/kg bw/d)** | |
| Vineyard | Small omnivorous bird | 95.3 | 1.6 | 22.64 | 88.34 |
| **Reprod. toxicity (mg/kg bw/d)** | 122.8 | | | | |
| **TER criterion** | 5 | | | | |
| **Crop scenario Growth stage** | **Indicator/generic focal species** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$ (mg/kg bw/d)** | **$TER_{lt}$** |
| Vineyard | Small omnivorous bird | 38.9 | 2.0 × 0.53 | 6.12 | 20.07 |

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

**Table 9.2-3: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in potatoes**

| **Intended use** | **Potatoes** | | | | |
|---|---|---|---|---|---|
| **Active substance/product** | **zoxamide** | | | | |
| **Application rate (g/ha)** | 3 × 148.5 | | | | |
| **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)** | **$TER_a$** |
| ~~Fruiting vegetables~~ Potatoes | Small omnivorous bird | 158.8 | 1.6 | 37.73 | 53.01 |
| **Reprod. toxicity (mg/kg bw/d)** | 122.8 | | | | |
| **TER criterion** | 5 | | | | |
| **Crop scenario Growth stage** | **Indicator/generic focal species** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$ (mg/kg bw/d)** | **$TER_{lt}$** |
| ~~Fruiting vegetables~~ Potatoes | Small omnivorous bird | 64.8 | 2.0 × 0.53 | 10.20 | 12.04 |

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

The screening risk calculations show an acceptable acute and long-term risk for birds from the use of zoxamide applied as 'CYMOXANIL 33% + ZOXAMIDE 33% WG' according to the proposed use pattern. A higher tier assessment is not necessary.

No evidence of any reproducible endocrine effects is expected for zoxamide (see EFSA Peer Review Conclusion for zoxamide, 2017).

Cymoxanil

According to EFSA guidance document, the Daily Dietary Doses (DDD) for reproductive exposure is calculated according to the formula:

$$DDD_{multiple\ applications} = \text{application rate [kg /ha]} \times \text{shortcut value} \times F_{twa} \times MAF_{mean}$$

As a first step, the maximum application rate (0.1485 kg a.s./ha), and the default shortcut, MAF, and TWA (0.53) values were used in the calculations. Since the application rate is the same for the two active ingredients, the exposures calculated for screening and Tier-1 assessments are applicable to both Cy-

moxanil and Zoxamide. The Daily Dietary Doses, calculated for screening and Tier 1 assessments, are reported in the below Tables:

To assess the long-term risk for the a.s. Cymoxanil, a screening assessment was performed as a first step; the results are reported in the table below.

**Table 9.2-4: Screening assessment of reproductive risk – a.s. Cymoxanil**

| Crop | Indicator species | Application rate (kg a.s./ha) | Shortcut value | $F_{twa}$ | $MAF_{mean}$ | DDD (mg a.s./kg bw/d) |
|---|---|---|---|---|---|---|
| Potato | Small omnivorous bird | 0.1485 | 64.8 | 0.53 | 2.99* | 15.2 |
| Grape | Small omnivorous bird | 0.1485 | 38.9 | 0.53 | 2.4** | 7.3 |

* 6 applications with 5-day interval; ** 5 applications with 7-day interval

| Crop | Indicator species | Toxicity endpoint (mg a.s./kg bw/d) | DDD (mg a.s./kg bw/d) | TER |
|---|---|---|---|---|
| Potato | Small omnivorous bird | 14.9 | 15.2 | ***1.0*** |
| Grape | Small omnivorous bird | 14.9 | 7.3 | ***2.0*** |

Since the trigger of 5 was not met at the screening, a first-Tier assessment was performed, considering the worst-case exposures for each scenario (risk envelope approach); the results are reported in the table below.

**Table 9.2-5: Tier-1 assessment of reproductive risk – a.s. Cymoxanil**

| Crop | Representative species | Stage | Shortcut value | $F_{twa}$ | $MAF_{mean}$ | DDD (mg a.s./kg bw/d) |
|---|---|---|---|---|---|---|
| Potato | Small omnivorous bird "lark" | BBCH 10 - 39 | 10.9 | 0.53 | 2.99 | 2.6 |
| | | BBCH ≥ 40 | 3.3 | 0.53 | 2.99 | 0.8 |
| | Small insectivorous bird "wagtail" | BBCH ≥ 20 | 9.7 | 0.53 | 2.99 | 2.3 |
| | | | | | | |
| Grape | Small omnivorous bird "lark" | BBCH 10 – 19 | 6.5 | 0.53 | 2.4 | 1.2 |
| | | BBCH 20 – 39 | 5.4 | 0.53 | 2.4 | 1.0 |
| | | BBCH ≥ 40 | 3.3 | 0.53 | 2.4 | 0.6 |
| | Small granivorous bird "finch" | BBCH 10 – 19 | 6.9 | 0.53 | 2.4 | 1.3 |
| | | BBCH 20 – 39 | 5.7 | 0.53 | 2.4 | 1.1 |
| | | BBCH ≥ 40 | 3.4 | 0.53 | 2.4 | 0.6 |
| | Small insectivorous bird "redstart" | BBCH 10 – 19 | 11.5 | 0.53 | 2.4 | 2.2 |

| Crop | Representative species | Stage | Shortcut value | $F_{twa}$ | $MAF_{mean}$ | DDD (mg a.s./kg bw/d) |
|---|---|---|---|---|---|---|
| | | BBCH ≥ 20 | 9.9 | 0.53 | 2.4 | 1.9 |
| | Frugivorous bird "trush/starling" | Ripening | 14.4 | 0.53 | 2.4 | 2.7 |

| Crop | Representative species | Stage | Application rate (kg a.s./ha) | DDD (mg a.s./kg bw/d) | NOEL mg a.s./kg b.w./day | TER |
|---|---|---|---|---|---|---|
| Potato | Small omnivorous bird "lark" | BBCH 10 - 39 | 0.1485 | 2.6 | 14.9 | 5.8 |
| | | BBCH ≥ 40 | 0.1485 | 0.8 | 14.9 | 19.2 |
| | Small insectivorous bird "wagtail" | BBCH ≥ 20 | 0.1485 | 2.3 | 14.9 | 6.5 |
| Grape | Small omnivorous bird "lark" | BBCH 10 – 19 | 0.1485 | 1.2 | 14.9 | 12.1 |
| | | BBCH 20 – 39 | 0.1485 | 1.0 | 14.9 | 14.6 |
| | | BBCH ≥ 40 | 0.1485 | 0.6 | 14.9 | 23.9 |
| | Small granivorous bird "finch" | BBCH 10 – 19 | 0.1485 | 1.3 | 14.9 | 11.4 |
| | | BBCH 20 – 39 | 0.1485 | 1.1 | 14.9 | 13.8 |
| | | BBCH ≥ 40 | 0.1485 | 0.6 | 14.9 | 23.2 |
| | Small insectivorous bird "redstart" | BBCH 10 – 19 | 0.1485 | 2.2 | 14.9 | 6.9 |
| | | BBCH ≥ 20 | 0.1485 | 1.9 | 14.9 | 8.0 |
| | Frugivorous bird "trush/starling" | Ripening | 0.1485 | 2.7 | 14.9 | 5.5 |

The results of the Tier-1 assessment showed acceptable risk for all the representative species and scenarios relevant for the proposed use on potato and grape.

### 9.2.2.2 Higher-tier risk assessment

Zoxamide

Not required.

Cymoxanil

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). The puddle scenario is relevant for the intended GAP uses.

**Puddle scenario**

Due to the different properties of cymoxanil and zoxamide, the risk via drinking water is assessed separately for the active substances.

Zoxamide

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 the 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). This is the case for zoxamide if the maximum yearly application rate (g a.s./ha/year) and the $LD_{50}$ and the NOEL value (mg/kg bw/day) are taken into account. Thus, no specific calculations of exposure and TER are necessary.

With a geometric mean (n=4) $K_{(f)oc}$ of 1179, zoxamide belongs to the group of more sorptive substances.

| | | | | | |
|---|---|---|---|---|---|
| Effective application rate (g/ha) | = | 445.5 | | | |
| Acute toxicity (mg/kg bw) | > | 2000 | quotient | < | 0.223 |
| Reprod. toxicity (mg/kg bw/d) | = | 122.8 | quotient | = | 3.6 |

Review Comments:

According EFSA B&M guidance the effective application rate should be calculated by multiplying the proposed application rates by MAF values based on the $DT_{50}$ in soil for the active substance. Nevertheless, taking to consideration short $DT_{50}$ of zoxamide in soil (slow-phase $DT_{50}$ of 46.9 days from the DFOP kinetics (k = 0.01477)), the risk assessment performed by the Applicant represent worst case scenario, thus is acceptable.

Since the ratio of effective application rate (in g/ha) to the relevant endpoint (in mg/kg bw/d) does not exceed the critical value of 3000 for the worst-case scenario, a quantitative risk assessment (calculation of TER values) is not necessary.

Cymoxanil

Exposure of birds will be predominantly dietary, through the consumption of residues on food items. Nevertheless, following the recommendations of EFSA guidance, the risk to birds via drinking water was estimated, considering the case of birds drinking the water collected in leaf whorls after application and subsequent rainfall or irrigation ("leaf scenario"), and the case of a bird drinking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application ("puddle scenario"). The first case is considered relevant only for acute risk, whereas the second scenario was used to assess both acute and long-term risk of birds.

The exposure for the "leaf scenario" was calculated as $PEC_{pool}$ according the following formula:

$$PEC_{pool} = \frac{C_{spray}}{5}$$

*where:*

$C_{spray}$ = *concentration of the a.s. in the spray solution, expressed in mg/L*

According to the intended uses proposed for the product, the maximum concentration for the a.s. in water (worst-case) is 743 mg/L (0.1485 kg a.s./ha in 200 L water), considering the maximum application rate of Cymoxanil and the minimum water volume. The resulting $PEC_{pool}$ is therefore 148.6 mg a.s./L.

The environmental concentration for the "puddle scenario" was calculated as $PEC_{puddle}$ according the following formula:

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

*where:*

*AR = Application rate, expressed as g a.s./ha*
*w (pore water term: volume) = 0.02*
*Koc = organic carbon adsorption coefficient of the a.s.*
*s (soil term: volume, density, organic carbon content) = 0.0015*

$$MAF_m \text{ (multiple application factor, mean)} = \frac{1 - e^{-nki}}{1 - e^{-ki}}$$

with: $k = ln(2)/DT_{50}$ *soil*
*n = number of applications*
*i = application interval*

Considering worst-case values from the product's intended uses (i.e.: 6 applications with 7 days interval on potato) and considering worst-case endpoints detailed in section 5 (i.e.: for the a.s. Cymoxanil: Koc = 43.6, and $DT_{50}$ soil = 7.3 days, which gives a $MAF_m$ = 2), the resulting worst-case $PEC_{puddle}$ of 0.35 mg a.s./L is coming from the Cymxoanil assessment.
The $PEC_{dw}$ value (i.e. the $PEC_{pool}$ and the $PEC_{puddle}$ for leaf scenario and puddle scenario respectively) for Cymoxanil was related to the Drinking Water Rate (DWR) typical for the representative bird species ("small granivorous bird" of 15.3 g bw), which is 0.46 L/kg bw/d.

The risk for birds was calculated as the ratio between the ecotoxicological endpoint of Cymoxanil from Table IIIA 10.1-1 and the product of DWR and $PEC_{dw}$. The respective TER calculation is summarised in the following table:

**Table 9.2-6: Risk for birds through drinking water – a.s. Cymoxanil**

| Risk | Generic focal species | Scenario | PECdw (mg/L) | DWR (L/kg bw/d) | Toxicity value (mg as/kg bw) | TER value |
|---|---|---|---|---|---|---|
| Acute | Granivorous bird (15.3 g) | leaf | 148.6 | 0.46 | 2000 | 29 |
| | | puddle | 0.35 | 0.46 | 2000 | 10001.5 |
| Long-term | Granivorous bird (15.3 g) | puddle | 0.35 | 0.46 | 14.9 | 74.5 |

Since the obtained TER value for Cymoxanil is well above the trigger of 10 for acceptable acute risk, and of 5 for acceptable long-term risk, it is concluded that a possible exposure of birds to Cymoxanil and Zoxamide via drinking water doesn't impose an unacceptable risk.

The calculations presented above indicate an acceptable risk for birds drinking water from puddles. Thus, birds are not at risk from drinking water exposure after application of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' to vines and potatoes.

### 9.2.2.4 Effects of secondary poisoning

Zoxamide

The log $P_{ow}$ of zoxamide amounts to 3.76 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required. In addition, metabolites RH-127450 and RH-24549 have log $P_{ow}$ values > 3 and were both identified as being relevant for the soil and aquatic risk assessments. They have the potential to bioaccumulate and therefore a secondary poisoning assessment is presented.

The assessments were performed considering default values and equations of the EFSA (2009) birds and mammals guidance document (2009)[4].

Cymoxanil

Following the results on the n-octanol/water partitioning co-efficient of **Cymoxanil** (log Pow 0.67 – 0.59), no bioconcentration potential is to be expected and consequently no effects of secondary poisoning to birds. No experimentally derived log Pow values are available for Cymoxanil metabolites. However, as exposed in the above mentioned EFSA Scientific Report for Cymoxanil, these substances are considered unlikely to accumulate in fat tissue, based on model calculations (KOWWIN, US-EPA).

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

According to EFSA/2009/1438 the risk for vermivorous birds is assessed for a bird of 100 g body weight with a daily food consumption of 104.6 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil. For earthworm eating birds, the "dry soil approach" was taken into account.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, always the scenario with the highest PEC value was taken into account for the risk assessment.

**Table 9.2-7: Assessment of the risk for earthworm-eating birds due to exposure to zoxamide via bioaccumulation in earthworms (secondary poisoning)**

| Parameter | Zoxamide | Comments |
|---|---|---|
| $PEC_{soil}$ (~~twa = 21 d~~ accu) (mg/kg soil) | ~~0.21343~~ 0.21599 | See Part B, Section 8.7.2.1 |
| log $P_{ow}$ / $P_{ow}$ | 3.76 / 5754.4 | EFSA (2017) |
| Koc | 1179 | Geomean (n = 4) EFSA LoEP |
| Foc | 0.02 | Default |
| $BCF_{worm}$ | 2.964 | $BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$ |
| $PEC_{worm}$ | ~~0.633~~ 0.64 | $PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$ |
| Daily dietary dose (mg/kg bw/d) | ~~0.664~~ 0.672 | $DDD = PEC_{worm} \times 1.05$ |
| NOEL (mg/kg bw/d) | 122.8 | |
| $TER_{lt}$ | ~~184.9~~ 182.7 | |

TER values shown in bold fall below the relevant trigger.

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

**Table 9.2-8: Assessment of the risk for earthworm-eating birds due to exposure to RH-127450 via bioaccumulation in earthworms (secondary poisoning)**

| Parameter | RH-127450 | Comments |
|---|---|---|
| $PEC_{soil}$ (twa = 21 d) (mg/kg soil) | ~~0.0235~~ 0.01537 | See Part B, Section 8.7.2.1 |
| log $P_{ow}$ / $P_{ow}$ | 3.5 / 3162 | RAR (2017) |
| Koc | 593 | Geomean (n = 3) EFSA LoEP |
| Foc | 0.02 | Default |
| $BCF_{worm}$ | 3.270 | $BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$ |
| $PEC_{worm}$ | ~~0.0768~~ 0.0503 | $PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$ |
| Daily dietary dose (mg/kg bw/d) | ~~0.081~~ 0.053 | $DDD = PEC_{worm} \times 1.05$ |
| NOEL (mg/kg bw/d) | 12.28 | Parent endpoint / 10* |
| $TER_{lt}$ | ~~151.6~~ 231.7 | |

TER values shown in bold fall below the relevant trigger.
* In the absence of toxicity data, the metabolite is considered as 10 times more toxic than the parent compound.

**Table 9.2-9: Assessment of the risk for earthworm-eating birds due to exposure to RH-24549 via bioaccumulation in earthworms (secondary poisoning)**

| Parameter | RH-24549 | Comments |
|---|---|---|
| $PEC_{soil}$ (twa = 21 d) (mg/kg soil) | ~~0.03495~~ 0.02214 | See Part B, Section 8.7.2.1 |
| log $P_{ow}$ / $P_{ow}$ | 3.83 / 6760 | RAR (2017) |
| Koc | ~~161~~ 90.55 | ~~Geometric mean (n=3)~~ worst case |
| Foc | 0.02 | Default |
| $BCF_{worm}$ | ~~25.45~~ 45.257 | $BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$ |
| $PEC_{worm}$ | ~~0.890~~ 1.002 | $PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$ |
| Daily dietary dose (mg/kg bw/d) | ~~0.934~~ 1.052 | $DDD = PEC_{worm} \times 1.05$ |
| NOEL (mg/kg bw/d) | 12.28 | Parent endpoint / 10* |
| $TER_{lt}$ | ~~13.15~~ 11.67 | |

TER values shown in bold fall below the relevant trigger.
* In the absence of toxicity data, the metabolite is considered as 10 times more toxic than the parent compound.

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

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, always the scenario with the highest PEC value was taken into account for the risk assessment.

**Table 9.2-10: Assessment of the risk for fish-eating birds due to exposure to zoxamide via bioaccumulation in fish (secondary poisoning)**

| Parameter | Zoxamide | Comments |
|---|---|---|
| $PEC_{sw}$ (twa = 21 d) (mg/L) | 0.00265 | Max FOCUS SW Step 2 PEC (twa = 21 d)<br>See Part B, Section 8.9.2.1 |
| $BCF_{fish}$ | 136 | EFSA (2017) |
| BMF | - | Biomagnification factor (relevant for BCF ≥ 2000) |
| $PEC_{fish}$ | 0.3604 | $PEC_{fish} = PEC_{water} \times BCF_{fish}$ |
| Daily dietary dose (mg/kg bw/d) | 0.057 | $DDD = PEC_{fish} \times 0.159$ |
| NOEL (mg/kg bw/d) | 122.8 | |
| $TER_{lt}$ | 2143.0 | |

TER values shown in bold fall below the relevant trigger.

**Table 9.2-11: Assessment of the risk for fish-eating birds due to exposure to RH-127450 via bioaccumulation in fish (secondary poisoning)**

| Parameter | RH-127450 | Comments |
|---|---|---|
| $PEC_{sw}$ (twa = 21 d) (mg/L) | 0.00476 | Max FOCUS SW Step 2 PEC (twa = 21 d)<br>See Part B, Section 8.9.2.1 |
| $BCF_{fish}$ | 136 | Parent value, EFSA (2017) |
| BMF | - | Biomagnification factor (relevant for BCF ≥ 2000) |
| $PEC_{fish}$ | 0.6474 | $PEC_{fish} = PEC_{water} \times BCF_{fish}$ |
| Daily dietary dose (mg/kg bw/d) | 0.103 | $DDD = PEC_{fish} \times 0.159$ |
| NOEL (mg/kg bw/d) | 12.28 | Parent endpoint / 10* |
| $TER_{lt}$ | 119.3 | |

TER values shown in bold fall below the relevant trigger.
* In the absence of a toxicity value, the metabolite is considered as 10 times more toxic than the parent compound.

**Table 9.2-12: Assessment of the risk for fish-eating birds due to exposure to RH-24549 via bioaccumulation in fish (secondary poisoning)**

| Parameter | RH-24549 | Comments |
|---|---|---|
| $PEC_{sw}$ (twa = 21 d) (mg/L) | 0.0041 | Max FOCUS SW Step 2 PEC (twa = 21 d)<br>See Part B, Section 8.9.2.1 |
| $BCF_{fish}$ | 136 | Parent value, EFSA (2017) |
| BMF | - | Biomagnification factor (relevant for BCF ≥ 2000) |
| $PEC_{fish}$ | 0.5576 | $PEC_{fish} = PEC_{water} \times BCF_{fish}$ |
| Daily dietary dose (mg/kg bw/d) | 0.089 | $DDD = PEC_{fish} \times 0.159$ |
| NOEL (mg/kg bw/d) | 12.28 | Parent endpoint / 10* |
| $TER_{lt}$ | 138.5 | |

TER values shown in bold fall below the relevant trigger.
* In the absence of a toxicity value, the metabolite is considered as 10 times more toxic than the parent compound.

As a result, birds are not at risk from secondary poisoning after application of ‘CYMOXANIL 33% + ZOXAMIDE 33% WG’ to vines and potatoes.

#### 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 assessment for birds was carried out according to EFSA/2009/1438.

An acceptable acute and chronic risk to birds was demonstrated for both active substances as well as for the potentially relevant metabolites of zoxamide and therefore also for 'CYMOXANIL 33% + ZOXAMIDE 33% WG'. Secondary poisoning for earthworm and fish-eating birds and via drinking water is not likely.

Review Comments:

The acute and chronic risks of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' to birds were assessed from toxicity exposure ratios between toxicity endpoints, estimated from study with active ingredients and maximum residues occurring on food items. No acute toxicity test with the formulation was required.

All TER values exceed the relevant triggers indicating that 'CYMOXANIL 33% + ZOXAMIDE 33% WG' does not pose an unacceptable risk to birds following applications according to recommended use pattern.

Evaluation of exposing to birds through the drinking water demonstrated the acceptable risk. The potential risk of secondary poisoning for zoxamide, RH-127450 and RH-24549 is low.

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

The risk assessment for birds was performed in accordance with the current "Guidance document on risk assessment for birds & mammals on request from EFSA" (EFSA Journal 2009; 7(12): 1438).

### 9.3.1 Toxicity data

Effects of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' on mammals were not evaluated as part of the EU assessment of active substances.

Mammals toxicity studies have been carried out with zoxamide and cymoxanil. Full details of these studies are provided in the EU review dossiers, for zoxamide in the RAR (2017 and the EFSA Peer Review Conclusion (2017) and for cymoxanil in the DAR (2007) as well as the EFSA Peer Review Conclusion (2008). New toxicity data submitted for zoxamide metabolites are summarised in the dRR Part B Section 6 document. Endpoints used for the risk assessment are summarised below. The selection of studies and endpoints for the risk assessment is in line with results of the EU review processes.

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

| Species | Substance | Exposure system | Results | Reference |
|---|---|---|---|---|
| **Zoxamide** | | | | |
| Rat | zoxamide | Oral<br>Acute | **$LD_{50}$ > 5 000 mg/kg bw** | EFSA (2017) |
| Rat | zoxamide | Long-term (parental) | NOAEL = 5 000 mg/kg bw (= 360 mg/kg bw/d) | EFSA (2017) |
| Rat | zoxamide | Reproductive | NOAEL > 20 000 mg/kg bw (= 1 474 mg/kg bw/d) | EFSA (2017) |
| Rat | zoxamide | Long-term (offspring) | NOAEL = 5 000 mg/kg bw (= 360 mg/kg bw/d) | EFSA (2017) |
| Rabbit | zoxamide | Long-term (development) | NOAEL = 1 000 mg/kg bw/d | EFSA (2017) |
| Rat | zoxamide | Long term (development) | NOAEL = 1 000 mg/kg bw/d | EFSA (2017) |
| Rat | zoxamide | | **NOAEL = 71*** | EFSA (2017) |
| **'CYMOXANIL 33% + ZOXAMIDE 33% WG'** | | | | |
| Sprague Dawley rats | 'CYMOXANIL 33% + ZOXAMIDE 33% WG' | Oral<br>Acute | $LD_{50}$ = 1469 mg prod./kg bw | Ferguson et al., 1999; report no. 99R-102 |

* Value agreed in the Peer review meeting 160 by experts
**Bold letters:** endpoints used for the risk assessment

**Table 9.3-2: EU endpoints - toxicity of Cymoxanil to mammals**

| Study | EU agreed endpoints (EFSA Scientific Report of Cymoxanil (2008)) |
|---|---|
| Acute toxicity | $LD_{50}$ = 760 mg / kg bw |
| Reproductive toxicity (long-term) | NOEL = 10.5 mg / kg bw /day |

* Corrected following zRMS comment reported in the RR: *The acute toxicity of cymoxanil to mammals should be 760 mg / kg bw instead of 960 mg / kg bw according with EFSA Scientific Report of Cymoxanil (2008).*

### 9.3.1.1 Justification for new endpoints

An acute oral study with the formulated product 'CYMOXANIL 33% + ZOXAMIDE 33% WG' is available (please refer to Ferguson et al., 1999, report no. 99R-102 with a $LD_{50}$ oral, rat of 1469 mg/kg bw). In this study, three groups of six males and six females of rats were exposed to CYMOXANIL 33% + ZOXAMIDE 33% WG (containing cymoxanil at 34.7% and zoxamide at 33.8%) at 500, 2000 and 5000 mg/kg body weight. Results indicate an acute lethal dose of 1469 mg/kg with 95% confidence limits of 814 to 2070 mg/kg.

## 9.3.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the EFSA (2009) birds and mammals guidance document (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).
The major route of exposure is by feeding on e.g. contaminated vegetation, small insects or earthworms.

According to the EFSA (2009) guidance document a short-term risk assessment is not necessary for spray applications due to the poor distinction between short and long-term exposure. The risk assessment for short-term periods is covered by the long-term risk assessment.

**Toxicity of active substances *vs.* formulation**

*Acute Toxicity*

The acute risk of the formulated product 'CYMOXANIL 33% + ZOXAMIDE 33% WG' on mammals was evaluated considering the worst-case intended uses
Following the provisions of the Annex III of Dir 91/414/EEC (now Annex of Reg. (EU) 545/2011), an acute oral toxicity test on rat was carried out with the formulated product 'CYMOXANIL 33% + ZOXAMIDE 33% WG' (for details, see Section B6); the results of this test, as well as the ecotoxicological endpoints for the active substances Cymoxanil and Zoxamide, are summarized in the table below.

**Table 9.3-3: Toxicity of Cymoxanil, Zoxamide, and 'CYMOXANIL 33% + ZOXAMIDE 33% WG' to terrestrial vertebrates other than birds**

| Endpoint | Cymoxanil * | Zoxamide ** | CYMOXANIL 33% + ZOXAMIDE 33% WG |
|---|---|---|---|
| Oral $LD_{50}$ (rat) | 760 mg a.s./kg bw | >5000 mg a.s./kg bw | 1469 mg prod./kg bw |

* source: EFSA Scientific Report (2008) 167, 1-116
** source: Zoxamide Review Report SANCO/10297/2003 - Final, dated 4th February 2004

Following recommendations of the EFSA Guidance, a "surrogate" $LD_{50}$ for the formulated product was calculated using the formula reported above under point IIIA 10.1; the calculation is hereunder exposed:

$$LD_{50\,(mix)} = \left( \frac{0.5}{760} + \frac{0.5}{5000} \right)^{-1} = \left( 0.00066 + 0.00010 \right)^{-1} = 1319.44 \text{ mg prod./kg b.w.}$$

The endpoints reported in the above tables clearly show that Cymoxanil is the active ingredient which gives the higher contribution to the toxicity of the formulated product. For this reason, the potential risk of the a.s. Cymoxanil and of the formulated product were compared in terms of "tox per fraction". According to the EFSA Guidance, if the quotients "tox per fraction (a.s.)" and "tox per fraction (mix)" deviate by ≤ 10%, then the active substance a.s. contributes to ≥ 90% to mixture toxicity, while the other components of the mixture have only a marginal impact on the predicted risk; in this case it is considered appropriate to perform the risk assessment for the most toxic active substance alone.

The "tox per fraction" values for the a.s. Cymoxanil and for the formulated product were calculated according to the following formula (where X (a.s.$_i$) is the fraction of the active substance [$i$] in the mixture,

$$\text{Tox per fraction (a.s.)} = \frac{LD_{50}\,(\text{a.s.}_i)}{X(\text{a.s.}_i)} = \frac{760}{0.5} = 1520.0$$

$$\text{Tox per fraction (mix)} = \frac{LD_{50}\,(\text{mix})}{\sum X(\text{a.s.}_i)} = \frac{1610.7}{1} = 1610.7$$

Since the deviation between "tox per fraction (a.s.)" and "tox per fraction (mix)" was higher than 10% (16.1%), the toxicity of the formulated product was considered the most appropriate endpoint for the acute risk assessment.
Following a worst-case approach, the $LD_{50}$ obtained from the test with the formulated product (1469 mg prod./kg bw) was used for risk assessment purpose, being slightly lower than the "surrogate" $LD_{50}$.

The assessment results showed an acceptable acute risk to mammals for the formulated product, with a $TER_A$ above the trigger of 10 already at the screening level, as summarized in in the following table.

**Table 9.3-4: Acute risk to mammals for 'CYMOXANIL 33% + ZOXAMIDE 33% WG'**

| Crop | Indicator species | $LD_{50}$ (mg prod./kg bw) | Daily Dietary Dose (mg prod./kg bw) | TER | Trigger |
|---|---|---|---|---|---|
| Grape | Small herbivorous mammal | ~~1318.5~~ 1469 | 76.97 | ~~17.0~~ 19.1 | *10* |
| Potato | Small herbivorous mammal | ~~1318.5~~ 1469 | 77.36 | ~~17.1~~ 19.0 | *10* |

*Reproductive Toxicity*

The combined risk of the two active ingredients was assessed assuming additive toxicity of cymoxanil and zoxamide. The formula

$$\sum \text{trigger/TER}$$

was used; if the sum is < 1, acceptable risk can be concluded.

The results of the Tier 1 assessment for zoxamide and cymoxanil, reported below at point 9.3.2.1, were used for this calculation. Following a risk-envelope approach, the worst-case scenario for each crop was considered, i.e. mouse for potato and lagomorph for grape (the scenarios that require higher-tier assessment result in TER values higher than the Tier-1 TERs of these organisms). The results of this combined assessment are here reported:

| | Potato Small omnivorous "mouse" | | Grape Large herbivorous "lagomorph" | |
|---|---|---|---|---|
| | **TER** | **trigger/TER** | **TER** | **trigger/TER** |
| ~~Zoxamide~~ | ~~9.8~~ | ~~0.510~~ | ~~6.1~~ | ~~0.820~~ |
| Zoxamide | 57.7 | 0.087 | 67.6 | 0.074 |
| Cymoxanil | 5.7 | 0.877 | 8.3 | 0.602 |
| **COMBINED RISK** | | ~~1.387~~ **0.964** | | ~~1.422~~ **0.676** |

Since the sum of trigger/TER values is ~~>1~~ <1, further consideration is not necessary. ~~The Tier-1 risk reported below for cymoxanil comes from the Registration Report of UK, where a worst-case use pattern was assessed (potato: 6 applications with 5-day interval; grape: 5 applications with 7-day interval). As a consequence, the MAF values used for Tier-1 reproductive assessment were 2.99 and 2.4 for potato and grape respectively. However, this dRR supports an intended use of 3 applications with 7-day interval, which gives a default MAF of 2.0. If this MAF is used in the Tier-1 assessment of cymoxanil, the following TER values are obtained:~~

| ~~Scenario~~ | ~~Appl. rate~~ | ~~Shortcut value~~ | ~~$F_{twa}$~~ | ~~MAF~~ | ~~DDD~~ | ~~NOEL~~ | ~~TER~~ |
|---|---|---|---|---|---|---|---|

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Potato<br>Small omnivorous "mouse" | 0.1485 | 7.8 | 0.53 | **2** | 1.2 | 10.5 | **8.6** |
| Grape<br>Large herbivorous "lagomorph" | 0.1485 | 6.7 | 0.53 | **2** | 1.1 | 10.5 | **10.0** |

Using these TER values in the combined risk assessment, the sum of trigger/ER values is still slightly above 1, requiring higher tier assessment:

| | Potato<br>Small omnivorous "mouse" | | Grape<br>Large herbivorous "lagomorph" | |
|---|---|---|---|---|
| | **TER** | **trigger/TER** | **TER** | **trigger/TER** |
| Zoxamide | 9. | 0.510 | 6.1 | 0.820 |
| Cymoxanil | 8.6 | 0.581 | 10.0 | 0.500 |
| **COMBINED RISK** | | **1.092** | | **1.320** |

For higher tier assessment, the refinement explained at point 9.3.2.2 below is adopted, i.e. DT50 of 3 days for plant material. This refinement was already accepted in the Registration Report of UK.
This DRT50 was used to calculate the MAFxTWA factor for 3 applications with 7-day interval); for this calculation the birds and mammal scenario template developed by the Northern Zone Ecotox Expert Group (Available at the Danish EPA web: http://eng.mst.dk/media/189945/bird-mammal-scenario-template-v10-5.xlsm) was used.
The exposure of mouse and lagomorph can be therefore refined associating the resulting MAFxTWA factor, 0.32, to the consumption of plant material in the diet.

| **Scenario** | **Appl. rate** | **Diet** | **PD** | **FIR/bw** | **RUD** | **MAF x TWA** | **DDD** | **NOEL** | **TER** |
|---|---|---|---|---|---|---|---|---|---|
| Potato<br>Small omnivorous "mouse" | 0.1485 | non-grass herbs | 0.25 | 0.27 | 28.7 | **0.32** | 0.43 | 10.5 | **24.4** |
| | | weed seeds | 0.5 | | 40.2 | **0.32** | | | |
| | | ground arthropods | 0.25 | | 7.5 | 2 x 0.53 | | | |
| Grape<br>Large herbivorous "lagomorph" | 0.1485 | 0.1485 | **Shortcut value:** 6.7 | | | **0.32** | 0.3 | 10.5 | **33.0** |

Using these TER values in the combined risk assessment, the sum of trigger/ER values is lower than 1 for both scenarios, indicated acceptable risk for mammals.

| | Potato<br>Small omnivorous "mouse" | | Grape<br>Large herbivorous "lagomorph" | |
|---|---|---|---|---|
| | **TER** | **trigger/TER** | **TER** | **trigger/TER** |
| Zoxamide | 9.8 | 0.510 | 6.1 | 0.820 |
| Cymoxanil | 24.4 | 0.205 | 33.0 | 0.152 |
| **COMBINED RISK** | | **0.715** | | **0.971** |

Review Comments:

The risk assessment performed above, based on the lowest TER 1 values for the representative species selected by the Applicant showed an acceptable risk. Nevertheless, it should be emphasized that no justification for the choice of mouse and lagomorph as a focal species was presented. Concerned Member States must decide on the consideration of this assumption on national level.

**Relevance of metabolites**

Please refer to information provided in chapter 9.2.2.

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

The results of the acute and reproductive first-tier (screening) risk assessment are summarised in the following tables.

Zoxamide

**Table 9.3-5: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in wine grapes**

| **Intended use** | | **Wine grapes** | | | |
|---|---|---|---|---|---|
| **Active substance/product** | | **Zoxamide** | | | |
| **Application rate (g/ha)** | | 3 × 148.5 | | | |
| **Acute toxicity (mg/kg bw)** | | > 5000 | | | |
| **TER criterion** | | 10 | | | |
| **Crop scenario Growth stage** | **Indicator/generic focal species** | **$SV_{90}$** | **$MAF_{90}$** | **$DDD_{90}$ (mg/kg bw/d)** | **$TER_a$** |
| Vineyard | Small herbivorous mammal | 136.4 | 1.6 | 32.41 | 154.27 |
| **Reprod. toxicity (mg/kg bw/d)** | | 71 | | | |
| **TER criterion** | | 5 | | | |
| **Crop scenario Growth stage** | **Indicator/generic focal species** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$ (mg/kg bw/d)** | **$TER_{lt}$** |
| Vineyard | Small herbivorous mammal | 72.3 | 2.0 × 0.53 | 11.38 | 6.24 |
| Tier 1* | | | | | |
| Vineyard BBCH 10-19 | Small omnivorous mammal "mouse" | 4.7 | 2.0 × 0.53 | 0.74 | 95.9 |
| Vineyard BBCH 10-19 | Large herbivorous "lagomorph" | 6.7 | 2.0 × 0.53 | 1.05 | 67.6 |

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
*scenarios needed for combined risk assessment

**Table 9.3-6: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in potatoes**

| **Intended use** | | **Potatoes** | | | |
|---|---|---|---|---|---|
| **Active substance/product** | | **Zoxamide** | | | |
| **Application rate (g/ha)** | | 3 × 148.5 | | | |
| **Acute toxicity (mg/kg bw)** | | > 5000 | | | |
| **TER criterion** | | 10 | | | |
| **Crop scenario Growth stage** | **Indicator/generic focal species** | **$SV_{90}$** | **$MAF_{90}$** | **$DDD_{90}$ (mg/kg bw/d)** | **$TER_a$** |
| ~~Fruiting vegetables~~ Potatoes | Small herbivorous mammal | 118.4 | 1.6 | 28.13 | 177.75 |
| **Reprod. toxicity (mg/kg bw/d)** | 71 | | | | |
| **TER criterion** | 5 | | | | |
| **Crop scenario Growth stage** | **Indicator/generic focal species** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$ (mg/kg bw/d)** | **$TER_{lt}$** |
| ~~Fruiting vegetables~~ Potatoes | Small herbivorous mammal | 48.3 | 2.0 × 0.53 | 7.60 | 9.34 |
| Tier 1* | | | | | |
| Potatoes BBCH 10-39 | Small omnivorous mammal "mouse" | 7.8 | 2.0 × 0.53 | 1.23 | 57.7 |

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

The screening risk calculations show an acceptable acute and long-term risk for mammals from the use of zoxamide applied as 'CYMOXANIL 33% + ZOXAMIDE 33% WG' according to the proposed use pattern. A higher tier assessment is not necessary.

No evidence of any reproducible endocrine effects is expected for zoxamide (see EFSA Peer Review Conclusion for zoxamide, 2017).

Cymoxanil

The acute risk to mammals of the two active substances alone has been assessed by following the screening assessment in TGD and the results given in the tables below:

**Table 9.3-7: Screening estimates of acute exposure to Cymoxanil**

| **Crop** | **Indicator sp** | **application rate (kg a.s./ha)** | **Short cut value** | **MAF** | **DDD** |
|---|---|---|---|---|---|
| Potato | Small herbivorous mammal | 0.1485 | 118.4 | 2.2 | 38.68 |
| Grape, tomato | Small herbivorous mammal | 0.1485 | 136.4 | 1.9 | 38.49 |

| **Crop** | **Indicator sp** | **application rate** | **DDD** | **$LD_{50}$** | **TER** | **Trigger** |
|---|---|---|---|---|---|---|

| | | (kg a.s./ha) | | | | |
|---|---|---|---|---|---|---|
| Potato | Small herbivorous mammal | 0.1485 | 38.68 | 760 | 19.6 | 10 |
| Grape | Small herbivorous mammal | 0.1485 | 38.49 | 760 | 19.7 | 10 |

To assess the long-term risk for the a.s. Cymoxanil, a screening assessment was performed as a first step; the results are reported in the table below.

**Table 9.3-8: Screening assessment of reproductive risk – a.s. Cymoxanil**

| Crop | Indicator species | Application rate (kg a.s./ha) | Shortcut value | $F_{twa}$ | $MAF_{mean}$ | DDD (mg a.s./kg bw/d) |
|---|---|---|---|---|---|---|
| Potato | Small herbivorous mammal | 0.1485 | 48.3 | 0.53 | 2.99 | 11.37 |
| Grape, tomato | Small herbivorous mammal | 0.1485 | 72.3 | 0.53 | 2.4 | 13.7 |

| Crop | Indicator species | Toxicity endpoint (mg a.s./kg bw/d) | DDD (mg a.s./kg bw/d) | TER |
|---|---|---|---|---|
| Potato | Small herbivorous mammal | 10.5 | 11.37 | 0.92 |
| Grape | Small herbivorous mammal | 10.5 | 13.7 | ***0.77*** |

Since the trigger of 5 was not met at the screening, a first-Tier assessment was performed, considering the highest exposures for each scenario; the results are reported in Table 9.3-9

**Table 9.3-9: Tier I assessment of reproductive risk – a.s. Cymoxanil**

| Crop | Representative species | Stage | Shortcut value | $MAF_{mean}$ | DDD (mg a.s./kg bw/d) |
|---|---|---|---|---|---|
| Potato | Small insectivorous mammal "shrew" | BBCH ≥ 20 | 1.9 | 2.99 | 0.45 |
| | Small omnivorous mammal "mouse" | BBCH 10 - 39 | 7.8 | 2.99 | 1.84 |
| | | BBCH ≥ 40 | 2.3 | 2.99 | 0.54 |
| | Large herbivorous mammal "lagomorph" | BBCH 10 - 39 | 14.3 | 2.99 | 3.37 |
| | | BBCH ≥ 40 | 4.3 | 2.99 | 1.01 |
| | Small herbivorous mammal "vole" | BBCH ≥ 40 | 21.7 | 2.99 | 5.11 |
| Grape | Small insectivorous mammal "shrew" | BBCH 10 - 19 | 4.2 | 2.4 | 0.8 |
| | | BBCH ≥ 20 | 1.9 | 2.4 | 0.4 |
| | Small omnivorous mammal "mouse" | BBCH 10 - 19 | 4.7 | 2.4 | 0.9 |
| | | BBCH 20 - 39 | 3.9 | 2.4 | 0.7 |
| | | BBCH ≥ 40 | 2.3 | 2.4 | 0.4 |
| | Large herbivorous mammal "lagomorph" | BBCH 10 - 19 | 6.7 | 2.4 | 1.3 |
| | | BBCH 20 - 39 | 5.5 | 2.4 | 1.0 |

| Crop | Representative species | Stage | Shortcut value | $MAF_{mean}$ | DDD (mg a.s./kg bw/d) |
|---|---|---|---|---|---|
| | | BBCH ≥ 40 | 3.3 | 2.4 | 0.6 |
| | Small herbivorous mammal "vole" | BBCH 10 - 19 | 43.4 | 2.4 | 8.2 |
| | | BBCH 20 - 39 | 36.1 | 2.4 | 6.8 |
| | | BBCH ≥ 40 | 21.7 | 2.4 | 4.1 |

| Crop | Representative species | Stage | Application rate (kg a.s./ha) | DDD (mg a.s./kg bw/d) | NOEL mg a.s./kg b.w./day | TER | Trigger |
|---|---|---|---|---|---|---|---|
| Potato | Small insectivorous mammal "shrew" | BBCH ≥ 20 | 0.1485 | 0.45 | 10.5 | 23.5 | 5 |
| | Small omnivorous mammal "mouse" | BBCH 10 - 39 | 0.1485 | 1.84 | 10.5 | 5.7 | 5 |
| | | BBCH ≥ 40 | 0.1485 | 0.54 | 10.5 | 19.4 | 5 |
| | Large herbivorous mammal "lago-morph" | BBCH 10 - 39 | 0.1485 | 3.37 | 10.5 | **3.1** | 5 |
| | | BBCH ≥ 40 | 0.1485 | 1.01 | 10.5 | 10.4 | 5 |
| | Small herbivorous mammal "vole" | BBCH ≥ 40 | 0.1485 | 5.11 | 10.5 | **2.1** | 5 |
| Grape | Small insectivorous mammal "shrew" | BBCH 10 - 19 | 0.1485 | 0.8 | 10.5 | 13.2 | 5 |
| | | BBCH ≥ 20 | 0.1485 | 0.4 | 10.5 | 29.3 | 5 |
| | Small omnivorous mammal "mouse" | BBCH 10 - 19 | 0.1485 | 0.9 | 10.5 | 11.8 | 5 |
| | | BBCH 20 - 39 | 0.1485 | 0.7 | 10.5 | 14.3 | 5 |
| | | BBCH ≥ 40 | 0.1485 | 0.4 | 10.5 | 24.2 | 5 |
| | Large herbivorous mammal "lago-morph" | BBCH 10 - 19 | 0.1485 | 1.3 | 10.5 | 8.3 | 5 |
| | | BBCH 20 - 39 | 0.1485 | 1.0 | 10.5 | 10.1 | 5 |
| | | BBCH ≥ 40 | 0.1485 | 0.6 | 10.5 | 16.8 | 5 |
| | Small herbivorous mammal "vole" | BBCH 10 - 19 | 0.1485 | 8.2 | 10.5 | **1.3** | 5 |
| | | BBCH 20 - 39 | 0.1485 | 6.8 | 10.5 | **1.5** | 5 |
| | | BBCH ≥ 40 | 0.1485 | 4.1 | 10.5 | **2.6** | 5 |

The results of Tier1 assessment show acceptable risk for the insectivorous and omnivorous mammals (representative species "mouse" and "shrew" respectively), whereas a refinement is triggered for the large herbivorous "lagomorph" in the scenario "Potato, BBCH 10-39", and for the small herbivorous "vole" in all the considered scenarios.

### 9.3.2.2 Higher-tier risk assessment

Zoxamide

Not required.

Cymoxanil

*Refined chronic risk for the crop Potato - a.s. Cymoxanil*

For refinement purpose, the individual parameters (FIR/bw, RUD, and Interception) used to derive the shortcut values of EFSA Guidance were considered, as indicated by the Appendix A of EFSA document. The default values for the individual food items on which the calculation of Tier-1 exposure is based, are reported in the table below.

**Table 9.3-10: Tier-1 chronic exposure for the scenario potato – details of calculation**

| Crop Stage (BBCH) | Representative species | Diet | FIR/bw | Mean RUD | Deposition factor | MAF | Twa factor | Daily Dietary Dose |
|---|---|---|---|---|---|---|---|---|
| Potato, BBCH 10-39 | Large herbivorous "lagomorph" | 100% non-grass herbs | 0.50 | 28.7 | 1.0 | 2.99 | 0.53 | 3.37 |
| Potato, BBCH ≥ 40 | Small herbivorous "vole" | 100% grass (grass + cereals) | 1.33 | 54.2 | 0.3 | 2.99 | 0.53 | 5.11 |

To refine the risk for this representative species, more realistic values for MAF and Ftwa were considered instead of the default ones provided by EFSA document.
As stated in Cymoxanil DAR, since the results of residue decline studies on potato and lettuce showed residue levels below the limit of detection, and no accumulation at all after multiple applications, an estimated $DT_{50}$ of 2 days can be considered an appropriate and still worst case assumption for the residue calculation in vegetation. As a consequence, the **MAF is set at 1** and the default time weighted average factor (Ftwa) is calculated based on an estimated $DT_{50}$ of 2 d, which results in a refined **Ftwa of 0.38** (application interval: 7 days). Using these values to refine the exposure for the herbivorous mammals "lagomorph" and "vole", the risk assessment results as follows:

**Table 9.3-11: Refined chronic risk assessment for herbivorous mammals in potato**

| Crop Stage (BBCH) | Representative species | FIR/bw | Mean RUD | Deposition factor | MAF | Twa factor | Daily Dietary Dose | Toxicity value | TER |
|---|---|---|---|---|---|---|---|---|---|
| Potato, BBCH 10-39 | Large herbivorous "lagomorph" | 0.50 | 28.7 | 1.0 | 1.0 | 0.38 | 0.81 | 10.5 | 13.0 |
| Potato, BBCH ≥ 40 | Small herbivorous "vole" | 1.33 | 54.2 | 0.3 | 1.0 | 0.38 | 1.22 | 10.5 | 8.6 |

Since the obtained TER values are above the trigger of 5 also for these representative species, it is possible to conclude that the exposure to Cymoxanil following applications of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' on potato, doesn't pose an unacceptable chronic risk to mammals.

*Refined chronic risk for the crops Grape - a.s. Cymoxanil*

For this crop, a refinement is required for the representative small herbivorous mammal "vole" in all the relevant BBCH stages. For this purpose, more realistic diet were considered for the focal species "vole" (*Microtus arvalis*), evaluating separately the consumption of grass and non-grass herbs. The results of this refined risk assessment are summarized in the below table.

**Table 9.3-12: Refined chronic risk assessment for common vole in Grape**

| Crop Stage (BBCH) | Diet | FIR/bw | Mean RUD | Deposition factor | PD | MAF x Twa | Daily Dietary Dose | DDD (total) | Tox. value | TER |
|---|---|---|---|---|---|---|---|---|---|---|
| Grape, BBCH 10-19 | 50% grass herbs | 1.49 | 54.2 | 0.6 | 0.245 | 0.404 | 0.712 | 1.87 | 10.5 | 5.6 |
| | 50% non-grass herbs | 1.49 | 28.7 | 0.6 | 0.755 | 0.404 | 1.162 | | | |
| Grape, BBCH 20-40 | 50% grass herbs | 1.49 | 54.2 | 0.5 | 0.245 | 0.404 | 0.594 | 1.56 | 10.5 | 6.7 |
| | 50% non-grass herbs | 1.49 | 28.7 | 0.5 | 0.755 | 0.404 | 0.968 | | | |
| Grape, BBCH ≥ 40 | 50% grass herbs | 1.49 | 54.2 | 0.3 | 0.245 | 0.404 | 0.356 | 0.94 | 10.5 | 11.2 |
| | 50% non-grass herbs | 1.49 | 28.7 | 0.3 | 0.755 | 0.404 | 0.581 | | | |

Since the obtained TERs value are above the trigger of 5 also for small herbivorous species, it is possible to conclude that the exposure to Cymoxanil following application of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' on Grape according to the proposed GAP, doesn't impose unacceptable chronic risks to mammals.

### 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).
Only the puddle scenario is relevant for mammals.

Zoxamide

**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) – what is the case for zoxamide - if the maximum yearly application rate (g a.s./ha/year) and the $LD_{50}$ and the NOEL values (mg/kg bw./day) are taken into account. Thus, no specific calculations of exposure and TER are necessary.

With a geometric mean (n=4) $K_{(f)oc}$ of 1179, zoxamide belongs to the group of more sorptive substances.

Review Comments:

According EFSA B&M guidance the effective application rate should be calculated by multiplying the proposed application rates by MAF values based on the $DT_{50}$ in soil for the active substance. Nevertheless, taking to consideration short $DT_{50}$ of zoxamide in soil (slow-phase $DT_{50}$ of 46.9 days from the DFOP kinetics (k = 0.01477)), the risk assessment performed by the Applicant represent worst case scenario, thus is acceptable.

| | | | | | | |
|---|---|---|---|---|---|---|
| Effective application rate (g/ha) | = | 445.5 | | | | |
| Acute toxicity (mg/kg bw) | > | 5000 | quotient | < | 0.089 | |
| Reprod. toxicity (mg/kg bw/d) | = | 71 | quotient | = | 6.275 | |

Since the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw) does not exceed the critical value of 3000 for the worst-case scenario, a quantitative risk assessment (calculation of TER values) is not necessary.

Cymoxanil

Considering worst-case values from the product's intended uses (i.e.: 6 applications with 7 days interval on potato) and considering worst-case endpoints detailed in section 5 (i.e.: for the a.s. Cymoxanil: Koc = 43.6, and $DT_{50}$ soil = 7.3 days, which gives a $MAF_m$ = 2; for the a.s. Zoxamide: Koc = 1224 and $DT_{50}$ soil = 2.8 days), the resulting worst-case $PEC_{puddle}$ of 0.35 mg a.s./L is coming from the Cymxoanil assessment, covering the Zoxamide use in a risk envelope.

**Table 9.3-13: Risk for mammals through drinking water – a.s. Cymoxanil**

| Risk | Generic focal species | Scenario | PECdw (mg/L) | DWR (L/kg bw/d) | Toxicity value (mg as/kg bw) | TER value |
|---|---|---|---|---|---|---|
| Acute | Granivorous mammal (21.7 g) | leaf | 148.6 | 0.24 | 760 | 27 |
| | | puddle | 0.35 | 0.24 | 760 | 7306.4 |
| Long-term | Granivorous mammal (21.7 g) | puddle | 0.35 | 0.24 | 10.5 | 100.9 |

Since the obtained TER values are well above the trigger of 10 for acute risk, and of 5 for acceptable long-term risk, it is possible to conclude that the exposure to the a.s. Cymoxanil and Zoxamide via drinking water doesn't pose unacceptable risks to mammals from the intended uses of 'CYMOXANIL 33% + ZOXAMIDE 33% WG'.

### 9.3.2.4 Effects of secondary poisoning

Zoxamide

The log $P_{ow}$ of zoxamide amounts to 3.76 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required. In addition, metabolites RH-127450 and RH-24549 have log $P_{ow}$ values > 3 and are were both identified as being relevant for the soil and aquatic risk assessments. They have the potential to bioaccumulate and therefore a secondary poisoning assessment is presented.

The assessments were performed considering default values and equations of the EFSA (2009) birds and mammals guidance document (2009)[5].

Cymoxanil

As exposed above, no risk of secondary poisoning is expected for the a.s. **Cymoxanil** or its metabolites.

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

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

According to EFSA/2009/1438, the risk for vermivorous mammals is assessed for a small mammal of 10 g body weight with a daily food consumption of 12.8 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil. For earthworm eating mammals the "dry soil approach" was taken into account.

**Table 9.3-14: Assessment of the risk for earthworm-eating mammals due to exposure to zoxamide via bioaccumulation in earthworms (secondary poisoning)**

| Parameter | Zoxamide | Comments |
|---|---|---|
| $PEC_{soil}$ (~~twa = 21 d~~ accu) (mg/kg soil) | ~~0.21343~~ 0.21599 | See Part B, Section 8.7.2.1 |
| log $P_{ow}$ / $P_{ow}$ | 3.76 / 5754.4 | EFSA (2017) |
| Koc | 1179 | Geomean (n = 4) EFSA LoEP |
| Foc | 0.02 | Default |
| $BCF_{worm}$ | 2.964 | $BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$ |
| $PEC_{worm}$ | ~~0.633~~ 0.64 | $PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$ |
| Daily dietary dose (mg/kg bw/d) | ~~0.810~~ 0.82 | $DDD = PEC_{worm} \times 1.28$ |
| NOEL (mg/kg bw/d) | 71 | |
| $TER_{lt}$ | ~~87.7~~ 86.6 | |

TER values shown in bold fall below the relevant trigger.

**Table 9.3-15: Assessment of the risk for earthworm-eating mammals due to exposure to RH-127450 via bioaccumulation in earthworms (secondary poisoning)**

| Parameter | RH-127450 | Comments |
|---|---|---|
| $PEC_{soil}$ (twa = 21 d) (mg/kg soil) | ~~0.0235~~ 0.01537 | See Part B, Section 8.7.2.1 |
| $P_{ow}$ | 3.5 / 3162 | RAR (2017) |
| Koc | 593 | Geomean (n = 3) EFSA LoEP |
| Foc | 0.02 | Default |
| $BCF_{worm}$ | 3.270 | $BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$ |
| $PEC_{worm}$ | ~~0.0768~~ 0.0503 | $PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$ |
| Daily dietary dose (mg/kg bw/d) | ~~0.098~~ 0.0644 | $DDD = PEC_{worm} \times 1.28$ |
| NOEL (mg/kg bw/d) | 7.1 | Parent endpoint / 10* |
| $TER_{lt}$ | ~~72.1~~ 110.2 | |

TER values shown in bold fall below the relevant trigger.
* In the absence of a toxicity value, the metabolite is considered as 10 times more toxic than the parent compound.

**Table 9.3-16: Assessment of the risk for earthworm-eating mammals due to exposure to RH-24549 via bioaccumulation in earthworms (secondary poisoning)**

| Parameter | RH-24549 | Comments |
|---|---|---|
| $PEC_{soil}$ (twa = 21 d) (mg/kg soil) | ~~0.03495~~ 0.02214 | See Part B, Section 8.7.2.1 |
| $P_{ow}$ | 3.83 / 6760 | RAR (2017) |
| Koc | ~~161~~ 90.55 | ~~Geometric mean (n=3)~~ worst case |
| Foc | 0.02 | Default |
| $BCF_{worm}$ | ~~25.45~~ 45.257 | $BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$ |
| $PEC_{worm}$ | ~~0.890~~ 1.002 | $PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$ |
| Daily dietary dose (mg/kg bw/d) | ~~1.139~~ 1.283 | $DDD = PEC_{worm} \times 1.28$ |
| NOEL (mg/kg bw/d) | 7.1 | Parent endpoint / 10* |
| $TER_{lt}$ | ~~6.2~~ 5.5 | |

TER values shown in bold fall below the relevant trigger.
* In the absence of a toxicity value, the metabolite is considered as 10 times more toxic than the parent compound.

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

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, always the scenario with the highest PEC value was taken into account for the risk assessment.

**Table 9.3-17: Assessment of the risk for fish-eating mammals due to exposure to zoxamide via bioaccumulation in fish (secondary poisoning)**

| Parameter | Zoxamide | Comments |
|---|---|---|
| $PEC_{sw}$ (twa = 21 d) (mg/L) | 0.002645 | Max FOCUS SW Step 2 PEC (twa = 21 d)<br>See Part B, Section 8.9.2.1 |
| $BCF_{fish}$ | 136 | EFSA (2017) |
| BMF | - | biomagnification factor (relevant for BCF ≥ 2000) |
| $PEC_{fish}$ | 0.360 | $PEC_{fish} = PEC_{water} \times BCF_{fish}$ |
| Daily dietary dose (mg/kg bw/d) | 0.051 | $DDD = PEC_{fish} \times 0.142$ |
| NOEL (mg/kg bw/d) | 71 | |
| $TER_{lt}$ | 1390.0 | |

TER values shown in bold fall below the relevant trigger.

**Table 9.3-18: Assessment of the risk for fish-eating mammals due to exposure to RH-127450 via bioaccumulation in fish (secondary poisoning)**

| Parameter | RH-127450 | Comments |
|---|---|---|
| $PEC_{sw}$ (twa = 21 d) (mg/L) | 0.004759 | Max FOCUS SW Step 2 PEC (twa = 21 d)<br>See Part B, Section 8.9.2.1 |
| $BCF_{fish}$ | 136 | Parent value, EFSA (2017) |
| BMF | - | biomagnification factor (relevant for BCF ≥ 2000) |
| $PEC_{fish}$ | 0.647 | $PEC_{fish} = PEC_{water} \times BCF_{fish}$ |
| Daily dietary dose (mg/kg bw/d) | 0.092 | $DDD = PEC_{fish} \times 0.142$ |
| NOEL (mg/kg bw/d) | 7.1 | Parent endpoint / 10* |
| $TER_{lt}$ | 77.3 | |

TER values shown in bold fall below the relevant trigger.
* In the absence of a toxicity value, the metabolite is considered as 10 times more toxic than the parent compound.

**Table 9.3-19: Assessment of the risk for fish-eating mammals due to exposure to RH-24549 via bioaccumulation in fish (secondary poisoning)**

| Parameter | RH-24549 | Comments |
|---|---|---|
| $PEC_{sw}$ (twa = 21 d) (mg/L) | 0.004063 | Max FOCUS SW Step 2 PEC (twa = 21 d)<br>See Part B, Section 8.9.2.1 |
| $BCF_{fish}$ | 136 | Parent value, EFSA (2017) |
| BMF | - | biomagnification factor (relevant for BCF ≥ 2000) |
| $PEC_{fish}$ | 0.553 | $PEC_{fish} = PEC_{water} \times BCF_{fish}$ |
| Daily dietary dose (mg/kg bw/d) | 0.078 | $DDD = PEC_{fish} \times 0.142$ |
| NOEL (mg/kg bw/d) | 7.1 | Parent endpoint / 10* |
| $TER_{lt}$ | 90.5 | |

TER values shown in bold fall below the relevant trigger.
* In the absence of a toxicity value, the metabolite is considered as 10 times more toxic than the parent compound.

As a result, mammals are not at risk from secondary poisoning after application of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' to vines and potatoes.

### 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 assessment for mammals was carried out according to EFSA/2009/1438.

An acceptable acute and chronic risk to mammals was demonstrated for both active substances as well as for the potentially relevant metabolites of zoxamide and therefore also for 'CYMOXANIL 33% + ZOX-

AMIDE 33% WG'. Secondary poisoning for earthworm and fish-eating mammals and via drinking water is not likely.

Review Comments:

The acute and chronic risks of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' to mammals were assessed from toxicity exposure ratios between toxicity endpoints, estimated from study with active ingredients and maximum residues occurring on food items.

All TER values exceed the relevant triggers indicating that 'CYMOXANIL 33% + ZOXAMIDE 33% WG' does not pose an unacceptable risk to mammals following applications according to recommended use pattern. Nevertheless, the chronic combined risk of the two active ingredients was assessed assuming that the mouse and lagomorph are a focal species. Concerned Member States must decide on the consideration of this assumption on national level.

Evaluation of exposing to mammals through the drinking water demonstrated the acceptable risk. The potential risk of secondary poisoning for zoxamide, RH-127450 and RH-24549 is low.

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

According to Reg. (EU) No. 284/20013: "Where it cannot be predicted from the active substance data and, if relevant, the risk to amphibians and reptiles from plant protection products shall be addressed".

There is currently no valid test guideline available to investigate toxic effects of pesticides on amphibians and reptiles. This datapoint is therefore addressed based on the available data for the active substances.

Available toxicological and ecotoxicological studies were evaluated in depth during the renewal process for zoxamide on EU level. However, specific studies on the toxicity of the active substance and its metabolites on other terrestrial vertebrate wildlife (i.e. reptiles and amphibians) were neither available nor requested (please refer to the EFSA Peer Review Conclusion for zoxamide (2017) and the EC Renewal Report (2018)). The evaluation on EU level included a comprehensive review of the open literature of zoxamide and its metabolites. Thee review included data with regard to KCA 8.1 (Effects on birds and other terrestrial vertebrates), covering also effects on birds, mammals, reptiles and amphibians. As a result of the literature survey, no data on the toxicity of zoxamide and its metabolites relevant for risk assessment were found (please refer to the final Renewal Assessment Report for zoxamide (2017), Vol. 3, CA, B.9.11).
Moreover, EFSA concluded in his recent Peer Review Conclusion for zoxamide (2017) that "*it is unlikely that zoxamide is an endocrine disruptor for mammals"*. And further: "*Zoxamide is not classified or proposed to be classified as toxic for reproduction category 2 or carcinogenic category 2, in accordance with the provisions of Regulation (EC) No 1272/2008, and therefore the conditions of the interim provisions of Annex II, Point 3.6.5 of Regulation (EC) No 1107/2009 concerning human health for the consideration of endocrine disrupting properties are not met. With regard to the scientific risk assessment, considering the high dose at which the thyroid effects were seen in the dog studies and the low magnitude of the effect together with the negative outcome in ToxCast/Tox21, the experts agreed that zoxamide is unlikely to have endocrine disrupting potential."*

A conclusion regarding endocrine effects or effects on amphibians and reptiles is not yet available for cymoxanil (see EFSA Peer Review Conclusion for cymoxanil, 2013). These data are expected during the active substance renewal on EU level.

## 9.5 Effects on aquatic organisms (KCP 10.2)

### 9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with zoxamide, cymoxanil and their relevant metabolites. Full details of these studies are provided in the respective EU DAR, EU RAR and related documents. Effects on aquatic organisms of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' were not evaluated as part of the EU assessment of active substances.
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 processes. In case of deviations, a justification is provided in chapter 9.5.1.1.

**Zoxamide**

New data submitted with this application are listed in Appendix 1 and are summarised in Appendix 2.
Studies on the toxicity to aquatic organisms have been carried out with zoxamide and its relevant metabolites. Full details on studies are provided in the EU RAR for zoxamide (2017) and the EFSA Peer Review Conclusion (2017)[6], as well as in Appendix 2 of this document (new studies).

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

| Species | Substance | Exposure system | Results | Reference |
|---|---|---|---|---|
| **Fish** | | | | |
| *Oncorhynchus mykiss* | Zoxamide | 96 h, ft | $LC_{50}$ = **0.16 mg a.s./L** $_{mm}$ | EFSA (2017) |
| *Lepomis macrochirus* | Zoxamide | 96 h, ft | $LC_{50}$ > 0.79 mg a.s./L $_{mm}$ | EFSA (2017) |
| *Pimephales promelas* | Zoxamide | 96 h, ft | $LC_{50}$ > 0.208 mg a.s./L $_{mm}$ # | EFSA (2017) |
| *Brachydanio rerio* | Zoxamide | 96 h, ft | $LC_{50}$ > 0.73 mg a.s./L $_{mm}$ | EFSA (2017) |
| *Cyprinodon variegatus* | Zoxamide | 96 h, ft | $LC_{50}$ > 0.85 mg a.s./L $_{mm}$ | EFSA (2017) |
| *Danio rerio* | Zoxium 240 SC ** | 96 h, s | $LC_{50}$ = 0.184 mg a.s./L $_{mm}$<br>$LC_{50}$ = 0.865 mg f.p./L $_{mm}$ | EFSA (2017) |
| *Oncorhynchus mykiss* | RH-139432 | 96 h, ft | $LC_{50}$ **= 2 mg a.s./L** $_{mm}$ | EFSA (2017) |
| *Oncorhynchus mykiss* | RH-24549 | 48 h, ss | $LC_{50}$ = **23 mg a.s./L** $_{mm}$ | EFSA (2017) |
| *Oncorhynchus mykiss* | RH-127450 | 96 h, ss | $LC_{50}$ = **4.17 mg a.s./L** $_{mm}$ | xxx. (2020) / 3202373 |
| *Oncorhynchus mykiss* | RH-163353 | 96 h, s | $LC_{50}$ **> 100 mg a.s./L** $_{nom}$ | xxx (2020) / 3202385 |
| *Oncorhynchus mykiss* | RH-141455 | 96 h, s | $LC_{50}$ **> 100 mg a.s./L** $_{nom}$ | xxx (2020) / 3202716 |
| *Oncorhynchus mykiss* | Zoxamide | 95 d, ft, ELS | NOEC = **0.00348 mg a.s./L** $_{mm}$ | EFSA (2017) |
| *Pimephales promelas* | Zoxamide | 202 d, ft, FLC | NOEC = 0.06 mg a.s./L $_{mm}$ | EFSA (2017) |
| *Danio rerio* | Zoxamide | 30 d, post-hatch, ft, ELS | NOEC ≥ 0.12 mg a.s./L $_{mm}$ | EFSA (2017) |

[6] EFSA (2017): Conclusion on the peer review of the pesticide risk assessment of the active substance zoxamide. EFSA Journal 2017, 5 (9):4980

| Species | Substance | Exposure system | Results | Reference |
|---|---|---|---|---|
| ~~*Cyprinodon variegatus*~~ | ~~Zoxamide~~ | ~~34 d, ft, ELS~~ | ~~NOEC = 0.04 mg a.s./L mm~~<br>~~$EC_{10}$ = 0.093 mg a.s./L~~<br>~~(fish wet weight)~~ | ~~xxx. (1998) / 97RC-0078 plus report addendum by Milligan et al. (2020)~~ |
| *Lepomis macrochirus* | Zoxamide | 28 d, ft, bioaccumulation | BCF = 95-136 mg a.s./L mm | EFSA (2017) |
| **Aquatic invertebrates** | | | | |
| *Daphnia magna* | Zoxamide | 48 h, ft | $LC_{50}$ > 0.78 mg a.s./L mm | EFSA (2017) |
| *Daphnia magna* | Zoxium 240 SC ** | 48 h, s | $EC_{50}$ > 0.69 mg a.s./L mm | EFSA (2017) |
| *Mysidopsis bahia* | Zoxamide | 96 h, ft | $LC_{50}$ = 0.076 mg a.s./L mm | EFSA (2017) |
| *Daphnia magna* | RH-139432 | 48 h, ss | $LC_{50}$ = 17 mg a.s./L mm | EFSA (2017) |
| *Americamysis bahia* | RH-139432 | 96 h, s | $LC_{50}$ = 6.95 mg a.s./L mm | Hugill, E. (2020) / 3202398 |
| *Daphnia magna* | RH-24549 | 48 h, s | $LC_{50}$ = 40 mg a.s./L mm | EFSA (2017) |
| *Americamysis bahia* | RH-24549 | 96 h, s | $LC_{50}$ = 23.2 mg a.s./L | Hugill, E. (2020) / 3202394 |
| *Americamysis bahia* | RH-127450 | 96 h, s | $LC_{50}$ = 2.93 mg a.s./L mm | Hugil, E. (2020) / 3202374 |
| ~~*Daphnia magna*~~ | ~~RH-141455~~ | ~~48 h, s~~ | ~~$LC_{50}$ > 100 mg a.s./L nom~~ | ~~Hugill, E. (2020) / 3202380~~ |
| *Americamysis bahia* | RH-141455 | 96 h, ss | $LC_{50}$ > 100 mg a.s./L nom | Hugil, E. (2020) / 3202381 |
| ~~*Daphnia magna*~~ | ~~RH-163353~~ | ~~48 h, s~~ | ~~$LC_{50}$ > 100 mg a.s./L nom~~ | ~~xxx. (2020) / 3202386~~ |
| *Americamysis bahia* | RH-163353 | 96 h, s | $LC_{50}$ > 100 mg a.s./L nom | Jarrom, R. (2020) / 3202387 |
| *Daphnia magna* | Zoxamide | 21 d, ft | NOEC = 0.039 mg a.s./L mm | EFSA (2017) |
| *Mysidopsis bahia* | Zoxamide | 27 d, ft | NOEC = **0.0072 mg a.s./L mm** | EFSA (2017) |
| *Chironomus riparius* | Zoxamide | 28 d, ft, spiked water | $NOEC_{(emergence\ rate)}$ = **0.38 mg a.s./L**[##]<br>$EC_{10\ (developmental\ rate)}$ = 0.223 mg a.s./L<br>$EC_{10\ (emergence\ rate)}$ = 0.318 mg a.s./L | EFSA (2017) |
| **Algae** ° | | | | |
| *Selenastrum capricornutum* | Zoxamide | 72, 96, 120 h, s | 72-96 h-$E_rC_{50}$ = **0.01413 mg a.s./L mm**<br>120 h-$E_bC_{50}$ = 0.023 mg a.s./L mm | RAR (2017)<br>Ziegler T.A., Stewart S. (1996) / 94RC-0238 |
| *Anabaena flos-aquae* | Zoxamide | 96 h, s | $E_rC_{50}$ > 0.86 mg a.s./L mm<br>$E_bC_{50}$ > 0.86 mg a.s./L mm | RAR (2017)<br>xxx. (1998) / 97RC-0130 |
| *Scenedesmus subspicatus* | Zoxamide | 96 h, s | $E_rC_{50}$ = 0.018 mg a.s./L mm<br>$E_bC_{50}$ = 0.011 mg a.s./L mm | RAR (2017)<br>xxx. (1998) / 97RC-0133 |
| *Navicula pelliculosa* | Zoxamide | 96 h, s | $E_rC_{50}$ > 0.93 mg a.s./L mm | RAR (2017) |

| Species | Substance | Exposure system | Results | Reference |
|---|---|---|---|---|
| | | | $E_bC_{50}$ > 0.93 mg a.s./L $_{mm}$ | xxx. (1998) / 97RC-0131 |
| *Skeletonema costatum* | Zoxamide | 96 h, s | $E_rC_{50}$ > 0.91 mg a.s./L $_{mm}$<br>$E_bC_{50}$ > 0.91 mg a.s./L $_{mm}$ | RAR (2017)<br>xxx. (1998) / 97RC-0132 |
| *Selenastrum capricornutum* | RH-117,281 2F ** | 96 h, s | $E_rC_{50}$ = 0.0582 mg a.s./L $_{mm}$ *<br>$E_bC_{50}$ = 0.0514 mg a.s./L $_{mm}$ * | EFSA (2017) |
| *Scenedesmus subspicatus* | RH-139432 | 72 h, s | $E_rC_{50}$ > **30 mg a.s./L $_{mm}$**<br>$E_bC_{50}$ = 26 mg a.s./L $_{mm}$ | RAR (2017)<br>Hoberg, J.R. (2002) / 12550.6288 |
| *Desmodesmus subspicatus* | RH-24549 | 72 h, s | $E_rC_{50}$ > **60 mg a.s./L $_{nom}$**<br>$E_bC_{50}$ > 60 mg a.s./L $_{nom}$ | EFSA (2017) |
| *Pseudokirchneriella subcapitata* | RH-141455 | 72 h, s | $EC_{50}$ > **100 mg a.s./L $_{nom}$** | EFSA (2017) |
| *Raphidocelis subcapitata* | RH-127450 | 72 h, s | $E_rC_{50}$ > **6.60 mg a.s./L $_{mm}$**<br>$E_yC_{50}$ = 5.98 mg a.s./L $_{mm}$ | Hugill, E. (2020) / 3202375 |
| *Raphidocelis subcapitata* | RH-163353 | 72 h, s | $E_rC_{50}$ > **100 mg a.s./L**<br>$E_yC_{50}$ > 100 mg a.s./L | Jarrom, R. (2020) / 3202388 |
| **Aquatic plants** ° | | | | |
| *Lemna gibba* | Zoxamide | 14 d, ss | 7-d $EC_{50}$ > 0.018 mg a.s./L $_{mm}$<br>14-d $EC_{50}$ = 0.017 mg a.s./L $_{mm}$<br>NOEC = 0.009 mg a.s./L $_{mm}$ | EFSA (2017) |
| ~~*Lemna gibba*~~ | ~~Zoxamide~~ | ~~7 d, ss~~ | ~~$E_rC_{50}$ = **0.0237 mg a.s./L** $_{nom}$~~<br>~~$E_yC_{50}$ = 0.0122 mg a.s./L $_{mm}$~~ | ~~Juckeland D. (2020) 18-48-ALE-0005~~ |
| **Higher-tier studies (micro- or mesocosm studies)** | | | | |
| | | | | |

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

* endpoints expressed as mg formulation/L are converted to mg a.s./L considering the purity of the formulation (21.24 %, w/w)

** RH-117,281 2F is a very similar formulation to Zoxium 240 SC. Refer to Document J-CP for details of both formulations. However, the toxicity endpoints for RH-117,281 2F / Zoxium 240 SC are not relevant for the risk assessment of 'CYMOXANIL 33% + ZOXAMIDE 33% WG'.

# Mistake in the EFSA Peer Review Conclusion (2017), which has been corrected based on xxx. (1998): RH-117,281 Technical: A flow-through life-cycle toxicity test with the fathead minnow (*Pimephales promelas*), report no. 97RC-0079, and based on the study summary in the RAR (2017).

## In line with the risk calculation during AIR / in the RAR (2017).

° According to the EFSA (2013) guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters, growth rate endpoints ($E_rRC_{50}$ values) are preferred.

**bold** = worst-case endpoint taken forward for the aquatic risk assessment

## Cymoxanil

The EU review concluded the following data for Cymoxanil as relevant for the aquatic risk assessment.

**Table 9.5-2: EU Endpoints - toxicity of Cymoxanil to aquatic organisms**

| Substance | Test species | EU agreed endpoints (EFSA Scientific Report of Cymoxanil (2008)) |
|---|---|---|
| ***Acute toxicity to fish*** | | |
| Cymoxanil | *Lepomis macrochirus* | $EC_{50}$ = 29 mg/L (s /96 h) |
| IN-U3204 | *Oncorhyncus mykiss* | $EC_{50}$ > 97 mg/L (ss /96 h) |
| IN-W3595 | *Oncorhyncus mykiss* | $EC_{50}$ > 130 mg/L (s /96 h) |
| IN-KQ960 | *Oncorhyncus mykiss* | $EC_{50}$ > 120 mg/L (s /96 h) |
| IN-T4226 | *Oncorhyncus mykiss* | $EC_{50}$ > 111 mg/L (ss /96 h) |
| ***Long-term toxicity to fish*** | | |
| Cymoxanil | *Oncorhyncus mykiss* | NOEC = 0.044 mg/L (f /90 d) |
| ***Acute toxicity to aquatic invertebrates*** | | |
| Cymoxanil | *Daphnia magna* | $EC_{50}$ = 27 mg/L (s /48 h) |
| IN-U3204 | *Daphnia magna* | $EC_{50}$ = 100 mg/L (ss /48 h) |
| IN-W3595 | *Daphnia magna* | $EC_{50}$ > 126 mg/L (s /48 h) |
| IN-KQ960 | *Daphnia magna* | $EC_{50}$ = 0.8 mg/L (s /48 h) |
| IN-T4226 | *Daphnia magna* | $EC_{50}$ > 116 mg/L (ss /48 h) |
| ***Long-term toxicity to aquatic invertebrates*** | | |
| Cymoxanil | *Daphnia magna* | NOEC = 0.067 mg/L (ss /21d) |
| IN-KQ960 | *Daphnia magna* | NOEC = 0.302 mg/L (ss /21 d) |
| ***Toxicity to green algae*** | | |
| Cymoxanil | *Anabaena flos-aquae* | $EC_{50}$ = 0.122 mg/L (s /96 h) |
| IN-W3595 | *Anabaena flos-aquae* | $EC_{50}$ = 12.7 mg/L (s /96 h) |
| IN-T4226 | *Anabaena flos-aquae* | $EC_{50}$ = 25.8 mg/L (s /96 h) |
| ***Toxicity to aquatic plants*** | | |
| Cymoxanil | *Lemna gibba* | $EC_{50}$ > 0.7 mg/L (s /14 h) |

**Table 9.5-3: Endpoints and effect values relevant for the risk assessment for aquatic organisms – 'CYMOXANIL 33% + ZOXAMIDE 33% WG'**

| Organism | Species | Endpoint | Value | Reference |
|---|---|---|---|---|
| Fish | *Oncorhynchus mykiss* | 96h $LC_{50}$ | 0.83 mg prod./L *(corresponding to 0.27 mg a.s./L)* | xxx.; report no. CH-E-023/2006 |
| Aquatic invertebrates | *Daphnia magna* | 48h $EC_{50}$ | > 44.64 mg prod./L *(corresponding to 14.73 mg a.s./L)* | xxx.; report no. CH-001/2007 |
| Algae | *Pseudokirchneriella subcapitata* | 72h $E_bC_{50}$ | 0.055 mg/L *(corresponding to 0.018 mg a.s./L)* | xxx.; report no. CH-E-002/2007 |
| | | 72h $E_rC_{50}$ | 0.151 mg/L ° | |

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

° According to the EFSA (2013) guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters, growth rate endpoints ($E_rRC_{50}$ values) are preferred.

#### 9.5.1.1 Justification for new endpoints

*Acute endpoints for zoxamide metabolites on fish*

EFSA (2017) requested in its Peer Review Conclusion: *"Further data or refinement (exposure and/or effects) for the acute risk assessment of fish for the metabolites RH-127450, RH-163353 and RH-141455 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Sections 4 and 5)."* To close these data gaps, acute toxicity studies with the metabolites RH-127450, RH-163353 and RH-141455 were provided. They are summarised in Appendix 2 of this document.

*Chronic endpoint for zoxamide on fish*

An additional fish ELS study with sheepshead minnow (xxx (1998), report no. 97RC-0078) is available from the authorisation of zoxamide and its products in the US. The study has been evaluated by US EPA, but not yet by European authorities. It is therefore provided with this submission to complete the picture on chronic toxicity of zoxamide to fish and to use it in the aquatic risk assessment (i.e. for a species sensitivity distribution).

The study has been performed at Wildlife International in the US. To support the report of xxx (1998), the legal successor of Wildlife International in the US has been asked to re-evaluate the findings with regard to current guidelines and based on the report data and the raw data still available in the laboratory. Please refer to the findings of the "Final Report Addendum for RH-117,281 Technical: An Early Life-stage Toxicity Test with The Sheepshead Minnow (*Cyprinodon variegatus*)" (Milligan et al., 2020) in Appendix 2; they are presented together with the summary of the original report.

In the sheepshead minnow ELS test conducted with RH-117,281 technical (synonym for zoxamide technical) in 1997 at Wildlife International in the US, up to a nominal concentration of 0.30 mg a.i./L (mean measured concentration of 0.25 mg a.i./L, or 83% of the nominal test concentration) were tested under flow-through conditions. As a result, the NOEC for this study was determined at 0.040 mg a.i./L. The LOEC, based on wet weight, was 0.078 mg a.i/L. The $EC_{10}$ value for fish wet weight was estimated to be 0.093 mg a.i./L, with a 95 % confidence interval of 0.054 to 0.14 mg a.i./L. $EC_{20}$ and $EC_{50}$ values for wet weight were not reportable since they were extrapolated above the highest mean measured test concentration.

*Acute endpoint for zoxamide on aquatic invertebrates*

Under EU Regulation (EC) No. 1107/2009 additional data on saltwater crustacean species may be required only for insecticides, therefore this is not an EU data requirement for zoxamide; however, data on the saltwater crustacean *Mysidopsis bahia* are available for the parent compound, so they are both considered in the risk assessment for safety reasons. In-line with the EFSA aquatic guidance document (2013) a refined acute toxicity endpoint can be used, taking into account the geometric mean $EC_{50}/LC_{50}$ of both invertebrates belonging to the same taxonomic group (crustaceans). This approach is considered appropriate as it has been indicated that sensitivity distributions of taxonomically similar freshwater and marine species to organic plant protection products do not differ significantly and thus the data can be combined (EFSA Aquatic Guidance, 2013). In addition, the resulting geomean $EC_{50}/LC_{50}$ (229 µg a.s./L) is less than an order of magnitude greater than the acute toxicity endpoint of the most sensitive species *Mysidopsis bahia* ($LC_{50}$ = 76 µg a.s./L); therefore, it is considered that the geomean approach in this case is not biased by using data on insensitive species. This approach was agreed for refinement in the RAR for zoxamide (2017; please refer to Vol. 3, CP, B.9).

As expected by EFSA (2017) and confirmed by the available toxicity endpoints for zoxamide and its metabolites, mysids give the more sensitive endpoints compared to daphnids. Therefore, for the zoxamide metabolite RH-127450 an aquatic risk assessment with the available mysid endpoint is regarded sufficient to conclude a safe use.

*Chronic toxicity endpoint for zoxamide on aquatic invertebrates*

Under EU Regulation (EC) No. 1107/2009 additional data on saltwater crustacean species may be required only for insecticides, therefore this is not an EU data requirement for zoxamide; however, data on the saltwater crustacean *Mysidopsis bahia* are available for the parent compound and the endpoint is lower than the *Daphnia* endpoint, so they are both taken into account in the risk assessment for safety reasons.
The available studies demonstrate a lower toxicity for the aquatic crustacean *Daphnia magna* and *Mysidopsis bahia* (NOEC = 39 µg a.s./L and 7.2 µg a.s/L, respectively) than for aquatic insects (NOEC for *Chironomus riparius* = 380 µg a.s./L). This is confirmed by the available data for terrestric non-target arthropods. Therefore, in line with the EFSA Aquatic Guidance (2013), a refined risk assessment is performed for the more sensitive taxonomic group of (aquatic) crustacean.

*Acute endpoints for zoxamide metabolites on aquatic invertebrates*

EFSA (2017) requested in its Peer Review Conclusion: "*Further data or refinement on aquatic invertebrates (Mysidopsis bahia) are needed to cover the risk for the metabolites RH-127450, RH-24549, RH-163353, RH-141455 and RH-139432 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5).*" To close this data gap, applicable studies on daphnids and mysids are presented. They are summarised in Appendix 2 of this document.

As expected by EFSA (2017) and confirmed by the available toxicity endpoints for zoxamide and its metabolites, mysids give the more sensitive endpoints compared to daphnids. Therefore, for the zoxamide metabolite RH-127450 an aquatic risk assessment with the available mysid endpoint is regarded sufficient to conclude a safe use.

*Alga endpoints for zoxamide and its metabolites*

EFSA (2017) requested in its Peer Review Conclusion: "Further algae studies following the latest OECD 201 guideline are needed or further detailed information on all validity criteria requested by the latest OECD 201 guideline from the studies provided in the RAR for zoxamide, RH-127450 and RH-163353 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5)." To close this data gap, new studies on the inhibition of alga growth by the zoxamide metabolites RH-127450 and RH-163353 have been performed and are presented (see study summary in Appendix 2).

With regard to the EFSA request on alga studies with the parent compound zoxamide and RH-139432: The alga studies with zoxamide and RH-139432 which are available in the RAR for zoxamide (2017) have been re-evaluated at a later stage during AIR based on additionally provided information. The results of the re-evaluation were included in the RAR, the alga studies were regarded valid. This was confirmed by Latvia as RMS for zoxamide. As such, the endpoints from the alga studies with the active substance zoxamide and RH-139432 available in the RAR (2017) are valid and applicable for the aquatic risk assessment.

*Lemna* endpoint for zoxamide

The *Lemna* study in the RAR (2017; xxx., 1998b; CA 8.2.7/01) was not conducted according to current guidelines. In this study, 7 and 14 days $IC_{50}$ values of >18 (highest test concentration) and 17 µg a.i./L,

respectively, were laid down for zoxamide in the EFSA Review Report (2004) and in the RAR (2017). However, due to lack of data around the $IC_{50}$ concentration, the endpoint for risk assessment with regard to growth rate was set to the NOEC of 9.0 µg a.s./L. Therefore, as replacement for the old study with several deficiencies, the applicant performed a new study according to current provisions on the toxicity of zoxamide to *Lemna gibba* (Juckeland, 2020; report no. 18 48 ALE 0005). This study contains a valid $E_rC_{50}$ value for biomass ($E_rC_{50}$ = 0.0237 mg a.s./L) based on nominal test concentrations, which is regarded applicable for risk assessment.

Review Comments:

In zRMS opinion, the endpoint agreed on EU level should be used in the risk assessment. Moreover, taking to consideration that NOEC of 9 µg/L is not the lowest value, overall won't have any impact on the evaluation.

<u>*Alga* and *Lemna* endpoints</u>

According to 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) 1107/2009" for the aquatic risk assessment on algae, *Lemna* and other higher aquatic plants growth rate (r) is the preferred endpoint according to EFSA guidance[7] and was therefore taken into account as far as available as endpoint for the 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) 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 are presented in section 5. 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 and presented below. Worst-case PEC values from single and multiple applications were considered.

<u>Zoxamide</u>

[7] EFSA (2013): Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290, 268 pp.

**Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for zoxamide for each organism group based on FOCUS Steps 1, 2 and 3 calculations (worst-case PECs) for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in vines, early**

| Group | | Fish acute | Fish prolonged | Inverteb. acute | Inverteb. prolonged | Algae | Sed. dwell. prolonged | ~~Aquatic plant~~ | Aquatic plant |
|---|---|---|---|---|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Oncorhynchus mykiss*** | **Geomean of available data*** | ***Mysidopsis bahia*** | ***Selenastrum capricornutum*** | ***Chironomus riparius*** | ~~*Lemna gibba*~~ | ***Lemna gibba*** |
| **Endpoint (µg/L)** | | $LC_{50}$ 160 | NOEC 3.48 | $EC_{50}$ 229 | NOEC 7.2 | $E_rC_{50}$ 14.13 | NOEC 380 | ~~$E_rC_{50}$ 23.7~~ | NOEC 9 |
| **AF** | | 100 | 10 | 100 | 10 | 10 | 10 | ~~10~~ | 10 |
| **RAC (µg/L)** | | 1.6 | 0.348 | 2.29 | 0.72 | 1.413 | 38 | ~~2.37~~ | 0.9 |
| **FOCUS Scenario** | **PEC $_{gl\text{-}max}$ (µg/L)** | | | | | | | - | |
| **Step 1** | | | | | | | | - | |
| | 61.745 | **38.591** | **177.428** | **26.963** | **85.757** | **43.698** | **1.625** | ~~**26.053**~~ | **68.6** |
| **Step 2** | | | | | | | | - | |
| N-Europe | 2.886 | **1.804** | **8.293** | **1.260** | **4.008** | **2.042** | 0.076 | ~~**1.218**~~ | **3.21** |
| S-Europe | 5.097 | **3.186** | **14.647** | **2.226** | **7.079** | **3.607** | 0.134 | ~~**2.151**~~ | **5.66** |
| **Step 3** | | | | | | | | - | |
| ~~D6/ditch~~ | ~~0.773~~ | ~~0.483~~ | ~~**2.221**~~ | ~~0.338~~ | ~~**1.074**~~ | ~~0.547~~ | ~~0.020~~ | ~~0.326~~ | |
| D6/ditch | 0.830 | 0.519 | **2.385** | 0.362 | **1.153** | 0.587 | - | | 0.922 |
| R1/pond | 0.059 | 0.037 | 0.170 | 0.026 | 0.082 | 0.042 | ~~0.002~~ | ~~0.025~~ | 0.066 |
| R1/stream | 0.708° | 0.443 | **2.034** | 0.309 | 0.983 | 0.501 | ~~0.019~~ | ~~0.299~~ | 0.787 |
| ~~R2/stream~~ | ~~0.743~~ | ~~0.464~~ | ~~**2.135**~~ | ~~0.324~~ | ~~**1.032**~~ | ~~0.526~~ | ~~0.020~~ | ~~0.314~~ | |
| R2/stream | 0.812 | 0.508 | **2.333** | 0.355 | **1.128** | 0.575 | - | | 0.902 |
| ~~R3/stream~~ | ~~0.784~~ | ~~0.490~~ | ~~**2.253**~~ | ~~0.342~~ | ~~**1.089**~~ | ~~0.555~~ | ~~0.021~~ | ~~0.331~~ | |
| R3/stream | 0.866 | 0.541 | **2.488** | 0.378 | **1.203** | 0.496 | - | | 0.962 |
| R4/stream | 1.153° | 0.721 | **3.313** | 0.503 | **1.601** | 0.816 | ~~0.030~~ | ~~0.486~~ | **1.282** |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration;
* See par. 9.5.1.1 for details; ° run-off/drainage induced peak
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 zoxamide for each organism group based on FOCUS Steps 1, 2 and 3 calculations (worst-case PECs) for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in vines, late**

| **Group** | | **Fish acute** | **Fish prolonged** | **Inverteb. acute** | **Inverteb. prolonged** | **Algae** | **Sed. dwell. prolonged** | **Aquatic plant** | **Aquatic plant** |
|---|---|---|---|---|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Oncorhynchus mykiss*** | **Geomean of available data*** | ***Mysidopsis bahia*** | ***Selenastrum capricornutum*** | ***Chironomus riparius*** | ***Lemna gibba*** | ***Lemna gibba*** |
| **Endpoint (µg/L)** | | $LC_{50}$ 160 | NOEC 3.48 | $EC_{50}$ 229 | NOEC 7.2 | $E_rC_{50}$ 14.13 | NOEC 380 | $E_rC_{50}$ 23.7 | NOEC 9 |
| **AF** | | 100 | 10 | 100 | 10 | 10 | 10 | 10 | 10 |
| **RAC (µg/L)** | | 1.6 | 0.348 | 2.29 | 0.72 | 1.413 | 38 | 2.37 | 0.9 |
| **FOCUS Scenario** | **PEC $_{gl\text{-}max}$ (µg/L)** | | | | | | | | |
| **Step 1** | | | | | | | | | |
| | 69.659 | **43.537** | **200.170** | **30.419** | **96.749** | **49.299** | **1.833** | **29.392** | **77.399** |
| **Step 2** | | | | | | | | | |
| N-Europe | 4.562 | **2.851** | **13.109** | **1.992** | **6.336** | **3.229** | 0.120 | **1.925** | **5.069** |
| S-Europe | 4.562 | **2.851** | **13.109** | **1.992** | **6.336** | **3.229** | 0.120 | **1.925** | **5.069** |
| **Step 3** | | | | | | | | | |
| D6/ditch | 3.156 | **1.973** | **9.069** | **1.378** | **4.383** | **2.234** | ~~0.083~~ | **1.332** | **3.507** |
| R1/pond | 0.192 | 0.120 | 0.552 | 0.084 | 0.267 | 0.136 | ~~0.005~~ | 0.081 | 0.213 |
| ~~R1/stream~~ | ~~1.592~~ | ~~0.995~~ | ~~4.575~~ | ~~0.695~~ | ~~2.211~~ | ~~1.127~~ | ~~0.042~~ | ~~0.672~~ | |
| R1/stream | 1.868 | **1.167** | **5.368** | 0.816 | **2.594** | **1.322** | - | | **2.076** |
| ~~R2/stream~~ | ~~2.134~~ | ~~1.334~~ | ~~6.132~~ | ~~0.932~~ | ~~2.964~~ | ~~1.510~~ | ~~0.056~~ | ~~0.900~~ | |
| R2/stream | 2.503 | **1.564** | **7.193** | **1.093** | **3.476** | **1.771** | - | | **2.781** |
| ~~R3/stream~~ | ~~2.244~~ | ~~1.403~~ | ~~6.448~~ | ~~0.980~~ | ~~3.117~~ | ~~1.588~~ | ~~0.059~~ | ~~0.947~~ | |
| R3/stream | 2.635 | **1.647** | **7.572** | **1.151** | **3.660** | **1.865** | - | | **2.928** |
| ~~R4/stream~~ | ~~1.591~~ | ~~0.994~~ | ~~4.572~~ | ~~0.695~~ | ~~2.210~~ | ~~1.126~~ | ~~0.042~~ | ~~0.671~~ | |
| R4/stream | 1.869 | **1.168** | **5.371** | 0.816 | **2.596** | **1.323** | - | | **2.077** |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration;
* See par. 9.5.1.1 for details. 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 zoxamide for each organism group based on FOCUS Steps 1, 2 and 3 calculations (worst-case PECs) for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in potatoes**

| Group | | Fish acute | Fish prolonged | Inverteb. acute | Inverteb. prolonged | Algae | Sed. dwell. prolonged | ~~Aquatic plant~~ | Aquatic plant |
|---|---|---|---|---|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Oncorhynchus mykiss*** | **Geomean of available data*** | ***Mysidopsis bahia*** | ***Selenastrum capricornutum*** | ***Chironomus riparius*** | ~~*Lemna gibba*~~ | ***Lemna gibba*** |
| **Endpoint (µg/L)** | | $LC_{50}$ 160 | NOEC 3.48 | $EC_{50}$ 229 | NOEC 7.2 | $E_rC_{50}$ 14.13 | NOEC 380 | ~~$E_rC_{50}$ 23.7~~ | NOEC 9 |
| **AF** | | 100 | 10 | 100 | 10 | 10 | 10 | ~~10~~ | 10 |
| **RAC (µg/L)** | | 1.6 | 0.348 | 2.29 | 0.72 | 1.413 | 38 | ~~2.37~~ | 0.9 |
| **FOCUS Scenario** | **PEC $_{gl\text{-}max}$ (µg/L)** | | | | | | | - | |
| **Step 1** | | | | | | | | - | |
| | 61.834 | **38.646** | **177.684** | **27.002** | **85.881** | **43.761** | **1.627** | ~~**26.090**~~ | **68.704** |
| **Step 2** | | | | | | | | - | |
| N-Europe | 3.669 | **2.293** | **10.543** | **1.602** | **5.096** | **2.597** | 0.097 | ~~**1.548**~~ | **4.077** |
| S-Europe | 6.802 | **4.251** | **19.546** | **2.970** | **9.447** | **4.814** | 0.179 | ~~**2.870**~~ | **7.558** |
| **Step 3** | | | | | | | | - | |
| ~~D3/ditch~~ | ~~0.565~~ | ~~0.353~~ | ~~**1.624**~~ | ~~0.247~~ | ~~0.785~~ | ~~0.400~~ | ~~0.015~~ | ~~0.238~~ | |
| D3/ditch | 0.778 | 0.486 | **2.236** | 0.340 | **1.081** | 0.551 | - | | 0.864 |
| D4/pond | 0.058 | 0.036 | 0.167 | 0.025 | 0.081 | 0.041 | ~~0.002~~ | ~~0.024~~ | 0.064 |
| ~~D4/stream~~ | ~~0.454~~ | ~~0.284~~ | ~~**1.305**~~ | ~~0.198~~ | ~~0.631~~ | ~~0.321~~ | ~~0.012~~ | ~~0.192~~ | |
| D4/stream | 0.608 | 0.380 | **1.747** | 0.266 | 0.844 | 0.430 | - | | 0.676 |
| ~~D6 1st/ditch~~ | ~~0.563~~ | ~~0.352~~ | ~~**1.618**~~ | ~~0.246~~ | ~~0.782~~ | ~~0.398~~ | ~~0.015~~ | ~~0.238~~ | |
| D6 1st/ditch | 0.770 | 0.481 | **2.213** | 0.336 | **1.069** | 0.545 | - | | 0.855 |
| ~~D6 2nd/ditch~~ | ~~0.559~~ | ~~0.349~~ | ~~**1.606**~~ | ~~0.244~~ | ~~0.776~~ | ~~0.396~~ | ~~0.015~~ | ~~0.236~~ | |
| D6 2nd/ditch | 0.764 | 0.477 | **2.195** | 0.334 | **1.061** | 0.541 | - | | 0.849 |
| R1/pond | 0.087 | 0.054 | 0.250 | 0.038 | 0.121 | 0.062 | ~~0.002~~ | ~~0.037~~ | 0.097 |
| ~~R1/stream~~ | ~~0.534°~~ | ~~0.334~~ | ~~**1.534**~~ | ~~0.233~~ | ~~0.742~~ | ~~0.378~~ | ~~0.014~~ | ~~0.225~~ | |

| **Group** | | **Fish acute** | **Fish prolonged** | **Inverteb. acute** | **Inverteb. prolonged** | **Algae** | **Sed. dwell. prolonged** | ~~**Aquatic plant**~~ | **Aquatic plant** |
|---|---|---|---|---|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Oncorhynchus mykiss*** | **Geomean of available data*** | ***Mysidopsis bahia*** | ***Selenastrum capricornutum*** | ***Chironomus riparius*** | ~~***Lemna gibba***~~ | ***Lemna gibba*** |
| **Endpoint (µg/L)** | | $LC_{50}$ 160 | NOEC 3.48 | $EC_{50}$ 229 | NOEC 7.2 | $E_rC_{50}$ 14.13 | NOEC 380 | ~~$E_rC_{50}$ 23.7~~ | NOEC 9 |
| **AF** | | 100 | 10 | 100 | 10 | 10 | 10 | ~~10~~ | 10 |
| **RAC (µg/L)** | | 1.6 | 0.348 | 2.29 | 0.72 | 1.413 | 38 | ~~2.37~~ | 0.9 |
| **FOCUS Scenario** | **PEC $_{gl\text{-}max}$ (µg/L)** | | | | | | | - | |
| R1/stream | 0.540 | 0.900 | **1.552** | 0.236 | 0.750 | 0.382 | | | 0.600 |
| ~~R2/stream~~ | ~~0.524~~ | ~~0.328~~ | ~~**1.506**~~ | ~~0.229~~ | ~~0.728~~ | ~~0.371~~ | ~~0.014~~ | ~~0.221~~ | |
| R2/stream | 0.712 | 0.445 | **2.046** | 0.311 | 0.989 | 0.504 | | | 0.791 |
| ~~R3/stream~~ | ~~0.688°~~ | ~~0.430~~ | ~~**1.977**~~ | ~~0.300~~ | ~~0.956~~ | ~~0.487~~ | ~~0.018~~ | ~~0.290~~ | |
| R3/stream | 0.760 | 0.475 | **2.184** | 0.332 | **1.056** | 0.538 | | | 0.667 |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration;
* See par. 9.5.1.1 for details; ° run-off/drainage induced peak
PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For several of the aquatic organism groups the PEC/RAC ratios for zoxamide are above 1 for the intended uses when max FOCUS Step 1, 2 and 3 $PEC_{sw}$ values are considered. To demonstrate that no unacceptable risk is expected following the application of 'CYMOXANIL 33% + ZOXAMIDE 33% WG', further refinements are required for zoxamide.
Further PEC/RAC ratios were calculated based on FOCUS SW Step 4 PEC values, taking into account more realistic scenarios and a substance specific geometric mean $DT_{50}$ value (n=32) for zoxamide on leaves. For PEC values see Part B.8 chapter 8.9.2.1.

**Table 9.5-7: Aquatic organisms: PEC/RAC ratios for <u>zoxamide</u> based on the RAC for fish long-term and FOCUS SW Step 4 calculations with mitigation of plant $DT_{50}$, spray drift and run-off for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in <u>vines, early, multiple applications</u>**

| **Intended use**<br>**Active substance**<br>**Application rate (g/ha)** | | Vines, early, multiple applications<br>Zoxamide<br>3 × 148.5 | | | |
|---|---|---|---|---|---|
| **RAC (µg/L) fish long-term** | | 0.348 | | | |
| **Nozzle reduction** | **No-spray buffer (m)** | 1/3 | 10 | 15 | 20 |
| | **Vegetated filter strip (m)** | FOCUS default | 10 | 15 | 20 |
| None | D6 ditch | **2.221** | 0.417 | 0.213 | 0.132 |
| 50% | | **1.112** | 0.210 | 0.106 | 0.066 |
| 75% | | 0.555 | 0.103 | 0.055 | 0.032 |
| 90% | | 0.221 | 0.043 | 0.020 | 0.014 |
| None | R1 pond | 0.170 | 0.103 | 0.066 | 0.049 |
| 50% | | 0.089 | 0.052 | 0.034 | 0.023 |
| 75% | | 0.055 | 0.029 | 0.020 | 0.014 |
| 90% | | 0.034 | 0.017 | 0.011 | 0.009 |
| None | R1 stream | **2.034** | 0.859 | 0.652 | 0.440 |
| 50% | | **2.034** | 0.859 | 0.652 | 0.440 |
| 75% | | **2.034** | 0.859 | 0.652 | 0.440 |
| 90% | | **2.034** | 0.859 | 0.652 | 0.440 |
| None | R2 stream | **2.135** | 0.491 | 0.250 | 0.155 |
| 50% | | **1.066** | 0.247 | 0.126 | 0.078 |
| 75% | | 0.534 | 0.124 | 0.063 | 0.040 |
| 90% | | 0.213 | 0.055 | 0.040 | 0.029 |
| None | R3 stream | **2.253** | 0.520 | 0.264 | 0.164 |
| 50% | | **1.126** | 0.259 | 0.132 | 0.083 |
| 75% | | 0.563 | 0.129 | 0.066 | 0.040 |
| 90% | | 0.224 | 0.052 | 0.026 | 0.017 |
| None | R4 stream | **3.313** | **1.448** | **1.106** | 0.756 |
| 50% | | **3.313** | **1.448** | **1.106** | 0.756 |
| 75% | | **3.313** | **1.448** | **1.106** | 0.756 |
| 90% | | **3.313** | **1.448** | **1.106** | 0.756 |

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: PEC/RAC ratios for <u>zoxamide</u> based on the RAC for fish long-term and FOCUS SW Step 4 calculations with mitigation of plant $DT_{50}$, spray drift and run-off for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in <u>vines, early, single application</u>**

| **Intended use** | | Vines, early, single applications | | | |
|---|---|---|---|---|---|
| **Active substance** | | Zoxamide | | | |
| **Application rate (g/ha)** | | 148.5 | | | |
| **RAC (µg/L) fish long-term** | | 0.348 | | | |
| **Nozzle reduction** | **No-spray buffer (m)** | 1/3 | 10 | 15 | 20 |
| | **Vegetated filter strip (m)** | FOCUS default | 10 | 15 | 20 |
| None | D6 ditch | **2.382** | 0.500 | 0.267 | 0.170 |
| 50% | | **1.193** | 0.250 | 0.132 | 0.086 |
| 75% | | 0.595 | 0.126 | 0.066 | 0.043 |
| 90% | | 0.239 | 0.049 | 0.026 | 0.017 |
| None | R1 pond | 0.083 | 0.052 | 0.034 | 0.026 |
| 50% | | 0.040 | 0.026 | 0.017 | 0.011 |
| 75% | | 0.020 | 0.014 | 0.009 | 0.006 |
| 90% | | 0.009 | 0.006 | 0.003 | 0.003 |
| None | R1 stream | **1.756** | 0.445 | 0.239 | 0.152 |
| 50% | | 0.879 | 0.224 | 0.118 | 0.078 |
| 75% | | 0.440 | 0.155 | 0.118 | 0.078 |
| 90% | | 0.365 | 0.155 | 0.118 | 0.078 |
| None | R2 stream | **2.333** | 0.592 | 0.316 | 0.201 |
| 50% | | **1.167** | 0.296 | 0.158 | 0.101 |
| 75% | | 0.583 | 0.149 | 0.080 | 0.052 |
| 90% | | 0.233 | 0.060 | 0.032 | 0.020 |
| None | R3 stream | **2.486** | 0.632 | 0.336 | 0.216 |
| 50% | | **1.241** | 0.316 | 0.170 | 0.106 |
| 75% | | 0.621 | 0.158 | 0.083 | 0.055 |
| 90% | | 0.247 | 0.063 | 0.034 | 0.020 |
| None | R4 stream | **1.739** | 0.480 | 0.368 | 0.250 |
| 50% | | **1.101** | 0.480 | 0.368 | 0.250 |
| 75% | | **1.101** | 0.480 | 0.368 | 0.250 |
| 90% | | **1.101** | 0.480 | 0.368 | 0.250 |

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

**Table 9.5-9: Aquatic organisms: PEC/RAC ratios for zoxamide based on the RAC for invertebrates prolonged and FOCUS SW Step 4 calculations with mitigation of plant DT50, spray drift and run-off for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in vines, early, multiple applications**

| **Intended use** | | Vines, early, multiple applications | | | |
|---|---|---|---|---|---|
| **Active substance** | | Zoxamide | | | |
| **Application rate (g/ha)** | | 3 × 148.5 | | | |
| **RAC (µg/L) inverteb. long-term** | | 0.72 | | | |
| **Nozzle reduction** | **No-spray buffer (m)** | 1/3 | 10 | 15 | 20 |
| | **Vegetated filter strip (m)** | FOCUS default | 10 | 15 | 20 |
| None | D6 ditch | **1.074** | 0.201 | 0.103 | 0.064 |
| 50% | | 0.538 | 0.101 | 0.051 | 0.032 |
| 75% | | 0.268 | 0.050 | 0.026 | 0.015 |
| 90% | | 0.107 | 0.021 | 0.010 | 0.007 |
| None | R1 pond | 0.082 | 0.050 | 0.032 | 0.024 |
| 50% | | 0.043 | 0.025 | 0.017 | 0.011 |
| 75% | | 0.026 | 0.014 | 0.010 | 0.007 |
| 90% | | 0.017 | 0.008 | 0.006 | 0.004 |
| None | R1 stream | 0.983 | 0.415 | 0.315 | 0.213 |
| 50% | | 0.983 | 0.415 | 0.315 | 0.213 |
| 75% | | 0.983 | 0.415 | 0.315 | 0.213 |
| 90% | | 0.983 | 0.415 | 0.315 | 0.213 |
| None | R2 stream | **1.032** | 0.238 | 0.121 | 0.075 |
| 50% | | 0.515 | 0.119 | 0.061 | 0.038 |
| 75% | | 0.258 | 0.060 | 0.031 | 0.019 |
| 90% | | 0.103 | 0.026 | 0.019 | 0.014 |
| None | R3 stream | **1.089** | 0.251 | 0.128 | 0.079 |
| 50% | | 0.544 | 0.125 | 0.064 | 0.040 |
| 75% | | 0.272 | 0.063 | 0.032 | 0.019 |
| 90% | | 0.108 | 0.025 | 0.013 | 0.008 |
| None | R4 stream | **1.601** | 0.700 | 0.535 | 0.365 |
| 50% | | **1.601** | 0.700 | 0.535 | 0.365 |
| 75% | | **1.601** | 0.700 | 0.535 | 0.365 |
| 90% | | **1.601** | 0.700 | 0.535 | 0.365 |

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 9.5-10: Aquatic organisms: PEC/RAC ratios for <u>zoxamide</u> based on the RAC for invertebrates prolonged and FOCUS SW Step 4 calculations with mitigation of plant $DT_{50}$, spray drift and run-off for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in <u>vines, early, single application</u>**

| **Intended use** | | Vines, early, single application | | | |
|---|---|---|---|---|---|
| **Active substance** | | Zoxamide | | | |
| **Application rate (g/ha)** | | 148.5 | | | |
| **RAC (µg/L) inverteb. long-term** | | 0.72 | | | |
| **Nozzle reduction** | **No-spray buffer (m)** | 1/3 | 10 | 15 | 20 |
| | **Vegetated filter strip (m)** | FOCUS default | 10 | 15 | 20 |
| None | D6 ditch | **1.151** | 0.242 | 0.129 | 0.082 |
| 50% | | 0.576 | 0.121 | 0.064 | 0.042 |
| 75% | | 0.288 | 0.061 | 0.032 | 0.021 |
| 90% | | 0.115 | 0.024 | 0.013 | 0.008 |
| None | R1 pond | 0.040 | 0.025 | 0.017 | 0.013 |
| 50% | | 0.019 | 0.013 | 0.008 | 0.006 |
| 75% | | 0.010 | 0.007 | 0.004 | 0.003 |
| 90% | | 0.004 | 0.003 | 0.001 | 0.001 |
| None | R1 stream | 0.849 | 0.215 | 0.115 | 0.074 |
| 50% | | 0.425 | 0.108 | 0.057 | 0.038 |
| 75% | | 0.213 | 0.075 | 0.057 | 0.038 |
| 90% | | 0.176 | 0.075 | 0.057 | 0.038 |
| None | R2 stream | **1.128** | 0.286 | 0.153 | 0.097 |
| 50% | | 0.564 | 0.143 | 0.076 | 0.049 |
| 75% | | 0.282 | 0.072 | 0.039 | 0.025 |
| 90% | | 0.113 | 0.029 | 0.015 | 0.010 |
| None | R3 stream | **1.201** | 0.306 | 0.163 | 0.104 |
| 50% | | 0.600 | 0.153 | 0.082 | 0.051 |
| 75% | | 0.300 | 0.076 | 0.040 | 0.026 |
| 90% | | 0.119 | 0.031 | 0.017 | 0.010 |
| None | R4 stream | 0.840 | 0.232 | 0.178 | 0.121 |
| 50% | | 0.532 | 0.232 | 0.178 | 0.121 |
| 75% | | 0.532 | 0.232 | 0.178 | 0.121 |
| 90% | | 0.532 | 0.232 | 0.178 | 0.121 |

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

**Table 9.5-11: Aquatic organisms: PEC/RAC ratios for <u>zoxamide</u> based on the RAC for fish acute and FOCUS SW Step 4 calculations with mitigation of plant $DT_{50}$, spray drift and run-off for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in <u>vines, late, multiple applications</u>**

| **Intended use** | | Vines, late, multiple applications | | | |
|---|---|---|---|---|---|
| **Active substance** | | Zoxamide | | | |
| **Application rate (g/ha)** | | 3 x 148.5 | | | |
| **RAC (µg/L) fish acute** | | 1.6 | | | |
| **Nozzle reduction** | **No-spray buffer (m)** | 1/3 | 10 | 15 | 20 |
| | **Vegetated filter strip (m)** | FOCUS default | 10 | 15 | 20 |
| None | D6 ditch | **1.973** | 0.423 | 0.228 | 0.146 |
| 50% | | 0.986 | 0.211 | 0.114 | 0.073 |
| 75% | | 0.493 | 0.106 | 0.057 | 0.036 |
| 90% | | 0.197 | 0.042 | 0.023 | 0.017 |
| None | R1 pond | 0.120 | 0.076 | 0.051 | 0.038 |
| 50% | | 0.060 | 0.038 | 0.026 | 0.019 |
| 75% | | 0.030 | 0.019 | 0.013 | 0.009 |
| 90% | | 0.012 | 0.008 | 0.005 | 0.004 |
| None | R1 stream | 0.995 | 0.258 | 0.139 | 0.089 |
| 50% | | 0.498 | 0.129 | 0.069 | 0.044 |
| 75% | | 0.249 | 0.064 | 0.035 | 0.023 |
| 90% | | 0.099 | 0.026 | 0.018 | 0.012 |
| None | R2 stream | **1.334** | 0.346 | 0.186 | 0.120 |
| 50% | | 0.667 | 0.173 | 0.093 | 0.060 |
| 75% | | 0.333 | 0.086 | 0.047 | 0.030 |
| 90% | | 0.133 | 0.034 | 0.019 | 0.012 |
| None | R3 stream | **1.403** | 0.364 | 0.196 | 0.126 |
| 50% | | 0.701 | 0.193 | 0.148 | 0.101 |
| 75% | | 0.424 | 0.193 | 0.148 | 0.101 |
| 90% | | 0.424 | 0.193 | 0.148 | 0.101 |
| None | R4 stream | 0.995 | 0.393 | 0.301 | 0.204 |
| 50% | | 0.878 | 0.393 | 0.301 | 0.204 |
| 75% | | 0.878 | 0.393 | 0.301 | 0.204 |
| 90% | | 0.878 | 0.393 | 0.301 | 0.204 |

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

**Table 9.5-12: Aquatic organisms: PEC/RAC ratios for <u>zoxamide</u> based on the RAC for fish acute and FOCUS SW Step 4 calculations with mitigation of plant $DT_{50}$, spray drift and run-off for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in <u>vines, late, single application</u>**

| **Intended use** | | Vines, late, single applications | | | |
|---|---|---|---|---|---|
| **Active substance** | | Zoxamide | | | |
| **Application rate (g/ha)** | | 148.5 | | | |
| **RAC (µg/L) fish acute** | | 1.6 | | | |
| **Nozzle reduction** | **No-spray buffer (m)** | 1/3 | 10 | 15 | 20 |
| | **Vegetated filter strip (m)** | FOCUS default | 10 | 15 | 20 |
| None | D6 ditch | **3.536** | 0.775 | 0.421 | 0.272 |
| 50% | | **1.768** | 0.388 | 0.211 | 0.136 |
| 75% | | 0.883 | 0.193 | 0.106 | 0.068 |
| 90% | | 0.354 | 0.078 | 0.042 | 0.028 |
| None | R1 pond | 0.126 | 0.081 | 0.054 | 0.040 |
| 50% | | 0.063 | 0.040 | 0.028 | 0.021 |
| 75% | | 0.032 | 0.019 | 0.014 | 0.010 |
| 90% | | 0.013 | 0.008 | 0.006 | 0.004 |
| None | R1 stream | **2.594** | 0.685 | 0.372 | 0.240 |
| 50% | | **1.297** | 0.342 | 0.186 | 0.119 |
| 75% | | 0.649 | 0.171 | 0.093 | 0.060 |
| 90% | | 0.260 | 0.068 | 0.038 | 0.024 |
| None | R2 stream | **3.476** | 0.918 | 0.499 | 0.322 |
| 50% | | **1.738** | 0.458 | 0.249 | 0.161 |
| 75% | | 0.869 | 0.229 | 0.125 | 0.081 |
| 90% | | 0.347 | 0.092 | 0.050 | 0.032 |
| None | R3 stream | **3.656** | 0.965 | 0.524 | 0.339 |
| 50% | | **1.828** | 0.482 | 0.263 | 0.169 |
| 75% | | 0.914 | 0.242 | 0.131 | 0.085 |
| 90% | | 0.365 | 0.096 | 0.053 | 0.033 |
| None | R4 stream | **2.593** | 0.685 | 0.372 | 0.240 |
| 50% | | **1.297** | 0.342 | 0.186 | 0.119 |
| 75% | | 0.649 | 0.171 | 0.099 | 0.067 |
| 90% | | 0.286 | 0.128 | 0.099 | 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 9.5-13: Aquatic organisms: PEC/RAC ratios for <u>zoxamide</u> based on the RAC for fish long-term and FOCUS SW Step 4 calculations with mitigation of plant $DT_{50}$, spray drift and run-off for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in <u>vines, late, multiple applications</u>**

| **Intended use** | | Vines, late, multiple applications | | | |
|---|---|---|---|---|---|
| **Active substance** | | Zoxamide | | | |
| **Application rate (g/ha)** | | 3 x 148.5 | | | |
| **RAC (µg/L) fish long-term** | | 0.348 | | | |
| **Nozzle reduction** | **No-spray buffer (m)** | 1/3 | 10 | 15 | 20 |
| | **Vegetated filter strip (m)** | FOCUS default | 10 | 15 | 20 |
| None | D6 ditch | **9.069** | **1.945** | **1.049** | 0.672 |
| 50% | | **4.532** | 0.971 | 0.523 | 0.336 |
| 75% | | **2.264** | 0.486 | 0.261 | 0.167 |
| 90% | | 0.905 | 0.193 | 0.103 | 0.078 |
| None | R1 pond | 0.552 | 0.351 | 0.236 | 0.175 |
| 50% | | 0.276 | 0.175 | 0.118 | 0.086 |
| 75% | | 0.138 | 0.086 | 0.057 | 0.043 |
| 90% | | 0.055 | 0.034 | 0.023 | 0.017 |
| None | R1 stream | **4.575** | **1.187** | 0.641 | 0.411 |
| 50% | | **2.287** | 0.595 | 0.319 | 0.204 |
| 75% | | **1.144** | 0.296 | 0.161 | 0.103 |
| 90% | | 0.457 | 0.118 | 0.080 | 0.055 |
| None | R2 stream | **6.132** | **1.592** | 0.856 | 0.552 |
| 50% | | **3.066** | 0.796 | 0.428 | 0.276 |
| 75% | | **1.532** | 0.397 | 0.216 | 0.138 |
| 90% | | 0.612 | 0.158 | 0.086 | 0.055 |
| None | R3 stream | **6.448** | **1.672** | 0.902 | 0.578 |
| 50% | | **3.224** | 0.888 | 0.681 | 0.466 |
| 75% | | **1.951** | 0.888 | 0.681 | 0.466 |
| 90% | | **1.951** | 0.888 | 0.681 | 0.466 |
| None | R4 stream | **4.575** | **1.807** | **1.382** | 0.940 |
| 50% | | **4.037** | **1.807** | **1.382** | 0.940 |
| 75% | | **4.037** | **1.807** | **1.382** | 0.940 |
| 90% | | **4.037** | **1.807** | **1.382** | 0.940 |

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

**Table 9.5-14: Aquatic organisms: PEC/RAC ratios for <u>zoxamide</u> based on the RAC for fish long-term and FOCUS SW Step 4 calculations with mitigation of plant $DT_{50}$, spray drift and run-off for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in <u>vines, late, single application</u>**

| **Intended use** | | Vines, late, single application | | | |
|---|---|---|---|---|---|
| **Active substance** | | Zoxamide | | | |
| **Application rate (g/ha)** | | 148.5 | | | |
| **RAC (µg/L) fish long-term** | | 0.348 | | | |
| **Nozzle reduction** | **No-spray buffer (m)** | 1/3 | 10 | 15 | 20 |
| | **Vegetated filter strip (m)** | FOCUS default | 10 | 15 | 20 |
| None | D6 ditch | **7.316** | **1.603** | 0.871 | 0.563 |
| 50% | | **3.658** | 0.802 | 0.437 | 0.282 |
| 75% | | **1.828** | 0.399 | 0.218 | 0.141 |
| 90% | | 0.733 | 0.161 | 0.086 | 0.057 |
| None | R1 pond | 0.261 | 0.167 | 0.112 | 0.083 |
| 50% | | 0.129 | 0.083 | 0.057 | 0.043 |
| 75% | | 0.066 | 0.040 | 0.029 | 0.020 |
| 90% | | 0.026 | 0.017 | 0.011 | 0.009 |
| None | R1 stream | **5.368** | **1.417** | 0.770 | 0.497 |
| 50% | | **2.684** | 0.707 | 0.385 | 0.247 |
| 75% | | **1.342** | 0.353 | 0.193 | 0.124 |
| 90% | | 0.537 | 0.141 | 0.078 | 0.049 |
| None | R2 stream | **7.193** | **1.899** | **1.032** | 0.667 |
| 50% | | **3.595** | 0.948 | 0.514 | 0.333 |
| 75% | | **1.799** | 0.474 | 0.259 | 0.167 |
| 90% | | 0.718 | 0.190 | 0.103 | 0.066 |
| None | R3 stream | **7.563** | **1.997** | **1.083** | 0.701 |
| 50% | | **3.782** | 0.997 | 0.543 | 0.351 |
| 75% | | **1.891** | 0.500 | 0.270 | 0.175 |
| 90% | | 0.756 | 0.198 | 0.109 | 0.069 |
| None | R4 stream | **5.365** | **1.417** | 0.770 | 0.497 |
| 50% | | **2.684** | 0.707 | 0.385 | 0.247 |
| 75% | | **1.342** | 0.353 | 0.204 | 0.138 |
| 90% | | 0.592 | 0.264 | 0.204 | 0.138 |

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

**Table 9.5-15: Aquatic organisms: PEC/RAC ratios for <u>zoxamide</u> based on the RAC for invertebrates acute and FOCUS SW Step 4 calculations with mitigation of plant $DT_{50}$, spray drift and run-off for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in <u>vines, late, multiple applications</u>**

| **Intended use** | | Vines, late, multiple applications | | | |
|---|---|---|---|---|---|
| **Active substance** | | Zoxamide | | | |
| **Application rate (g/ha)** | | 3 x 148.5 | | | |
| **RAC (µg/L) invert. acute** | | 2.29 | | | |
| **Nozzle reduction** | **No-spray buffer (m)** | 1/3 | 10 | 15 | 20 |
| | **Vegetated filter strip (m)** | FOCUS default | 10 | 15 | 20 |
| None | D6 ditch | **1.378** | 0.296 | 0.159 | 0.102 |
| 50% | | 0.689 | 0.148 | 0.079 | 0.051 |
| 75% | | 0.344 | 0.074 | 0.040 | 0.025 |
| 90% | | 0.138 | 0.029 | 0.016 | 0.012 |
| None | R1 pond | 0.084 | 0.053 | 0.036 | 0.027 |
| 50% | | 0.042 | 0.027 | 0.018 | 0.013 |
| 75% | | 0.021 | 0.013 | 0.009 | 0.007 |
| 90% | | 0.008 | 0.005 | 0.003 | 0.003 |
| None | R1 stream | 0.695 | 0.180 | 0.097 | 0.062 |
| 50% | | 0.348 | 0.090 | 0.048 | 0.031 |
| 75% | | 0.174 | 0.045 | 0.024 | 0.016 |
| 90% | | 0.069 | 0.018 | 0.012 | 0.008 |
| None | R2 stream | 0.932 | 0.242 | 0.130 | 0.084 |
| 50% | | 0.466 | 0.121 | 0.065 | 0.042 |
| 75% | | 0.233 | 0.060 | 0.033 | 0.021 |
| 90% | | 0.093 | 0.024 | 0.013 | 0.008 |
| None | R3 stream | 0.980 | 0.254 | 0.137 | 0.088 |
| 50% | | 0.490 | 0.135 | 0.103 | 0.071 |
| 75% | | 0.297 | 0.135 | 0.103 | 0.071 |
| 90% | | 0.297 | 0.135 | 0.103 | 0.071 |
| None | R4 stream | 0.695 | 0.275 | 0.210 | 0.143 |
| 50% | | 0.614 | 0.275 | 0.210 | 0.143 |
| 75% | | 0.614 | 0.275 | 0.210 | 0.143 |
| 90% | | 0.614 | 0.275 | 0.210 | 0.143 |

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

**Table 9.5-16: Aquatic organisms: PEC/RAC ratios for <u>zoxamide</u> based on the RAC for invertebrates acute and FOCUS SW Step 4 calculations with mitigation of plant $DT_{50}$, spray drift and run-off for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in <u>vines, late, single application</u>**

| **Intended use** | | Vines, late, single application | | | |
|---|---|---|---|---|---|
| **Active substance** | | Zoxamide | | | |
| **Application rate (g/ha)** | | 148.5 | | | |
| **RAC (µg/L) invert. acute** | | 2.29 | | | |
| **Nozzle reduction** | **No-spray buffer (m)** | 1/3 | 10 | 15 | 20 |
| | **Vegetated filter strip (m)** | FOCUS default | 10 | 15 | 20 |
| None | D6 ditch | **1.112** | 0.244 | 0.132 | 0.086 |
| 50% | | 0.556 | 0.122 | 0.066 | 0.043 |
| 75% | | 0.278 | 0.061 | 0.033 | 0.021 |
| 90% | | 0.111 | 0.024 | 0.013 | 0.009 |
| None | R1 pond | 0.040 | 0.025 | 0.017 | 0.013 |
| 50% | | 0.020 | 0.013 | 0.009 | 0.007 |
| 75% | | 0.010 | 0.006 | 0.004 | 0.003 |
| 90% | | 0.004 | 0.003 | 0.002 | 0.001 |
| None | R1 stream | 0.816 | 0.215 | 0.117 | 0.076 |
| 50% | | 0.408 | 0.107 | 0.059 | 0.038 |
| 75% | | 0.204 | 0.054 | 0.029 | 0.019 |
| 90% | | 0.082 | 0.021 | 0.012 | 0.007 |
| None | R2 stream | **1.093** | 0.289 | 0.157 | 0.101 |
| 50% | | 0.546 | 0.144 | 0.078 | 0.051 |
| 75% | | 0.273 | 0.072 | 0.039 | 0.025 |
| 90% | | 0.109 | 0.029 | 0.016 | 0.010 |
| None | R3 stream | **1.149** | 0.303 | 0.165 | 0.107 |
| 50% | | 0.575 | 0.152 | 0.083 | 0.053 |
| 75% | | 0.287 | 0.076 | 0.041 | 0.027 |
| 90% | | 0.115 | 0.030 | 0.017 | 0.010 |
| None | R4 stream | 0.815 | 0.215 | 0.117 | 0.076 |
| 50% | | 0.408 | 0.107 | 0.059 | 0.038 |
| 75% | | 0.204 | 0.054 | 0.031 | 0.021 |
| 90% | | 0.090 | 0.040 | 0.031 | 0.021 |

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

**Table 9.5-17: Aquatic organisms: PEC/RAC ratios for <u>zoxamide</u> based on the RAC for invertebrates long-term and FOCUS SW Step 4 calculations with mitigation of plant $DT_{50}$, spray drift and run-off for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in <u>vines, late, multiple applications</u>**

| **Intended use** | | Vines, late, multiple applications | | | |
|---|---|---|---|---|---|
| **Active substance** | | Zoxamide | | | |
| **Application rate (g/ha)** | | 3 x 148.5 | | | |
| **RAC (µg/L) invert. long-term** | | 0.72 | | | |
| **Nozzle reduction** | **No-spray buffer (m)** | 1/3 | 10 | 15 | 20 |
| | **Vegetated filter strip (m)** | FOCUS default | 10 | 15 | 20 |
| None | D6 ditch | **4.383** | 0.940 | 0.507 | 0.325 |
| 50% | | **2.190** | 0.469 | 0.253 | 0.163 |
| 75% | | **1.094** | 0.235 | 0.126 | 0.081 |
| 90% | | 0.438 | 0.093 | 0.050 | 0.038 |
| None | R1 pond | 0.267 | 0.169 | 0.114 | 0.085 |
| 50% | | 0.133 | 0.085 | 0.057 | 0.042 |
| 75% | | 0.067 | 0.042 | 0.028 | 0.021 |
| 90% | | 0.026 | 0.017 | 0.011 | 0.008 |
| None | R1 stream | **2.211** | 0.574 | 0.310 | 0.199 |
| 50% | | **1.106** | 0.288 | 0.154 | 0.099 |
| 75% | | 0.553 | 0.143 | 0.078 | 0.050 |
| 90% | | 0.221 | 0.057 | 0.039 | 0.026 |
| None | R2 stream | **2.964** | 0.769 | 0.414 | 0.267 |
| 50% | | **1.482** | 0.385 | 0.207 | 0.133 |
| 75% | | 0.740 | 0.192 | 0.104 | 0.067 |
| 90% | | 0.296 | 0.076 | 0.042 | 0.026 |
| None | R3 stream | **3.117** | 0.808 | 0.436 | 0.279 |
| 50% | | **1.558** | 0.429 | 0.329 | 0.225 |
| 75% | | 0.943 | 0.429 | 0.329 | 0.225 |
| 90% | | 0.943 | 0.429 | 0.329 | 0.225 |
| None | R4 stream | **2.211** | 0.874 | 0.668 | 0.454 |
| 50% | | **1.951** | 0.874 | 0.668 | 0.454 |
| 75% | | **1.951** | 0.874 | 0.668 | 0.454 |
| 90% | | **1.951** | 0.874 | 0.668 | 0.454 |

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

**Table 9.5-18: Aquatic organisms: PEC/RAC ratios for <u>zoxamide</u> based on the RAC for invertebrates long-term and FOCUS SW Step 4 calculations with mitigation of plant $DT_{50}$, spray drift and run-off for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in <u>vines, late, single application</u>**

| **Intended use** | | Vines, late, single application | | | |
|---|---|---|---|---|---|
| **Active substance** | | Zoxamide | | | |
| **Application rate (g/ha)** | | 148.5 | | | |
| **RAC (µg/L) invert. long-term** | | 0.72 | | | |
| **Nozzle reduction** | **No-spray buffer (m)** | 1/3 | 10 | 15 | 20 |
| | **Vegetated filter strip (m)** | FOCUS default | 10 | 15 | 20 |
| None | D6 ditch | **3.536** | 0.775 | 0.421 | 0.272 |
| 50% | | **1.768** | 0.388 | 0.211 | 0.136 |
| 75% | | 0.883 | 0.193 | 0.106 | 0.068 |
| 90% | | 0.354 | 0.078 | 0.042 | 0.028 |
| None | R1 pond | 0.126 | 0.081 | 0.054 | 0.040 |
| 50% | | 0.063 | 0.040 | 0.028 | 0.021 |
| 75% | | 0.032 | 0.019 | 0.014 | 0.010 |
| 90% | | 0.013 | 0.008 | 0.006 | 0.004 |
| None | R1 stream | **2.594** | 0.685 | 0.372 | 0.240 |
| 50% | | **1.297** | 0.342 | 0.186 | 0.119 |
| 75% | | 0.649 | 0.171 | 0.093 | 0.060 |
| 90% | | 0.260 | 0.068 | 0.038 | 0.024 |
| None | R2 stream | **3.476** | 0.918 | 0.499 | 0.322 |
| 50% | | **1.738** | 0.458 | 0.249 | 0.161 |
| 75% | | 0.869 | 0.229 | 0.125 | 0.081 |
| 90% | | 0.347 | 0.092 | 0.050 | 0.032 |
| None | R3 stream | **3.656** | 0.965 | 0.524 | 0.339 |
| 50% | | **1.828** | 0.482 | 0.263 | 0.169 |
| 75% | | 0.914 | 0.242 | 0.131 | 0.085 |
| 90% | | 0.365 | 0.096 | 0.053 | 0.033 |
| None | R4 stream | **2.593** | 0.685 | 0.372 | 0.240 |
| 50% | | **1.297** | 0.342 | 0.186 | 0.119 |
| 75% | | 0.649 | 0.171 | 0.099 | 0.067 |
| 90% | | 0.286 | 0.128 | 0.099 | 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 9.5-19: Aquatic organisms: PEC/RAC ratios for <u>zoxamide</u> based on the RAC for algae and FOCUS SW Step 4 calculations with mitigation of plant $DT_{50}$, spray drift and run-off for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in <u>vines, late, multiple applications</u>**

| **Intended use**<br>**Active substance**<br>**Application rate (g/ha)** | | Vines, late, multiple applications<br>Zoxamide<br>3 x 148.5 | | | |
|---|---|---|---|---|---|
| **RAC (µg/L) algae** | | 1.413 | | | |
| **Nozzle reduction** | **No-spray buffer (m)** | 1/3 | 10 | 15 | 20 |
| | **Vegetated filter strip (m)** | FOCUS default | 10 | 15 | 20 |
| None | D6 ditch | **2.234** | 0.479 | 0.258 | 0.166 |
| 50% | | **1.116** | 0.239 | 0.129 | 0.083 |
| 75% | | 0.558 | 0.120 | 0.064 | 0.041 |
| 90% | | 0.223 | 0.047 | 0.025 | 0.019 |
| None | R1 pond | 0.136 | 0.086 | 0.058 | 0.043 |
| 50% | | 0.068 | 0.043 | 0.029 | 0.021 |
| 75% | | 0.034 | 0.021 | 0.014 | 0.011 |
| 90% | | 0.013 | 0.008 | 0.006 | 0.004 |
| None | R1 stream | **1.127** | 0.292 | 0.158 | 0.101 |
| 50% | | 0.563 | 0.146 | 0.079 | 0.050 |
| 75% | | 0.282 | 0.073 | 0.040 | 0.025 |
| 90% | | 0.113 | 0.029 | 0.020 | 0.013 |
| None | R2 stream | 1.510 | 0.392 | 0.211 | 0.136 |
| 50% | | 0.755 | 0.196 | 0.105 | 0.068 |
| 75% | | 0.377 | 0.098 | 0.053 | 0.034 |
| 90% | | 0.151 | 0.039 | 0.021 | 0.013 |
| None | R3 stream | **1.588** | 0.412 | 0.222 | 0.142 |
| 50% | | 0.794 | 0.219 | 0.168 | 0.115 |
| 75% | | 0.481 | 0.219 | 0.168 | 0.115 |
| 90% | | 0.481 | 0.219 | 0.168 | 0.115 |
| None | R4 stream | **1.127** | 0.445 | 0.340 | 0.231 |
| 50% | | 0.994 | 0.445 | 0.340 | 0.231 |
| 75% | | 0.994 | 0.445 | 0.340 | 0.231 |
| 90% | | 0.994 | 0.445 | 0.340 | 0.231 |

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

**Table 9.5-20: Aquatic organisms: PEC/RAC ratios for <u>zoxamide</u> based on the RAC for algae and FOCUS SW Step 4 calculations with mitigation of plant $DT_{50}$, spray drift and run-off for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in <u>vines, late, single application</u>**

| **Intended use** | | Vines, late, single application | | | |
|---|---|---|---|---|---|
| **Active substance** | | Zoxamide | | | |
| **Application rate (g/ha)** | | 148.5 | | | |
| **RAC (µg/L) algae** | | 1.413 | | | |
| **Nozzle reduction** | **No-spray buffer (m)** | 1/3 | 10 | 15 | 20 |
| | **Vegetated filter strip (m)** | FOCUS default | 10 | 15 | 20 |
| None | D6 ditch | **1.802** | 0.395 | 0.214 | 0.139 |
| 50% | | 0.901 | 0.197 | 0.108 | 0.069 |
| 75% | | 0.450 | 0.098 | 0.054 | 0.035 |
| 90% | | 0.180 | 0.040 | 0.021 | 0.014 |
| None | R1 pond | 0.064 | 0.041 | 0.028 | 0.021 |
| 50% | | 0.032 | 0.021 | 0.014 | 0.011 |
| 75% | | 0.016 | 0.010 | 0.007 | 0.005 |
| 90% | | 0.006 | 0.004 | 0.003 | 0.002 |
| None | R1 stream | **1.322** | 0.349 | 0.190 | 0.122 |
| 50% | | 0.661 | 0.174 | 0.095 | 0.061 |
| 75% | | 0.331 | 0.087 | 0.047 | 0.030 |
| 90% | | 0.132 | 0.035 | 0.019 | 0.012 |
| None | R2 stream | **1.771** | 0.468 | 0.254 | 0.164 |
| 50% | | 0.885 | 0.234 | 0.127 | 0.082 |
| 75% | | 0.443 | 0.117 | 0.064 | 0.041 |
| 90% | | 0.177 | 0.047 | 0.025 | 0.016 |
| None | R3 stream | **1.863** | 0.492 | 0.267 | 0.173 |
| 50% | | 0.931 | 0.246 | 0.134 | 0.086 |
| 75% | | 0.466 | 0.123 | 0.067 | 0.043 |
| 90% | | 0.186 | 0.049 | 0.027 | 0.017 |
| None | R4 stream | **1.321** | 0.349 | 0.190 | 0.122 |
| 50% | | 0.661 | 0.174 | 0.095 | 0.061 |
| 75% | | 0.331 | 0.087 | 0.050 | 0.034 |
| 90% | | 0.146 | 0.065 | 0.050 | 0.034 |

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

**Table 9.5-21: Aquatic organisms: PEC/RAC ratios for zoxamide based on the RAC for aquatic plant and FOCUS SW Step 4 calculations with mitigation of plant $DT_{50}$, spray drift and run-off for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in vines, late, multiple applications**

| Intended use<br>Active substance<br>Application rate (g/ha) | | Vines, late, multiple applications<br>Zoxamide<br>3 x 148.5 | | | |
|---|---|---|---|---|---|
| RAC (µg/L) *Lemna* | | 2.37 | | | |
| Nozzle reduction | No spray buffer (m) | 1/3 | 10 | 15 | 20 |
| | Vegetated filter strip (m) | FOCUS default | 10 | 15 | 20 |
| None | D6 ditch | **1.332** | 0.286 | 0.154 | 0.099 |
| 50% | | 0.665 | 0.143 | 0.077 | 0.049 |
| 75% | | 0.332 | 0.071 | 0.038 | 0.024 |
| 90% | | 0.133 | 0.028 | 0.015 | 0.011 |
| None | R1 pond | 0.081 | 0.051 | 0.035 | 0.026 |
| 50% | | 0.041 | 0.026 | 0.017 | 0.013 |
| 75% | | 0.020 | 0.013 | 0.008 | 0.006 |
| 90% | | 0.008 | 0.005 | 0.003 | 0.003 |
| None | R1 stream | 0.672 | 0.174 | 0.094 | 0.060 |
| 50% | | 0.336 | 0.087 | 0.047 | 0.030 |
| 75% | | 0.168 | 0.043 | 0.024 | 0.015 |
| 90% | | 0.067 | 0.017 | 0.012 | 0.008 |
| None | R2 stream | 0.900 | 0.234 | 0.126 | 0.081 |
| 50% | | 0.450 | 0.117 | 0.063 | 0.041 |
| 75% | | 0.225 | 0.058 | 0.032 | 0.020 |
| 90% | | 0.090 | 0.023 | 0.013 | 0.008 |
| None | R3 stream | 0.947 | 0.246 | 0.132 | 0.085 |
| 50% | | 0.473 | 0.130 | 0.100 | 0.068 |
| 75% | | 0.286 | 0.130 | 0.100 | 0.068 |
| 90% | | 0.286 | 0.130 | 0.100 | 0.068 |
| None | R4 stream | 0.672 | 0.265 | 0.203 | 0.138 |
| 50% | | 0.593 | 0.265 | 0.203 | 0.138 |
| 75% | | 0.593 | 0.265 | 0.203 | 0.138 |
| 90% | | 0.593 | 0.265 | 0.203 | 0.138 |

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

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | | Vines, late, multiple applications | | | |
| **Active substance** | | Zoxamide | | | |
| **Application rate (g/ha)** | | 3 x 148.5 | | | |
| **RAC (µg/L) *Lemna*** | | 0.9 | | | |
| **Nozzle re-duction** | **No-spray buffer (m)** | 1/3 | 10 | 15 | 20 |
| | **Vegetated filter strip (m)** | FOCUS default | 10 | 15 | 20 |
| None | D6 ditch | **3.5** | 0.75 | - | - |
| 50% | | **1.75** | - | - | - |
| 75% | | 0.88 | - | - | - |
| 90% | | - | - | - | - |
| None | R1 pond | 0.21 | - | - | - |
| 50% | | - | - | - | - |
| 75% | | - | - | - | - |
| 90% | | - | - | - | - |
| None | R1 stream | **1.77** | 0.46 | - | - |
| 50% | | 0.88 | - | - | - |
| 75% | | - | - | - | - |
| 90% | | - | - | - | - |
| None | R2 stream | **2.37** | 0.62 | - | - |
| 50% | | **1.19** | - | - | - |
| 75% | | 0.59 | - | - | - |
| 90% | | - | - | - | - |
| None | R3 stream | **2.49** | 0.65 | - | - |
| 50% | | **1.25** | - | - | - |
| 75% | | 0.75 | - | - | - |
| 90% | | - | - | - | - |
| None | R4 stream | **1.77** | 0.70 | - | - |
| 50% | | **1.56** | - | - | - |
| 75% | | **1.56** | - | - | - |
| 90% | | **1.56** | - | - | - |

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 9.5-22: Aquatic organisms: PEC/RAC ratios for zoxamide based on the RAC for aquatic plant and FOCUS SW Step 4 calculations with mitigation of plant $DT_{50}$, spray drift and run-off for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in vines, late, single application**

| Intended use<br>Active substance<br>Application rate (g/ha) | | Vines, late, single application<br>Zoxamide<br>148.5 | | | |
|---|---|---|---|---|---|
| RAC (µg/L) *Lemna* | | 2.37 | | | |
| Nozzle reduction | No spray buffer (m) | 1/3 | 10 | 15 | 20 |
| | Vegetated filter strip (m) | FOCUS default | 10 | 15 | 20 |
| None | D6 ditch | **1.074** | 0.235 | 0.128 | 0.083 |
| 50% | | 0.537 | 0.118 | 0.064 | 0.041 |
| 75% | | 0.268 | 0.059 | 0.032 | 0.021 |
| 90% | | 0.108 | 0.024 | 0.013 | 0.008 |
| None | R1 pond | 0.038 | 0.024 | 0.016 | 0.012 |
| 50% | | 0.019 | 0.012 | 0.008 | 0.006 |
| 75% | | 0.010 | 0.006 | 0.004 | 0.003 |
| 90% | | 0.004 | 0.003 | 0.002 | 0.001 |
| None | R1 stream | 0.788 | 0.208 | 0.113 | 0.073 |
| 50% | | 0.394 | 0.104 | 0.057 | 0.036 |
| 75% | | 0.197 | 0.052 | 0.028 | 0.018 |
| 90% | | 0.079 | 0.021 | 0.011 | 0.007 |
| None | R2 stream | **1.056** | 0.279 | 0.151 | 0.098 |
| 50% | | 0.528 | 0.139 | 0.076 | 0.049 |
| 75% | | 0.264 | 0.070 | 0.038 | 0.024 |
| 90% | | 0.105 | 0.028 | 0.015 | 0.010 |
| None | R3 stream | **1.111** | 0.293 | 0.159 | 0.103 |
| 50% | | 0.555 | 0.146 | 0.080 | 0.051 |
| 75% | | 0.278 | 0.073 | 0.040 | 0.026 |
| 90% | | 0.111 | 0.029 | 0.016 | 0.010 |
| None | R4 stream | 0.788 | 0.208 | 0.113 | 0.073 |
| 50% | | 0.394 | 0.104 | 0.057 | 0.036 |
| 75% | | 0.197 | 0.052 | 0.030 | 0.020 |
| 90% | | 0.087 | 0.039 | 0.030 | 0.020 |

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

| **Intended use** | | Vines, late, single applications | | | |
|---|---|---|---|---|---|
| **Active substance** | | Zoxamide | | | |
| **Application rate (g/ha)** | | 3 x 148.5 | | | |
| **RAC (µg/L) *Lemna*** | | 0.9 | | | |
| **Nozzle re-duction** | **No-spray buffer (m)** | 1/3 | 10 | 15 | 20 |
| | **Vegetated filter strip (m)** | FOCUS default | 10 | 15 | 20 |
| None | D6 ditch | **2.83** | 0.62 | - | - |
| 50% | | **1.41** | - | - | - |
| 75% | | 0.71 | - | - | - |
| 90% | | - | - | - | - |
| None | R1 pond | 0.10 | - | - | - |
| 50% | | - | - | - | - |
| 75% | | - | - | - | - |
| 90% | | - | - | - | - |
| None | R1 stream | **2.08** | 0.55 | - | - |
| 50% | | **1.04** | - | - | - |
| 75% | | 0.52 | - | - | - |
| 90% | | - | - | - | - |
| None | R2 stream | **2.78** | 0.73 | - | - |
| 50% | | **1.39** | - | - | - |
| 75% | | 0.70 | - | - | - |
| 90% | | - | - | - | - |
| None | R3 stream | **2.92** | 0.77 | - | - |
| 50% | | **1.46** | - | - | - |
| 75% | | 0.73 | - | - | - |
| 90% | | - | - | - | - |
| None | R4 stream | **2.07** | 0.55 | - | - |
| 50% | | **1.04** | - | - | - |
| 75% | | 0.52 | - | - | - |
| 90% | | - | - | - | - |

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

For refinement, FOCUS SW Step 4 calculations with the reduced max. dose in grapes authorised in certain EU zone countries are included in blue for completeness. A risk assessment with these scenarios is presented in the following for fish prolonged as worst-case.

**Table 9.5-23: Aquatic organisms: PEC/RAC ratios for zoxamide based on the RAC for fish long-term and FOCUS SW Step 4 calculations with mitigation of plant $DT_{50}$, spray drift and run-off for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in vines, early, multiple applications**

| **Intended use** | | Vines, early, multiple applications | | | |
|---|---|---|---|---|---|
| **Active substance** | | Zoxamide | | | |
| **Application rate (g/ha)** | | 3 × 132 | | | |
| **RAC (µg/L) fish long-term** | | 0.348 | | | |
| **Nozzle reduction** | **No-spray buffer (m)** | 1/3 | 10 | 15 | 20 |
| | **Vegetated filter strip (m)** | FOCUS default | 10 | 15 | 20 |
| None | D6 ditch | **1.974** | 0.371 | 0.190 | 0.118 |
| 50% | | 0.989 | 0.187 | 0.095 | 0.057 |
| 75% | | 0.494 | 0.092 | 0.046 | 0.029 |
| 90% | | 0.198 | 0.037 | 0.020 | 0.011 |
| None | R1 pond | 0.152 | 0.092 | 0.060 | 0.043 |
| 50% | | 0.080 | 0.046 | 0.029 | 0.020 |
| 75% | | 0.049 | 0.026 | 0.017 | 0.011 |
| 90% | | 0.032 | 0.014 | 0.011 | 0.009 |
| None | R1 stream | **1.802** | 0.761 | 0.578 | 0.388 |
| 50% | | **1.802** | 0.761 | 0.578 | 0.388 |
| 75% | | **1.802** | 0.761 | 0.578 | 0.388 |
| 90% | | **1.802** | 0.761 | 0.578 | 0.388 |
| None | R2 stream | **1.897** | 0.437 | 0.224 | 0.138 |
| 50% | | 0.948 | 0.218 | 0.112 | 0.069 |
| 75% | | 0.474 | 0.109 | 0.055 | 0.034 |
| 90% | | 0.190 | 0.046 | 0.037 | 0.026 |
| None | R3 stream | **2.003** | 0.463 | 0.236 | 0.147 |
| 50% | | **1.000** | 0.230 | 0.118 | 0.072 |
| 75% | | 0.500 | 0.115 | 0.060 | 0.037 |
| 90% | | 0.201 | 0.046 | 0.023 | 0.014 |
| None | R4 stream | **2.940** | **1.284** | 0.983 | 0.670 |
| 50% | | **2.940** | **1.284** | 0.983 | 0.670 |
| 75% | | **2.940** | **1.284** | 0.983 | 0.670 |
| 90% | | **2.940** | **1.284** | 0.983 | 0.670 |

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 9.5-24: Aquatic organisms: PEC/RAC ratios for zoxamide based on the RAC for fish long-term and FOCUS SW Step 4 calculations with mitigation of plant $DT_{50}$, spray drift and run-off for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in vines, late, multiple applications**

| **Intended use** | | Vines, late, multiple applications | | | |
|---|---|---|---|---|---|
| **Active substance** | | Zoxamide | | | |
| **Application rate (g/ha)** | | 3 x 132 | | | |
| **RAC (µg/L) fish long-term** | | 0.348 | | | |
| **Nozzle reduction** | **No-spray buffer (m)** | 1/3 | 10 | 15 | 20 |
| | **Vegetated filter strip (m)** | FOCUS default | 10 | 15 | 20 |
| None | D6 ditch | **8.060** | **1.727** | 0.931 | 0.598 |
| 50% | | **4.026** | 0.865 | 0.466 | 0.299 |
| 75% | | **2.011** | 0.431 | 0.233 | 0.149 |
| 90% | | 0.805 | 0.172 | 0.092 | 0.069 |
| None | R1 pond | 0.489 | 0.310 | 0.210 | 0.155 |
| 50% | | 0.244 | 0.155 | 0.103 | 0.078 |
| 75% | | 0.124 | 0.078 | 0.052 | 0.037 |
| 90% | | 0.049 | 0.032 | 0.020 | 0.014 |
| None | R1 stream | **4.066** | **1.055** | 0.569 | 0.365 |
| 50% | | **2.032** | 0.529 | 0.284 | 0.184 |
| 75% | | **1.017** | 0.264 | 0.141 | 0.092 |
| 90% | | 0.408 | 0.106 | 0.072 | 0.049 |
| None | R2 stream | **5.448** | **1.414** | 0.761 | 0.489 |
| 50% | | **2.724** | 0.707 | 0.382 | 0.244 |
| 75% | | **1.362** | 0.353 | 0.190 | 0.124 |
| 90% | | 0.546 | 0.141 | 0.078 | 0.049 |
| None | R3 stream | **5.730** | **1.489** | 0.802 | 0.514 |
| 50% | | **2.865** | 0.787 | 0.603 | 0.411 |
| 75% | | **1.730** | 0.787 | 0.603 | 0.411 |
| 90% | | **1.730** | 0.787 | 0.603 | 0.411 |
| None | R4 stream | **4.066** | **1.601** | **1.224** | 0.833 |
| 50% | | **4.066** | **1.601** | **1.224** | 0.833 |
| 75% | | **4.066** | **1.601** | **1.224** | 0.833 |
| 90% | | **4.066** | **1.601** | **1.224** | 0.833 |

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 9.5-25: Aquatic organisms: PEC/RAC ratios for zoxamide based on the RAC for fish long-term and FOCUS SW Step 4 calculations with mitigation of plant $DT_{50}$, spray drift and run-off for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in vines, late, single application**

| **Intended use** | | Vines, late, single application | | | |
|---|---|---|---|---|---|
| **Active substance** | | Zoxamide | | | |
| **Application rate (g/ha)** | | 132 | | | |
| **RAC (µg/L) fish long-term** | | 0.348 | | | |
| **Nozzle reduction** | **No-spray buffer (m)** | 1/3 | 10 | 15 | 20 |
| | **Vegetated filter strip (m)** | FOCUS default | 10 | 15 | 20 |
| None | D6 ditch | **6.503** | **1.425** | 0.773 | 0.500 |
| 50% | | **3.253** | 0.713 | 0.388 | 0.250 |
| 75% | | **1.626** | 0.356 | 0.193 | 0.124 |
| 90% | | 0.649 | 0.144 | 0.078 | 0.049 |
| None | R1 pond | 0.233 | 0.149 | 0.101 | 0.075 |
| 50% | | 0.115 | 0.075 | 0.049 | 0.037 |
| 75% | | 0.057 | 0.037 | 0.026 | 0.017 |
| 90% | | 0.023 | 0.014 | 0.009 | 0.009 |
| None | R1 stream | **4.770** | **1.259** | 0.684 | 0.443 |
| 50% | | **2.385** | 0.629 | 0.342 | 0.221 |
| 75% | | **1.193** | 0.316 | 0.170 | 0.109 |
| 90% | | 0.477 | 0.126 | 0.069 | 0.043 |
| None | R2 stream | **6.394** | **1.687** | 0.917 | 0.592 |
| 50% | | **3.195** | 0.845 | 0.460 | 0.296 |
| 75% | | **1.598** | 0.422 | 0.230 | 0.147 |
| 90% | | 0.641 | 0.170 | 0.092 | 0.060 |
| None | R3 stream | **6.724** | 1.776 | 0.963 | 0.624 |
| 50% | | **3.362** | 0.888 | 0.483 | 0.310 |
| 75% | | **1.681** | 0.443 | 0.241 | 0.155 |
| 90% | | 0.672 | 0.178 | 0.098 | 0.063 |
| None | R4 stream | **4.770** | **1.259** | 0.684 | 0.443 |
| 50% | | **2.385** | 0.629 | 0.342 | 0.221 |
| 75% | | **1.193** | 0.316 | 0.181 | 0.124 |
| 90% | | 0.526 | 0.236 | 0.181 | 0.124 |

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 9.5-26: Aquatic organisms: PEC/RAC ratios for <u>zoxamide</u> based on the RAC for fish prolonged and FOCUS SW Step 4 calculations with mitigation of plant $DT_{50}$, spray drift and run-off for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in <u>potatoes, multiple applications</u>**

| **Intended use** | | Potatoes, multiple applications | | | | |
|---|---|---|---|---|---|---|
| **Active substance** | | Zoxamide | | | | |
| **Application rate (g/ha)** | | 3 x 148.5 | | | | |
| **RAC (µg/L) fish prolonged** | | 0.348 | | | | |
| **Nozzle reduction** | **No-spray buffer (m)** | 1/3 | 5 | 10 | 15 | 20 |
| | **Vegetated filter strip (m)** | FOCUS default | 5 | 10 | 15 | 20 |
| None | D3 ditch | **1.624** | 0.523 | 0.273 | 0.187 | 0.141 |
| 50% | | 0.813 | 0.261 | 0.138 | 0.092 | 0.069 |
| 75% | | 0.405 | 0.129 | 0.069 | 0.046 | 0.034 |
| 90% | | 0.164 | 0.052 | 0.029 | 0.017 | 0.014 |
| None | D4 pond | 0.167 | 0.149 | 0.106 | 0.083 | 0.069 |
| 50% | | 0.083 | 0.075 | 0.052 | 0.043 | 0.034 |
| 75% | | 0.040 | 0.037 | 0.026 | 0.020 | 0.017 |
| 90% | | 0.017 | 0.014 | 0.011 | 0.009 | 0.006 |
| None | D4 stream | **1.305** | 0.540 | 0.284 | 0.193 | 0.147 |
| 50% | | 0.652 | 0.270 | 0.141 | 0.095 | 0.072 |
| 75% | | 0.328 | 0.135 | 0.072 | 0.049 | 0.037 |
| 90% | | 0.129 | 0.055 | 0.029 | 0.020 | 0.014 |
| None | D6 ditch 1st | **1.618** | 0.520 | 0.273 | 0.184 | 0.141 |
| 50% | | 0.810 | 0.259 | 0.135 | 0.092 | 0.069 |
| 75% | | 0.405 | 0.129 | 0.069 | 0.046 | 0.034 |
| 90% | | 0.161 | 0.052 | 0.026 | 0.017 | 0.014 |
| None | D6 ditch 2nd | **1.606** | 0.514 | 0.270 | 0.184 | 0.138 |
| 50% | | 0.802 | 0.259 | 0.135 | 0.092 | 0.069 |
| 75% | | 0.402 | 0.129 | 0.069 | 0.046 | 0.034 |
| 90% | | 0.161 | 0.052 | 0.026 | 0.017 | 0.014 |
| None | R1 pond | 0.250 | 0.210 | 0.138 | 0.106 | 0.083 |
| 50% | | 0.172 | 0.141 | 0.086 | 0.069 | 0.052 |
| 75% | | 0.132 | 0.106 | 0.063 | 0.049 | 0.034 |
| 90% | | 0.109 | 0.086 | 0.049 | 0.037 | 0.026 |
| None | R1 stream | **1.534** | **1.213** | 0.698 | 0.534 | 0.365 |
| 50% | | **1.534** | **1.213** | 0.698 | 0.534 | 0.365 |
| 75% | | **1.534** | **1.213** | 0.698 | 0.534 | 0.365 |
| 90% | | **1.534** | **1.213** | 0.698 | 0.534 | 0.365 |
| None | R2 stream | **1.503** | 0.790 | 0.448 | 0.342 | 0.233 |
| 50% | | **1.006** | 0.790 | 0.448 | 0.342 | 0.233 |
| 75% | | **1.006** | 0.790 | 0.448 | 0.342 | 0.233 |
| 90% | | **1.006** | 0.790 | 0.448 | 0.342 | 0.233 |

| | | | | | | |
|---|---|---|---|---|---|---|
| None | R3 stream | **1.977** | **1.560** | 0.897 | 0.687 | 0.468 |
| 50% | | **1.977** | **1.560** | 0.897 | 0.687 | 0.468 |
| 75% | | **1.977** | **1.560** | 0.897 | 0.687 | 0.468 |
| 90% | | **1.977** | **1.560** | 0.897 | 0.687 | 0.468 |

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 9.5-27: Aquatic organisms: PEC/RAC ratios for <u>zoxamide</u> based on the RAC for fish prolonged and FOCUS SW Step 4 calculations with mitigation of plant $DT_{50}$, spray drift and run-off for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in <u>potatoes; single application</u>**

| **Intended use** | | Potatoes, single application | | | | |
|---|---|---|---|---|---|---|
| **Active substance** | | Zoxamide | | | | |
| **Application rate (g/ha)** | | 148.5 | | | | |
| **RAC (µg/L) fish prolonged** | | 0.348 | | | | |
| **Nozzle reduction** | **No-spray buffer (m)** | 1/3 | 5 | 10 | 15 | 20 |
| | **Vegetated filter strip (m)** | FOCUS default | 5 | 10 | 15 | 20 |
| None | D3 ditch | **2.236** | 0.733 | 0.388 | 0.264 | 0.201 |
| 50% | | **1.118** | 0.365 | 0.195 | 0.132 | 0.101 |
| 75% | | 0.557 | 0.184 | 0.098 | 0.066 | 0.052 |
| 90% | | 0.224 | 0.072 | 0.040 | 0.026 | 0.020 |
| None | D4 pond | 0.089 | 0.080 | 0.057 | 0.046 | 0.037 |
| 50% | | 0.046 | 0.040 | 0.029 | 0.023 | 0.020 |
| 75% | | 0.023 | 0.020 | 0.014 | 0.011 | 0.009 |
| 90% | | 0.009 | 0.009 | 0.006 | 0.006 | 0.003 |
| None | D4 stream | **1.744** | 0.736 | 0.391 | 0.267 | 0.201 |
| 50% | | 0.874 | 0.368 | 0.195 | 0.132 | 0.101 |
| 75% | | 0.437 | 0.184 | 0.098 | 0.066 | 0.052 |
| 90% | | 0.175 | 0.075 | 0.040 | 0.026 | 0.020 |
| None | D6 ditch 1st | **2.210** | 0.724 | 0.385 | 0.261 | 0.198 |
| 50% | | **1.106** | 0.362 | 0.193 | 0.132 | 0.101 |
| 75% | | 0.552 | 0.181 | 0.095 | 0.066 | 0.049 |
| 90% | | 0.221 | 0.072 | 0.037 | 0.026 | 0.020 |
| None | D6 ditch 2nd | **2.193** | 0.718 | 0.382 | 0.261 | 0.198 |
| 50% | | **1.098** | 0.359 | 0.190 | 0.129 | 0.098 |
| 75% | | 0.549 | 0.181 | 0.095 | 0.066 | 0.049 |
| 90% | | 0.218 | 0.072 | 0.037 | 0.026 | 0.020 |
| None | R1 pond | 0.149 | 0.124 | 0.078 | 0.060 | 0.046 |
| 50% | | 0.112 | 0.089 | 0.055 | 0.040 | 0.032 |
| 75% | | 0.092 | 0.072 | 0.043 | 0.032 | 0.023 |
| 90% | | 0.080 | 0.063 | 0.034 | 0.026 | 0.017 |

| | | | | | | |
|---|---|---|---|---|---|---|
| None | R1 stream | **1.549** | 0.897 | 0.517 | 0.397 | 0.270 |
| 50% | | **1.135** | 0.897 | 0.517 | 0.397 | 0.270 |
| 75% | | **1.135** | 0.897 | 0.517 | 0.397 | 0.270 |
| 90% | | **1.135** | 0.897 | 0.517 | 0.397 | 0.270 |
| None | R2 stream | **2.046** | 0.862 | 0.457 | 0.313 | 0.239 |
| 50% | | **1.023** | 0.431 | 0.230 | 0.155 | 0.118 |
| 75% | | 0.511 | 0.267 | 0.152 | 0.118 | 0.080 |
| 90% | | 0.342 | 0.267 | 0.152 | 0.118 | 0.080 |
| None | R3 stream | **2.181** | 0.920 | 0.489 | 0.333 | 0.253 |
| 50% | | **1.092** | 0.460 | 0.244 | 0.167 | 0.126 |
| 75% | | 0.546 | 0.264 | 0.152 | 0.118 | 0.080 |
| 90% | | 0.336 | 0.264 | 0.152 | 0.118 | 0.080 |

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

Cymoxanil

The risk assessment of Cymoxanil following the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' on potato is covered by the assessment reported in the DAR, where the use of Cymoxanil on potato from BBCH 21 to 95 with 6-8 application up to 0.175 kg a.s./ha (7 days of interval between applications) resulted to be safe. Therefore, to assess the risk to aquatic organisms of Cymoxanil and its metabolites, only the use on grape and tomato is considered in the present document.

For grape, the $PEC_{sw}$ for the a.s. Cymoxanil and its water-relevant metabolites were calculated with FOCUS modelling considering the maximum application rate of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' (0.1485 kg Cymoxanil/ha); the PECsw values resulting from Step 2 model were sufficient to demonstrate acceptable risk for aquatic organisms. Details of the calculation are reported in Section 5 of this Registration Report. In addition, according to SANCO document, the risk associated to the exposure to leachate when groundwater reaches surface water, needs to be considered. As detailed in Section 5, PECgw following application of Cymoxanil 33% + Zoxamide 33% WG on tomato and grape were calculated for Cymoxanil and for the metabolites with a leaching potential. Since the $PEC_{gw}$ were significantly lower than the $PEC_{sw}$, only the $PEC_{sw}$ were considered in the present risk assessment (risk-envelope approach).

The highest Step 2 $PEC_{sw}$ values for the a.s. Cymoxanil and its metabolites were obtained from late application of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' on grape; these values were used to assess the risk to aquatic organisms.

The results of the acute risk assessment for fish, carried out considering the endpoints reported in the below table and the PECsw summarized in Section B8, are reported in the table below:

**Table 9.5-28: Acute risk assessment for Fish – a.s. Cymoxanil**

| Substance | Timescale | Toxicity endpoint (µg/L) | $PEC_{sw}$ (µg a.s./L) | TER | Trigger |
|---|---|---|---|---|---|
| Cymoxanil | Acute | $EC_{50}$ 29000 | 3.285 | 8828 | 100 |
| | Chronic | NOEC 44 | | 13.4 | 10 |
| IN-U3204 | Acute | $EC_{50}$ > 97000 | 0.811 | > 119605 | 100 |
| IN-W3595 | Acute | $EC_{50}$ > 130000 | 0.690 | > 188406 | 100 |
| IN-KQ960 | Acute | $EC_{50}$ > 120000 | 2.249 | > 53357 | 100 |
| IN-T4226 | Acute | $EC_{50}$ > 111000 | 1.088 | > 102022 | 100 |

Since the resulting acute TER values for fish are well above the trigger of 100, it is possible to conclude that the active ingredient Cymoxanil and its metabolites pose an acceptable acute risk to fish.

No studies on fish are available for metabolites IN-JX915, IN-R3273, IN-KP533, and the metabolite fraction M5. However, as stated in the ESA Scientific report for Cymoxanil, in an additional static acute study on the toxicity of Cymoxanil to fish, metabolites arising from the mainly abiotic degradation of the parent compound under test conditions were identified and quantified (in parallel test solutions without fish). Moreover, the PECsw values estimated for the metabolites IN-JX915, IN-R3273, IN-KP533 and the metabolite fraction M5 were several orders of magnitude lower than the concentrations at which no fish died in the static test. Therefore, the risk to fish from these metabolites was considered to be low.

The results of the acute risk assessment for Daphnia carried out considering the endpoints reported in Table 9.5-2 and the PECsw summarized in Table 9.5-29, are reported in the table below:

**Table 9.5-29: Acute risk assessment for *Daphnia* – a.s. Cymoxanil**

| Substance | Timescale | Toxicity endpoint (µg/L) | $PEC_{sw}$ (µg/L) | TER | Trigger |
|---|---|---|---|---|---|
| Cymoxanil | Acute | $EC_{50}$ 27000 | 3.285 | 8219 | 100 |
| | Chronic | NOEC 67 | | 20 | 10 |
| IN-U3204 | Acute | $EC_{50}$ 100000 | 0.811 | 123305 | 100 |
| IN-W3595 | Acute | $EC_{50}$ > 126000 | 0.690 | > 182609 | 00 |
| IN-KQ960 | Acute | $EC_{50}$ 800 | 2.249 | 356 | 100 |
| | Chronic | NOEC 302 | | 134 | 10 |
| IN-T4226 | Acute | $EC_{50}$ > 116000 | 1.088 | > 106618 | 100 |

Since the resulting acute TER values for daphnia are well above the trigger of 100, it is possible to conclude that the active ingredient and its metabolites pose an acceptable acute risk to aquatic invertebrates.
No studies on daphnia are available for metabolites IN-JX915, IN-R3273, IN-KP533, and the metabolite fraction M5. However, as stated in the EFSA Scientific report for Cymoxanil, if the toxicity of these metabolites to daphnids were higher than the toxicity of the active substance by a factor of 100, TER values would still be above the respective Annex VI trigger, indicating a low acute risk to invertebrates.

**Table 9.5-29: Risk assessment for Algae – a.s. Cymoxanil**

| Substance | Timescale | $EC_{50}$ (µg/L) | PECsw (µg/L) | TER |
|---|---|---|---|---|
| Cymoxanil | Acute | 122 | 3.285 | 37.1 |
| IN-W3595 | Acute | 12700 | 0.690 | 18406 |
| IN-T4226 | Acute | 25800 | 1.088 | 23713 |

Since the resulting TER values for algae are well above the trigger of 10, it is possible to conclude that the active ingredient and its metabolites pose an acceptable risk to Algae.
No studies on algae are available for metabolites IN-U3204, IN-KQ960, IN-JX915, IN-R3273, IN-KP533, and the metabolite fraction M5. However, as stated in the ESA Scientific report for Cymoxanil, and accepted by member state experts at PRAPeR 48, all studies with algae were performed under static conditions at relatively high pH levels. At alkaline pH levels the degradation of Cymoxanil is mainly driven by abiotic processes (basically hydrolysis). Hence from fate and behaviour information on Cymoxanil it is reasonable to assume that the metabolites INKQ960, IN-U3204, IN-JX915, IN-R3273, IN-KP533 and the metabolite fraction M5 have been present in the test solutions of the algae studies with Cymoxanil to a sufficient extent to have influenced the outcome of the studies. The risk to aquatic algae from exposure to these metabolites was therefore considered to be low, as it was covered by the assessment of the parent substance.

**Table 9.5-31: Risk assessment for aquatic plants – a.s. Cymoxanil**

| Substance | Timescale | Toxicity endpoint (µg/L) | $PEC_{sw}$ (µg/L) | TER | Trigger |
|---|---|---|---|---|---|
| Cymoxanil | Acute | NOEC 700 | 3.285 | 213.09 | 10 |

The results show that the risks to aquatic plants (*Lemna gibba*) from cymoxanil are acceptable.

**Metabolites of zoxamide**

For zoxamide metabolites the risk assessment is based on acute toxicity endpoints presented in Table 9.5-1 and FOCUS SW Step 1 and 2 PEC values available from Part B.8.
For aquatic invertebrates only worst-case toxicity endpoints for *Americamysis bahia* were taken into account.

**Table 9.5-30: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the zoxamide metabolite RH-127450 for each organism group based on FOCUS Step 1 and 2 calculations for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in vines, early**

| Group | | Fish acute | Inverteb. acute | Algae |
|---|---|---|---|---|
| Test species | | *Oncorhynchus mykiss* | *Americamysis bahia* | *Raphidocelis subcapitata* |
| Endpoint (µg/L) | | $LC_{50}$ 4170 | $LC_{50}$ 2930 | $E_rC_{50}$ > 6600 |
| AF | | 100 | 100 | 10 |
| RAC (µg/L) | | 41.7 | 29.3 | > 660 |
| FOCUS Scenario | PEC gl-max (µg/L) | | | |
| Step 1 | | | | |
| | 41.904 | **1.005** | **1.430** | < 0.063 |

| | | | | |
|---|---|---|---|---|
| **Step 2** | | | | |
| N-Europe | 2.375 | 0.057 | 0.081 | < 0.004 |
| S-Europe | 3.903 | 0.094 | 0.133 | < 0.006 |

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 9.5-31: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the zoxamide metabolite RH-127450 for each organism group based on FOCUS Step 1 and 2 calculations for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in vines, late**

| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** |
|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Americamysis bahia*** | ***Raphidocelis subcapitata*** |
| **Endpoint** | | $LC_{50}$ | $LC_{50}$ | $E_rC_{50}$ |
| **(µg/L)** | | 4170 | 2930 | > 6600 |
| **AF** | | 100 | 100 | 10 |
| **RAC (µg/L)** | | 41.7 | 29.3 | > 660 |
| **FOCUS Scenario** | **PEC $_{gl\text{-}max}$ (µg/L)** | | | |
| **Step 1** | | | | |
| | 44.696 | **1.072** | **1.525** | < 0.068 |
| **Step 2** | | | | |
| N-Europe | 3.311 | 0.079 | 0.113 | < 0.005 |
| S-Europe | 3.820 | 0.092 | 0.130 | < 0.006 |

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 9.5-32: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the zoxamide metabolite RH-127450 for each organism group based on FOCUS Step 1 and 2 calculations for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in potatoes**

| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** |
|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Americamysis bahia*** | ***Raphidocelis subcapitata*** |
| **Endpoint** | | $LC_{50}$ | $LC_{50}$ | $E_rC_{50}$ |
| **(µg/L)** | | 4170 | 2930 | > 6600 |
| **AF** | | 100 | 100 | 10 |
| **RAC (µg/L)** | | 41.7 | 29.3 | > 660 |
| **FOCUS Scenario** | **PEC $_{gl\text{-}max}$ (µg/L)** | | | |
| **Step 1** | | | | |
| | 41.936 | **1.006** | **1.431** | < 0.064 |
| **Step 2** | | | | |
| N-Europe | 2.838 | 0.068 | 0.097 | < 0.004 |
| S-Europe | 5.004 | 0.120 | 0.171 | < 0.008 |

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 9.5-33: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the zoxamide metabolite RH-24549 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in vines, early**

| **Group** | | **Fish acute** | **Inverteb. Acute** | **Algae** |
|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Americamysis bahia*** | ***Desmodesmus subspicatus*** |
| **Endpoint (µg/L)** | | $LC_{50}$ 23000 | $LC_{50}$ 23200 | $E_rC_{50}$ > 60000 |
| **AF** | | 100 | 100 | 10 |
| **RAC (µg/L)** | | 230 | 232 | > 6000 |
| **FOCUS Scenario** | **PEC $_{gl\text{-}max}$ (µg/L)** | | | |
| **Step 1** | | | | |
| | 31.428 | 0.137 | 0.135 | < 0.005 |
| **Step 2** | | | | |
| N-Europe | 1.521 | 0.007 | 0.007 | < 0.001 |
| S-Europe | 2.937 | 0.013 | 0.013 | < 0.001 |

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

**Table 9.5-34: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the zoxamide metabolite RH-24549 for each organism group based on FOCUS Step 1 and 2 calculations for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in vines, late**

| **Group** | | **Fish acute** | **Inverteb. Acute** | **Algae** |
|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Americamysis bahia*** | ***Desmodesmus subspicatus*** |
| **Endpoint (µg/L)** | | $LC_{50}$ 23000 | $LC_{50}$ 23200 | $E_rC_{50}$ > 60000 |
| **AF** | | 100 | 100 | 10 |
| **RAC (µg/L)** | | 230 | 232 | > 6000 |
| **FOCUS Scenario** | **PEC $_{gl\text{-}max}$ (µg/L)** | | | |
| **Step 1** | | | | |
| | 31.669 | 0.138 | 0.137 | < 0.005 |
| **Step 2** | | | | |
| N-Europe | 1.230 | 0.005 | 0.005 | < 0.001 |
| S-Europe | 1.702 | 0.007 | 0.007 | < 0.001 |

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

**Table 9.5-35: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the zoxamide metabolite RH-24549 for each organism group based on FOCUS Step 1 and 2 calculations for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in potatoes**

| **Group** | | **Fish acute** | **Inverteb. Acute** | **Algae** |
|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Americamysis bahia*** | ***Desmodesmus subspicatus*** |
| **Endpoint (µg/L)** | | $LC_{50}$ 23000 | $LC_{50}$ 23200 | $E_rC_{50}$ > 60000 |
| **AF** | | 100 | 100 | 10 |
| **RAC (µg/L)** | | 230 | 232 | > 6000 |
| **FOCUS Scenario** | **PEC gl-max (µg/L)** | | | |
| **Step 1** | | | | |
| | 31.431 | 0.137 | 0.135 | < 0.005 |
| **Step 2** | | | | |
| N-Europe | 2.090 | 0.009 | 0.009 | < 0.001 |
| S-Europe | 4.095 | 0.018 | 0.018 | < 0.001 |

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

**Table 9.5-36: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the zoxamide metabolite RH-163353 for each organism group based on FOCUS Step 1 and 2 calculations for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in vines, early**

| **Group** | | **Fish acute** | **Inverteb. Acute** | **Algae** |
|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Americamysis bahia*** | ***Raphidocelis subcapitata*** |
| **Endpoint (µg/L)** | | $LC_{50}$ > 100000 | $LC_{50}$ > 100000 | $E_rC_{50}$ > 100000 |
| **AF** | | 100 | 100 | 10 |
| **RAC (µg/L)** | | > 1000 | > 1000 | > 10000 |
| **FOCUS Scenario** | **PEC gl-max (µg/L)** | | | |
| **Step 1** | | | | |
| | 48.638 | < 0.049 | < 0.049 | < 0.005 |
| **Step 2** | | | | |
| N-Europe | 3.054 | < 0.003 | < 0.003 | < 0.001 |
| S-Europe | 5.389 | < 0.005 | < 0.005 | < 0.001 |

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

**Table 9.5-37: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the zoxamide metabolite RH-163353 for each organism group based on FOCUS Step 1 and 2 calculations for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in vines, late**

| **Group** | | **Fish acute** | **Inverteb. Acute** | **Algae** |
|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Americamysis bahia*** | ***Raphidocelis Subcapitata*** |
| **Endpoint (µg/L)** | | $LC_{50}$ > 100000 | $LC_{50}$ > 100000 | $E_rC_{50}$ > 100000 |
| **AF** | | 100 | 100 | 10 |
| **RAC (µg/L)** | | > 1000 | > 1000 | > 10000 |
| **FOCUS Scenario** | **PEC $_{gl\text{-}max}$ (µg/L)** | | | |
| **Step 1** | | | | |
| | 50.246 | < 0.050 | < 0.050 | < 0.005 |
| **Step 2** | | | | |
| N-Europe | 3.505 | < 0.004 | < 0.004 | < 0.001 |
| S-Europe | 4.284 | < 0.004 | < 0.004 | < 0.001 |

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

**Table 9.5-38: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the zoxamide metabolite RH-163353 for each organism group based on FOCUS Step 1 and 2 calculations for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in potatoes**

| **Group** | | **Fish acute** | **Inverteb. Acute** | **Algae** |
|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Americamysis bahia*** | ***Raphidocelis subcapitata*** |
| **Endpoint (µg/L)** | | $LC_{50}$ > 100000 | $LC_{50}$ > 100000 | $E_rC_{50}$ > 100000 |
| **AF** | | 100 | 100 | 10 |
| **RAC (µg/L)** | | > 1000 | > 1000 | > 10000 |
| **FOCUS Scenario** | **PEC $_{gl\text{-}max}$ (µg/L)** | | | |
| **Step 1** | | | | |
| | 48.656 | < 0.049 | < 0.049 | < 0.005 |
| **Step 2** | | | | |
| N-Europe | 3.880 | < 0.004 | < 0.004 | < 0.001 |
| S-Europe | 7.188 | < 0.007 | < 0.007 | < 0.001 |

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

**Table 9.5-39: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the zoxamide metabolite RH-141455 for each organism group based on FOCUS Step 1 and 2 calculations for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in vines, early**

| **Group** | | **Fish acute** | **Inverteb. Acute** | **Algae** |
|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Americamysis bahia*** | ***Pseudokirchneriella subcapitata*** |
| **Endpoint (µg/L)** | | $LC_{50}$ > 100000 | $LC_{50}$ > 100000 | $E_rC_{50}$ > 100000 |
| **AF** | | 100 | 100 | 10 |
| **RAC (µg/L)** | | > 1000 | > 1000 | > 10000 |
| **FOCUS Scenario** | **PEC $_{gl\text{-}max}$ (µg/L)** | | | |
| **Step 1** | | | | |
| | 10.904 | < 0.011 | < 0.011 | < 0.001 |
| **Step 2** | | | | |
| N-Europe | 0.568 | < 0.006 | < 0.006 | < 0.001 |
| S-Europe | 1.082 | < 0.001 | < 0.001 | < 0.001 |

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

**Table 9.5-40: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the zoxamide metabolite RH-141455 for each organism group based on FOCUS Step 1 and 2 calculations for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in vines, late**

| **Group** | | **Fish acute** | **Inverteb. Acute** | **Algae** |
|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Americamysis bahia*** | ***Pseudokirchneriella subcapitata*** |
| **Endpoint (µg/L)** | | $LC_{50}$ > 100000 | $LC_{50}$ > 100000 | $E_rC_{50}$ > 100000 |
| **AF** | | 100 | 100 | 10 |
| **RAC (µg/L)** | | > 1000 | > 1000 | > 10000 |
| **FOCUS Scenario** | **PEC $_{gl\text{-}max}$ (µg/L)** | | | |
| **Step 1** | | | | |
| | 11.020 | < 0.011 | < 0.011 | < 0.001 |
| **Step 2** | | | | |
| N-Europe | 0.491 | < 0.001 | < 0.001 | < 0.001 |
| S-Europe | 0.662 | < 0.001 | < 0.001 | < 0.001 |

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

**Table 9.5-41: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the zoxamide metabolite RH-141455 for each organism group based on FOCUS Step 1 and 2 calculations for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in potatoes**

| **Group** | | **Fish acute** | **Inverteb. Acute** | **Algae** |
|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Americamysis bahia*** | ***Pseudokirchneriella subcapitata*** |
| **Endpoint** | | $LC_{50}$ | $LC_{50}$ | $E_rC_{50}$ |
| **(µg/L)** | | > 100000 | > 100000 | > 100000 |
| **AF** | | 100 | 100 | 10 |
| **RAC (µg/L)** | | > 1000 | > 1000 | > 10000 |
| **FOCUS Scenario** | **PEC gl-max (µg/L)** | | | |
| **Step 1** | | | | |
| | 10.905 | < 0.011 | < 0.011 | < 0.001 |
| **Step 2** | | | | |
| N-Europe | 0.771 | < 0.001 | < 0.001 | < 0.001 |
| S-Europe | 1.498 | < 0.001 | < 0.001 | < 0.001 |

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

**Table 9.5-42: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the zoxamide metabolite RH-139432 for each organism group based on FOCUS Step 1 and 2 calculations for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in vines, early**

| **Group** | | **Fish acute** | **Inverteb. Acute** | **Algae** |
|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Americamysis bahia*** | ***Scenedesmus subcapitatus*** |
| **Endpoint** | | $LC_{50}$ | $LC_{50}$ | $E_rC_{50}$ |
| **(µg/L)** | | 2000 | 6950 | > 30000 |
| **AF** | | 100 | 100 | 10 |
| **RAC (µg/L)** | | 20 | 69.5 | > 3000 |
| **FOCUS Scenario** | **PEC gl-max (µg/L)** | | | |
| **Step 1** | | | | |
| | 23.882 | **1.194** | 0.344 | < 0.008 |
| **Step 2** | | | | |
| N-Europe | 1.729 | 0.086 | 0.025 | < 0.001 |
| S-Europe | 2.975 | 0.149 | 0.043 | < 0.001 |

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

**Table 9.5-43: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the zoxamide metabolite RH-139432 for each organism group based on FOCUS Step 1 and 2 calculations for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in vines, late**

| **Group** | | **Fish acute** | **Inverteb. Acute** | **Algae** |
|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Americamysis bahia*** | ***Scenedesmus subcapitatus*** |
| **Endpoint (µg/L)** | | $LC_{50}$ 2000 | $LC_{50}$ 6950 | $E_rC_{50}$ > 30000 |
| **AF** | | 100 | 100 | 10 |
| **RAC (µg/L)** | | 20 | 69.5 | > 3000 |
| **FOCUS Scenario** | **PEC $_{gl-max}$ (µg/L)** | | | |
| **Step 1** | | | | |
| | 24.908 | **1.245** | 0.358 | < 0.008 |
| **Step 2** | | | | |
| N-Europe | 2.138 | 0.107 | 0.031 | < 0.001 |
| S-Europe | 2.554 | 0.123 | 0.037 | < 0.001 |

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

**Table 9.5-44: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the zoxamide metabolite RH-139432 for each organism group based on FOCUS Step 1 and 2 calculations for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in potatoes**

| **Group** | | **Fish acute** | **Inverteb. Acute** | **Algae** |
|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Americamysis bahia*** | ***Scenedesmus subcapitatus*** |
| **Endpoint (µg/L)** | | $LC_{50}$ 2000 | $LC_{50}$ 6950 | $E_rC_{50}$ > 30000 |
| **AF** | | 100 | 100 | 10 |
| **RAC (µg/L)** | | 20 | 69.5 | > 3000 |
| **FOCUS Scenario** | **PEC $_{gl-max}$ (µg/L)** | | | |
| **Step 1** | | | | |
| | 23.893 | **1.195** | 0.344 | < 0.008 |
| **Step 2** | | | | |
| N-Europe | 2.149 | 0.107 | 0.031 | < 0.001 |
| S-Europe | 3.915 | 0.196 | 0.056 | < 0.001 |

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

Resulting PEC/RAC values for all zoxamide metabolites and all the intended uses are below 1 when FOCUS Step 1 or 2 $PEC_{sw}$ values are considered. Therefore, no unacceptable risk to aquatic organisms caused by the exposure to zoxamide metabolites is expected following the application of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' according to the intended use pattern.

**Mixture toxicity**

Regulation (EC) No. 1107/2009 requires in Article 29 that interaction between the a.s., safeners, synergists and co-formulants shall be taken into account.

Studies on aquatic organisms were carried out with the formulated product 'CYMOXANIL 33% + ZOXAMIDE 33% WG' according to the provisions of Reg. (EU) 545/2011 and have been presented during product authorisation. The resulting endpoints are hereunder summarised.

**Table 9.5-45: 'CYMOXANIL 33% + ZOXAMIDE 33% WG' - summary of the toxicity values for aquatic organisms**

| Organism | Species | Endpoint | Value | Reference |
|---|---|---|---|---|
| Fish | *Oncorhynchus mykiss* | 96h $LC_{50}$ | 0.83 mg prod./L *(corresponding to 0.27 mg a.s./L)* | xxx (2007) Repot no. CH-E-023/2006 |
| Aquatic invertebrates | *Daphnia magna* | 48h $EC_{50}$ | > 44.64 mg prod./L *(corresponding to 14.73 mg a.s./L)* | xxx (2007) Repot no. CH-001/2007 |
| Algae | *Pseudokirchneriella subcapitata* | 72h $E_bC_{50}$ | 0.055 mg/L *(corresponding to 0.018 mg a.s./L)* | xxx (2007) Repot no. CH-002/2007 |

In order to identify the worst-case toxicity endpoints on aquatic organisms for the risk assessment, the measured acute toxicity of the formulation was compared to the predicted acute mixture toxicity assuming dose additivity. A higher measured toxicity value compared to the predicted $LC_{50}/EC_{50(mix)}$ value (i.e. measured/predicted toxicity ration > 1) indicates that the formulation is less toxic than predicted from the toxicity of the active substances. In this case, TER calculations based on toxicity data for the active substances can be regarded as relevant. This has been demonstrated in the following table, where measured/predicted toxicity ratios were calculated for each aquatic group:

**Table 9.5-46: Measured toxicity/predicted toxicity ratios**

| Substance | Measured toxicity a.s. - $LC_{50}/EC_{50}$(a.s.) [µg a.s./L] | Nominal content of a.s. in the product [g a.s./kg] | Fraction in the product x(a.s.)* | Predicted toxicity - $LC_{50}/EC_{50}$(mix) [µg prod./L] | Measured toxicity product - $LC_{50}/EC_{50}$(prod.) [µg prod./L] | Measured/ predicted toxicity ratio |
|---|---|---|---|---|---|---|
| **Fish (*Oncorhynchus mykiss*), acute** | | | | | | |
| Zoxamide | 160 | 330 | 0.5 | 318.24 | 830 | 2.61 |
| Cymoxanil | 29 000 | 330 | 0.5 | | | |
| **Aquatic invertebrates (*Daphnia magna*), acute** | | | | | | |
| Zoxamide | 780 | 330 | 0.5 | 1 516.20 | > 44 640 | 29.44 |
| Cymoxanil | 27 000 | 330 | 0.5 | | | |
| **Algae** | | | | | | |
| Zoxamide | 14.13 # | 330 | 0.5 | 25.33 | 55 | 2.17 |
| Cymoxanil | 122 [1] | 330 | 0.5 | | | |

* sum of x(a.s.) is equal to 1

# *Selenastrum capricornutum*

[1] *Anabaena flos-aquae.*

For comparison of single active substance and mixture toxicity in terms of potential risk, a "tox per fraction" value can be calculated for each active substance in the mixture. If one of the active substances

clearly drives the toxicity of the formulation (e.g. toxic unit > 90%), a sufficient protection level might already be achieved by simply basing the risk assessment on the toxicity data for that single driver.

**Table 9.5-47: Toxic Unit of active substances as contained in 'CYMOXANIL 33% + ZOXAMIDE 33% WG'**

| **Product:** | 'CYMOXANIL 33% + ZOXAMIDE 33% WG' | | | | |
|---|---|---|---|---|---|
| **a.s. 1 content** | zoxamide, 330 g/kg | | | | |
| **a.s. 2 content** | cymoxanil, 330 g/kg | | | | |
| **Organisms** | **Endpoint** | **Measured toxicity of zoxamide** | **Measured toxicity of cymoxanil** | **Toxic Unit of zoxamide** | **Toxic Unit of cymoxanil** |
| | | **[mg a.s./L]** | **[mg a.s./L]** | **[%]** | **[%]** |
| *Oncorhynchus mykiss* | 96h-$LC_{50}$ | 0.160 | 29 | 94.45 | 5.55 |
| *Oncorhynchus mykiss* | NOEC* | 0.003 | 0.044 | 92.67 | 7.33 |
| *Daphnia magna* | 48h-$EC_{50}$ | 0.780 | 27 | 97.19 | 2.81 |
| *Daphnia magna* | 21d-NOEC | 0.039 | 0.067 | 63.21 | 36.79 |
| *Algae* * | 72h-$E_rC_{50}$ | 0.014 | 0.122 | 89.71 | 10.29 |
| *Chironomus riparius* | 28d-NOEC | 0.380 | -- | -- | -- |

* *Selenastrum capricornutu* for zoxamide and *Anabaena flos-aquae* for cymoxanil as worst-case.

As indicated in the table above, the acute and chronic toxicity of the mixture to fish, aquatic invertebrates and algae can be largely explained by the toxicity of zoxamide. Zoxamide is driving the aquatic risk assessment. Consequently, the risk from the mixture is largely covered by the risk calculations for zoxamide.

Overall, no synergistic effects are expected when aquatic organisms are exposed to 'CYMOXANIL 33% + ZOXAMIDE 33% WG'. Moreover, the formulated product 'CYMOXANIL 33% + ZOXAMIDE 33% WG' is less toxic than predicted based on the toxicity of the active substances. The active substance zoxamide clearly drives the toxicity of the formulated product. Therefore, it is applicable to conduct the risk assessment for aquatic organisms based on the toxicity data for the active substances zoxamide and cymoxanil.

**Risk assessment based on data for the formulated product 'CYMOXANIL 33% + ZOXAMIDE 33% WG'**

The PEC values for the formulated product were taken from Section 8. Single application values are considered relevant; multiple applications and long-term PECs are better described by active substance data.

**Table 9.5-48: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the formulation 'CYMOXANIL 33% + ZOXAMIDE 33% WG' for each organism group based on spray drift entry**

| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** |
|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Daphnia magna*** | ***Pseudokirchneriella subcapitata*** |
| **Endpoint** | | $LC_{50}$ | $EC_{50}$ | $E_rC_{50}$ |
| **(µg/L)** | | 830 | > 44 640 | 55 |
| **AF** | | 100 | 100 | 10 |
| **RAC (µg/L)** | | 8.3 | 446.4 | 5.5 |

| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** |
|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Daphnia magna*** | ***Pseudokirchneriella subcapitata*** |
| **Endpoint** | | $LC_{50}$ | $EC_{50}$ | $E_rC_{50}$ |
| **(µg/L)** | | 830 | > 44 640 | 55 |
| **AF** | | 100 | 100 | 10 |
| **RAC (µg/L)** | | 8.3 | 446.4 | 5.5 |
| | **$PEC_{gl\text{-}max}$ (µg/L)** | | | |
| Vine early, 3 m (standard) | 2.578 | 0.31 | 0.006 | 0.469 |
| Vine late, 3 m (standard) | 7.760 | 0.93 | 0.017 | **1.41** |
| Vine late, 10 m buffer | 1.699 | -- | -- | 0.31 |
| Potato field, 1-2 m (standard) | 2.891 | 0.35 | 0.006 | 0.53 |

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

As a result, PEC/RAC ratios are lower than 1.0 for all organisms if a buffer zone of 10 m is applied.

### 9.5.3 Overall conclusions

The aquatic risk assessment was carried out according to the EFSA (2013) aquatic guidance document (EFSA Journal 2013;11(7):3290).

New studies are available for zoxamide and its metabolites, which were either requested by EFSA (2017) during AIR zoxamide or are required according to Regulation (EC) No. 283/2013, respectively. The studies on 'CYMOXANIL 33% + ZOXAMIDE 33% WG' are all available from the product authorisation. A comparison of the results for 'CYMOXANIL 33% + ZOXAMIDE 33% WG' with the results for the active substances revealed no additive or synergistic effects. Instead, the assessment confirms that zoxamide drives the aquatoxicity of the mixture product.

The metabolites of zoxamide are of lower toxicity than the parent active substance.

For zoxamide metabolites, PEC/RAC ratios are below 1 for all aquatic organism when FOCUS SW Step 1+2 PECs are considered, indicating an acceptable risk at a lower tier. However, for the active substance zoxamide FOCUS SW Step 4 PECs are required.

For the intended worst-case GAP uses of zoxamide on potato fields and in vineyards (with **3 x 0.45 kg prod./ha**) the calculated PEC/RAC ratios (lower than 1.0) indicate an acceptable risk for aquatic organisms. However, several scenarios needed refinement. Therefore, further PEC/RAC ratios were calculated based on FOCUS SW Step 4 PECs considering a more realistic half-life of zoxamide on/in plants as well as run-off reducing vegetated buffer zones and drift reducing measures (drift reducing buffer zones and equipment/nozzles), leading to a reduction of the exposure of surface water bodies. As a result, the implementation of the following measures is necessary:

- For potatoes a 10 m non-sprayed, vegetated buffer zone.
- For vineyards 20 m non-sprayed, vegetated buffer zone.

FOCUS SW Step 4 calculations with the reduced max. dose in grapes authorised in certain EU zone countries were included for completeness. Also for worst-case GAP uses with 3x 0.4 kg prod./ha in vines

a vegetated buffer zone of 20 m to receive a PEC/RAC ratio lower than 1.0 in case that the R4 scenario is applicable.

Review Comments:

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

The standard RAs demonstrate that applications of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' according to good agricultural practice are of low risk to the aquatic ecosystem with appropriate mitigation measures.

## 9.6 Effects on bees (KCP 10.3.1)

An acute risk assessment for bees has been performed. The bee risk assessment is based on the EPPO (2010) bee guidance (EPPO Standard PP3/10 (3) Environmental risk assessment scheme for plant protection products. Chapter 10: Honeybees), as updated from the EPPO 2001 guidance, which is referred to in the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev. 2 (final), dated October 17 2002).

Besides acute oral and contact toxicity of GWN-10392 on adult honey and bumble bees, and chronic toxicity on adult honey bees and effects on honey bee larvae have been studied with the formulated product – as requested by Regulation (EC) No. 284/2013. However, since the EFSA bee guidance document (2014) has not yet been voted, a risk assessment based on the acute bumble bee toxicity results and the adult honey bee and honey bee larvae data is regarded as not relevant.

### 9.6.1 Toxicity data

Studies on the toxicity to honey bees have been carried out with zoxamide and cymoxanil. Full details on the studies are provided in the respective EU DARs/RARs and related documents.

Effects of the formulated product 'CYMOXANIL 33% + ZOXAMIDE 33% WG' on bees were not evaluated as part of the EU assessment of active substances, and are therefore presented in this dossier. New data submitted with this application are listed in the Table 9.6-1 and are summarised in Appendix 2.

For the a.s. Cymoxanil, reference is done to the EU review of this active ingredient, in agreement with the "risk envelope approach" (SANCO 11244/2011[8]).

~~An additional study on the toxicity to honey bee larvae has been carried out with zoxamide. Full details of the study are summarised in Appendix 2 of this document.~~

All relevant endpoints for the pollinator's risk assessment are listed in the tables below.

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

[8] Guidance document on the preparation and submission of dossiers for plant protection products according to the "risk envelope approach". SANCO/11244/2011 rev.5, 14 March 2011

| Study | Test species | EU agreed endpoints (EFSA Scientific Report of Cymoxanil (2008)) |
|---|---|---|
| Acute oral toxicity | Honey bee | $LD_{50}$ > 85.3 µg/bee |
| Acute contact toxicity | Honey bee | $LD_{50}$ > 100 µg/bee |

**Table 9.6-2: Endpoints and effect values relevant for the risk assessment for bees – zoxamide and the formulated product**

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| **Zoxamide** | | | | |
| *Apis mellifera* | RH-117,281 2F * | Oral acute | $LD_{50}$ >147 µg prod./bee (corresponding to 33 µg a.s./bee) | EFSA (2017) |
| *Apis mellifera* | RH-117,281 2F * | Contact acute | $LD_{50}$ > 200 µg prod./bee (corresponding to 43.2 µg a.s./bee) | EFSA (2017) |
| *Apis mellifera* | Zoxamide | Contact acute | $LD_{50}$ > 100 µg/bee | EFSA (2017) |
| *Apis mellifera* | Zoxium 240 SC | 10 d-oral chronic | $LDD_{50}$ = 174.8 µg a.s./bee/day<br>$LC_{50}$ > 5000 mg a.s./g feed | EFSA (2017) |
| ~~*Apis mellifera*~~ | ~~Zoxamide~~ | ~~22 d-oral larvae, repeated dose~~ | ~~$LD_{50}$ > 110 µg a.s/larvae (larval and pupal survival, adult emergence and adult weight at emergence)~~<br>~~NOED = 110 µg a.s./larvae (larval and pupal survival, adult emergence)~~<br>~~NOED = 49 µg a.s./larvae (adult weight at emergence)~~ | ~~xxx. (2018) Report no. 12791.6307~~ |
| *Apis mellifera* | Zoxium 240 SC | Semi-field bee brood test | No effects on bee brood development up to 3.47 g Zoxium 240 SC/L feeding solution corresponding to 0.75 g a.s./L feeding solution | EFSA (2017) |
| **'CYMOXANIL 33% + ZOXAMIDE 33% WG'** | | | | |
| *Apis mellifera* | 'CYMOXANIL 33% + ZOXAMIDE 33% WG' | Oral | $LD_{50}$ > 100 µg prod./bee (equivalent to > 33 µg a.s./bee for zoxamide and cymoxanil) | Colli M., 2006; report no. BT026/06 |
| | | Contact | $LD_{50}$ > 100 µg prod./bee (equivalent to > 33 µg a.s./bee for zoxamide and cymoxanil) | |
| *Bombus terrestris* | 'CYMOXANIL 33% + ZOXAMIDE 33% WG' | Oral | $LD_{50}$ > 608.0 µg prod./bee (equivalent to > 200 µg a.s./bee for zoxamide and cymoxanil) | Amsel K., 2017; report no. 17 48 BBA 0003 |
| | | Contact | $LD_{50}$ > 547.2 µg prod./bee (equivalent to > 180 µg | |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| | | | a.s./bee for zoxamide and cymoxanil) | |
| *Apis mellifera* | 'CYMOXANIL 33% + ZOXAMIDE 33% WG' | 10 d-oral chronic | $LDD_{50}$ = 34.2 µg consumed prod./bee/day (equivalent to 11.25 µg consumed a.s./bee/day for zoxamide and cymoxanil)<br>$LC_{50}$ = 1.356 g prod/kg food (equivalent to 0.446 g a.s./kg food for zoxamide and cymoxanil) | Ruhland S., 2018; report no. 17 48 BAC ~~BRC~~ 0005 |
| *Apis mellifera* | 'CYMOXANIL 33% + ZOXAMIDE 33% WG' | 22 d-oral larvae, repeated dose | $ED_{50}$ = 14.1 µg prod./larvae (equivalent to 4.65 µg a.s./larvae for zoxamide and cymoxanil)<br>NOED = 3.6 µg prod./larvae (equivalent to 1.2 µg a.s./larvae for zoxamide and cymoxanil) | Scheller K., 2020: report no. 17 48 BLC 0005 |

**Higher-tier studies (tunnel test, field studies)**

**Schnurr A., 2020; report no. 18 48 BFB 0001:** Effects of Cymoxanil 33 % + Zoxamide 33 % WG (containing nominally 33 % of each active ingredient cymoxanil and zoxamide) on the honeybee (*Apis mellifera*) have been studied in the field under central European growing conditions (in Germany) after two consecutive foliar applications of each 1.35 kg product/ha (the 3-fold max. single application rate of 0.45 kg product/ha) at an interval of 7 days during flowering of Phacelia (*Phacelia tanacetifolia*) during bee flight. The second application was conducted at the beginning of full-flowering of Phacelia (BBCH 65) and during bee flight. Main endpoints were the mortality, foraging activity, bee behaviour, colony and brood development. Special attention was paid on detailed brood development during two brood cycles via photo documentation of initially labelled eggs. Additionally, residues of cymoxanil and zoxamide were determined in spray solution, flowers, pollen (pollen from pollen traps and in-hive pollen), nectar (nectar from forager bees and in-hive nectar), dead bees and alive bees with an analytical method fully validated according to SANCO/3029/99 rev. 4 and SANCO/825/00 (2010). As a result, two consecutive foliar applications of Cymoxanil 33% + Zoxamide 33% WG at a rate of each 1.35 kg product/ha (the 3-fold max. single application rate of 0.45 kg product/ha) at an interval of 7 days under field conditions on full-flowering *Phacelia tanacetifolia* B. caused no adverse effects on honeybee behavior, mortality, foraging activity, colony weight development, colony strength, condition of the colonies, overall bee brood development as well as the detailed brood development over two consecutive, investigated brood cycles.

**Schnurr A., 2020; report no. 19 48 BFB 0001:** Effects of Cymoxanil 33 % + Zoxamide 33 % WG (containing nominally 33 % of each active ingredient cymoxanil and zoxamide) on the honeybee (*Apis mellifera*) have been studied in the field under southern European growing conditions (in Spain) after two consecutive foliar applications of each 1.35 kg product/ha (the 3-fold max. single application rate of 0.45 kg product/ha) at an interval of 7 days during flowering of Phacelia (*Phacelia tanacetifolia*) and during bee flight under field conditions. The first application was conducted at the beginning of flowering (BBCH 61) during daytime and the second application at full-flowering (BBCH 65) during daily bee flight. Main endpoints were mortality, foraging activity, bee behaviour, colony and brood development. Special attention was paid on detailed brood development during two brood cycles via photo documentation of initially labelled eggs. Additionally, residues of cymoxanil and zoxamide were determined in spray solution, flowers, pollen (pollen from pollen traps and in-hive pollen), nectar (nectar from forager bees and in-hive nectar), dead bees and alive bees with an analytical method fully validated according to SANCO/3029/99 rev. 4 and SANCO/825/00 (2010). As a result, two consecutive foliar applications of Cymoxanil 33% + Zoxamide 33% WG at a rate of each 1.35 kg product/ha (the 3-fold max. single application rate of 0.45 kg product/ha) at an interval of 7 days under field conditions on full-flowering *Phacelia tanacetifolia* B. caused no adverse effects on honeybee behavior, mortality, foraging activity, colony weight development, colony strength, condition of the colonies, overall bee brood development as well as the detailed brood development over two consecutive, investigated brood cycles.

* RH-117,281 2F is a very similar formulation to Zoxium 240 SC. Refer to Document J-CP for details of both formulations.

### 9.6.1.1 Justification for new endpoints

~~For zoxamide, EFSA (2017) requested *"Further information to address the risk to bee larvae (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5)."* A study to address this data requirement is presented in Appendix 2.~~

~~In addition,~~ new studies carried out with 'CYMOXANIL 33% + ZOXAMIDE 33% WG' are presented - as required according to Regulation (EC) No. 284/2013.

## 9.6.2 Risk assessment

### 9.6.2.1 Hazard quotients for bees

Hazard quotients for honey bees for the two active substances zoxamide and cymoxanil and the formulated product are presented hereafter.

The acute risk to honeybees from use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' was assessed calculating the hazard quotients HQ for oral ($Q_{HO}$) and contact ($Q_{HC}$) exposure as follows:

$$Hazard\ Quotient = \frac{Maximum\ application\ rate\ (g\ formulation/ha)}{Acute\ LD_{50}\ (\mu g\ formulation/bee)}$$

For the active ingredient Cymoxanil, the risk to bees was already assessed in the context of the EU review of Cymoxanil, which concluded acceptable risk ($HQ_{oral}$ < 1.8; $HQ_{contact}$ < 2.1) following application on potato at 175 g a.s./ha, and considering the endpoints reported in Table 9 .6.1. Since the rate assessed at the EU level is clearly worst-case compared to the one proposed for 'CYMOXANIL 33% + ZOXAMIDE 33% WG', in agreement with the "risk envelope approach" (SANCO 11244/2011) it is possible to conclude that the exposure to Cymoxanil following application of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' poses an acceptable risk to honeybees.

**No further risk assessment for Cymoxanil is required, however for additional information, the first-tier assessment was reported in the below table.**

**Table 9.6-3: First-tier assessment of the risk for bees due to the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG'**

| | | | |
|---|---|---|---|
| **Intended use** | Potatoes and wine grapes | | |
| **Active substance** | Zoxamide | | |
| **Application rate (g/ha)** | 3 × 148.5 | | |
| **Test design** | **$LD_{50}$ (lab.) (µg/bee)** | **Single application rate (g/ha)** | **$Q_{HO}$, $Q_{HC}$ criterion: $Q_H \leq 50$** |
| Oral toxicity | > 33 | 148.5 | < 4.50 |
| Contact toxicity | > 43.2 | | < 3.44 |
| **Active substance** | Cymoxanil | | |
| **Application rate (g/ha)** | 3 × 148.5 | | |

| **Test design** | **$LD_{50}$ (lab.) (µg/bee)** | **Single application rate (g/ha)** | **$Q_{HO}$, $Q_{HC}$ criterion: $Q_H \leq 50$** |
|---|---|---|---|
| Oral toxicity | 85.3 | 148.5 | < 1.74 |
| Contact toxicity | > 100 | | < 1.49 |
| **Product** | 'CYMOXANIL 33% + ZOXAMIDE 33% WG' | | |
| **Application rate (g/ha)** | 3 × 0.45 kg prod./ha | | |
| **Test design** | **$LD_{50}$ (lab.) (µg/bee)** | **Single application rate (g/ha)** | **$Q_{HO}$, $Q_{HC}$ criterion: $Q_H \leq 50$** |
| Oral toxicity | > 100 | 450 | < 4.5 |
| Contact toxicity | > 100 | | < 4.5 |

$Q_{HO}$, $Q_{HC}$: Hazard quotients for oral and contact exposure. $Q_H$ values shown in bold breach the relevant trigger.
* Based on the application rate of 0.7 L prod./ha and a product density of 1.1 g/mL.

For zoxamide, cymoxanil and 'CYMOXANIL 33% + ZOXAMIDE 33% WG' the HQ values for acute oral and contact toxicity to adult honeybees are below the relevant trigger of 50, indicating that no unacceptable risk is expected following the application of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' according to the intended use pattern.

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

Effects of Cymoxanil 33 % + Zoxamide 33 % WG (containing nominally 33 % of each active ingredient cymoxanil and zoxamide) on the honeybee (*Apis mellifera*) have been studied in two field studies, under central European growing conditions (in Germany) and under southern European growing conditions (in Spain) (see **Schnurr A., 2020; report no. 18 48 BFB 0001** and **Schnurr A., 2020; report no. 19 48 BFB 0001).** The product was applied with two consecutive foliar applications of each 1.35 kg product/ha (the 3-fold max. single application rate of 0.45 kg product/ha) at an interval of 7 days during flowering of Phacelia (*Phacelia tanacetifolia*) and during bee flight. The second application was conducted during full-flowering of Phacelia (BBCH 65) during daily bee flight. Main endpoints were the mortality, foraging activity, bee behaviour, colony and brood development. Special attention was paid on detailed brood development during two brood cycles via photo documentation of initially labelled eggs. Additionally, residues of cymoxanil and zoxamide were determined in spray solution, flowers, pollen (pollen from pollen traps and in-hive pollen), nectar (nectar from forager bees and in-hive nectar), dead bees and alive bees with an analytical method fully validated according to SANCO/3029/99 rev. 4 and SANCO/825/00 (2010). As a result, two consecutive foliar applications of Cymoxanil 33% + Zoxamide 33% WG at a rate of each 1.35 kg product/ha (the 3-fold max. single application rate of 0.45 kg product/ha) at an interval of 7 days under field conditions on full-flowering *Phacelia tanacetifolia* B. caused no adverse effects on honeybee behavior, mortality, foraging activity, colony weight development, colony strength, condition of the colonies, overall bee brood development as well as the detailed brood development over two consecutive, investigated brood cycles.

### 9.6.3 Effects on bumble bees

A study on the acute oral and topical toxicity to bumble bees with 'CYMOXANIL 33% + ZOXAMIDE 33% WG' has been performed and is presented above, a summary of the study is given in Appendix 2. The study of Amsel (2017; report no. 17 48 BBA 0003) indicates a low acute oral and contact toxicity of GWN-10392 for bumble bees. However, since the EFSA bee guidance document (2014) has not yet been voted, a risk assessment based on the acute bumble bee toxicity results is regarded not relevant.

### 9.6.4 Effects on solitary bees

No further information on solitary bees is deemed to be required.
The EFSA bee guidance document (2014) is not yet voted and therefore not taken into account.

### 9.6.5 Overall conclusions

The bee risk assessment is based on the EPPO (2010) bee guidance (EPPO Standard PP3/10 (3) Environmental risk assessment scheme for plant protection products. Chapter 10: Honeybees), as updated from the EPPO 2001 guidance, which is referred to in the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002).

For zoxamide, cymoxanil and 'CYMOXANIL 33% + ZOXAMIDE 33% WG' the HQ values for acute oral and contact toxicity to adult honeybees are below the relevant trigger of 50, indicating that no unacceptable risk is expected following the application of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' according to the intended use pattern in the field.

Two honey bee field studies from 2018 and 2019 with additional assessments on colony and brood development are available, which have been performed under southern EU (Spanish) and central EU (German) conditions. As a result, two consecutive foliar applications of 'Cymoxanil 33% + Zoxamide 33% WG' at a rate of each 1.35 kg product/ha (the 3-fold max. single application rate of 0.45 kg product/ha) with an interval of 7 days under field conditions on full-flowering *Phacelia tanacetifolia* B. caused no adverse effects on honeybee behaviour, mortality, foraging activity, colony weight development, colony strength, conditions of the colonies, overall bee brood development as well as detailed brood development over two consecutive investigated brood cycles.

~~An additional study on the toxicity to honey bee larvae has been carried out with zoxamide.~~

Additional studies with the formulated product on the chronic adult honey bee and honey bee larvae toxicity are available or are submitted with this dossier, but without risk assessment. The EFSA bee guidance document (2014) is not yet voted and therefore not taken into account.

Review comments:

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

The risk assessment performed for zoxamide, cymoxanil and 'CYMOXANIL 33% + ZOXAMIDE 33% WG' is agreed by the zRMS.

All hazard quotients calculated are lower than 50, indicating that the acute oral and contact risk to bees is acceptable according to the proposed use pattern of 'CYMOXANIL 33% + ZOXAMIDE 33% WG'.

Moreover, the available field studies confirm that 'CYMOXANIL 33% + ZOXAMIDE 33% WG' caused no adverse effects on the behaviour, the mortality, the foraging activity, the weight development, the condition of the colonies and the bee brood development over an observation period of 42 days.

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

The risk to arthropods other than bees from the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' has been evaluated according to the recommendations of ESCORT 2 (2000), the 'Guidance document on

terrestrial ecotoxicology under Council Directive 91/414/EEC' (Anonymous 2002)[9] and the requirements of Regulation (EC) No. 284/2013 for the plant protection product 'CYMOXANIL 33% + ZOXAMIDE 33% WG'.

### 9.7.1 Toxicity data

Effects of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' to non-target arthropods were not evaluated as part of the EU assessment for the active substances zoxamide and cymoxanil. New data are therefore submitted with this application.

In order to address properly the risk of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' to non-target arthropods, laboratory tests were carried out with the formulated product on the representative non-target arthropods *Aphidius rhopalosiphi* and *Typhlodromus pyri*.

The effects of the formulated product on *Aphidius rhopalosiphi* were investigated in a glass-plate laboratory test carried out at the single rate of 2.25 g product/ha (limit test). The product showed no adverse effects on mortality or reproduction at the tested rate. The study is summarized under point IIIA 10.5.1.

The effects of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' on the mortality and reproduction of *Typhlodromus pyri* were investigated in an extended laboratory test (rate-response-test). The results of this study showed a $LR_{50}$ > 2.25 kg formulated product/ha. Significant effects on the reproduction of *T. pyri* were observed at the highest tested rate (2.25 kg product/ha), but not at lower rates. A reduction in reproduction of 50% compared to control ($ER_{50}$) was observed at the dose of 1.57 kg prod./ha, so this rate was used as endpoint in the risk assessment. Moreover, the study investigated the duration of the adverse effects on reproduction of *T. pyri*. No statistically significant effects were observed at the highest rate of 2.25 kg prod./ha when *T. pyri* were exposed to leaves after 7 days of aging, showing that adverse effects are restricted to a very short time period, and indicating an acceptable potential of re-colonization/recovery within an ecologically relevant period.

In addition, new studies on *Chrysoperla carnea* and *Orius laevigatus* have been performed.

All are listed in Appendix 1 and summarised in Appendix 2. A summary of the available data is given in the tables below.

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

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Aphidius rhopalosiphi* (adults) | 'CYMOXANIL 33% + ZOXAMIDE 33% WG' | Laboratory test, glass plate | $LR_{50}$ > 2.25 kg product/ha<br>NOEC = 2.25 kg product/ha *<br>*corresponding to:* ***742.5 g a.s./ha*** | Colli M., 2006; report no. BT029/06 |
| *Typhlodromus pyri* (protonymphs) | 'CYMOXANIL 33% + ZOXAMIDE 33% WG' | Laboratory test, extended | $LR_{50}$ > 2.25 kg product/ha<br>*corresponding to: 742.5 g a.s./ha*<br>$ER_{50}$ = 1.57 kg product/ha<br>*corresponding to:* ***518.1 g a.s./ha*** | Colli M., 2006; report no. BT031/06 |
| | 'CYMOXANIL 33% + ZOXAMIDE 33% WG' | Laboratory test, aged residues | Exposure to leaves after 7-days of aging:<br>NOEC = 2.25 kg product/ha ** | |

[9] Guidance document on terrestrial ecotoxicology under Council Directive 91/414/EEC, SANCO/10329/2002 rev. 2 dated 17th October 2002

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| | | | *corresponding to: 742.5 g a.s./ha* | |
| *Chrysoperla carnea* (larvae) | 'CYMOXANIL 33% + ZOXAMIDE 33% WG' | Laboratory test, extended; application to bean leaves (2D) | $LR_{50}$ > 2 880 g prod./ha<br>$ER_{50}$ > 2 880 g prod./ha<br>*corresponding to: > 950 g a.s./ha for zoxamide and cymoxanil* | Vaughan R., 2017; report no. GWW-17-1 |
| *Orius laevigatus (second instar nymphs)* | 'CYMOXANIL 33% + ZOXAMIDE 33% WG' | Laboratory test, extended; application to bean leave discs (2D) | $LR_{50}$ > 1 440 g prod./ha<br>$ER_{50}$ > 1 440 g prod./ha<br>*corresponding to: > 475.2 g a.s./ ha for zoxamide and cymoxanil*<br>NOER = 1440 g prod./ha | Vinall S., 2018; report no. GWW-17-2 |
| **Field or semi-field tests** | | | | |
| - | | | | |

* * No effects on reproduction at the rate of 2.25 kg prod./ha.
** No significant effects on mortality and reproduction at the rate of 2.25 kg prod./ha.

In the EFSA Scientific Report of Cymoxanil (2008), the results of laboratory tests on *Chrysoperla carnea* (foliar dwelling) and Poecilus cupreus (soil dwelling) are reported. These studies were carried out with a product containing 500 g/kg of Cymoxanil as the sole active ingredient, formulated as wettable powder (WP). Since the ingredients contained in this formulation are not expected to display any activi-ty towards non-target arthropods, the effects observed in these studies are to be ascribed to the a.s. Cymoxanil.
The results of these studies, summarized in the table below, showed lack of significant effects of the a.s. Cymoxanil to *Chrysoperla carnea* and *Poecilus cupreus* at the rate of 0.48 kg a.s./ha, which is more than three times higher than the one proposed for 'CYMOXANIL 33% + ZOXAMIDE 33% WG'.

**Table 9.7-2: Tests on additional species of non-target arthropods – a.s. Cymoxanil**

| Test species (Life stage) | Test item | Study | Resulting endpoint |
|---|---|---|---|
| *Chrysoperla carnea* (larvae) | Cymoxanil 50% WP | Laboratory test, glass plate | $LR_{50}$ > 480 g a.s./ha<br>$ER_{50}$ > 480 g a.s./ha |
| *Poecilus cupreus* (adult) | Cymoxanil 50% WP | Laboratory test, glass plate | $LR_{50}$ > 480 g a.s./ha |

These data indicate that Cymoxanil poses low risk for non-target arthropods, with a wide safety margin to take into account the use of a test item different from 'CYMOXANIL 33% + ZOXAMIDE 33% WG'.

In addition, other tests on various species of non-target arthropods were assessed for the EU review of Cymoxanil, as reported in the EFSA Scientific Report. All these studies, carried out with products containing Cymoxanil in mixture with another fungicide, the a.s. Famoxadone, further support the conclusion of low risk of Cymoxanil to non-target arthropods, as stated in the EFSA document.

### 9.7.1.1 Justification for new endpoints

The toxicity of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' to non-target arthropods has been investigated by carrying out two extended (Tier II) laboratory studies with the sensitive indicator species,

*Aphidius rhopalosiphi* and *Typhlodromus pyri*. In accordance with the requirements of the ESCORT 2 guidance (Candolfi et al., 2000); additional extended laboratory tests on further two relevant non-target arthropod species (*Orius laevigatus* and *Chrysoperla carnea*) were performed and are provided.

The risk assessment is based on toxicity data with the formulated product 'CYMOXANIL 33% + ZOXAMIDE 33% WG'. Studies with other formulated products, as available on EU level for both zoxamide and cymoxanil, are regarded not applicable for this risk assessment

The product 'CYMOXANIL 33% + ZOXAMIDE 33% WG' shows in general a low toxicity to non-target arthropods.

### 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 (Candolfi et al., 2000)[10]. It is based on the hazard quotient (HQ) approach, assessing the risk to non-target arthropods in-field and off-field and considering either the $LR_{50}$ (lethal rate) or the $ER_{50}$ (effect rate) on reproduction.

#### 9.7.2.1 Risk assessment for in-field exposure

For in-field risk assessment, where exposure is given by the effective application rate, a risk envelope approach is applied in order to achieve a concise risk assessment. Here, the worst-case GAP of all three crops is 3 × 0.45 kg product/ha.

In the following, $PER_{in\text{-}field}$ values have been calculated according to ESCORT 2 as:

$$PER_{in\text{-}field} = application\ rate \times MAF$$

**Table 9.7-3: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' in potatoes and wine grapes**

| | | | |
|---|---|---|---|
| **Intended use** | Potatoes and wine grapes | | |
| **Active substance/product** | 'CYMOXANIL 33% + ZOXAMIDE 33% WG' | | |
| **Application rate (g/ha)** | 3 × 450 | | |
| **MAF** | 2.3 (corresponding to 3 applications) | | |
| **Test species Higher-tier (extended laboratory studies)** | **$LR_{50}$ (lab.) (g/ha)** | **$PER_{in\text{-}field}$ (g/ha)** | **$PER_{in\text{-}field}$ below rate with ≤ 50 % effect?** |
| *Typhlodromus pyri* | 1 570 | 1035 | yes |
| *Aphidius rhopalosiphi* | > 2250 | | yes |
| *Orius laevigatus* | > 1 440 | | yes |
| *Chrysoperla carnea* | > 2 880 | | yes |

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

[10] Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods: from the ESCORT 2 Workshop (European standard characteristics of non-target arthropod regulatory testing): a joint BART, EPPO/CoE, OECD, and IOBC Workshop organised in conjunction with SETAC Europe and EC: held at Wageningen International Conference Centre, Wageningen, the Netherlands : 21-23 March 2000.

### 9.7.2.2 Risk assessment for off-field exposure

Since for all representative uses and based on the results of the extended laboratory studies with *T. pyri*, *Aphidius*, *Chrysoperla* and *Orius* the $PER_{in\text{-}field}$ was below the rate with ≤ 50 % effect, also off-field no risk for non-target arthropods is expected from the intended uses of 'CYMOXANIL 33% + ZOXAMIDE 33% WG'. The risk assessment for the off-field area is therefore covered by the risk assessment for the in-field area.

In addition, the aged residue test carried out with the product on *Typhlodromus pyri* (Colli M., 2006; report no. BT031/06) showed no adverse effects at the rate of 2.25 kg product/ha after 7 days, indicating an acceptable potential of re-colonisation/recovery within an ecologically relevant period.

It is therefore possible to conclude that the GAP uses of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' do not pose an unacceptable risk to non-target arthropods.

'CYMOXANIL 33% + ZOXAMIDE 33% WG' shows a low risk to *T. pyri*, *Aphidius*, *Chrysoperla* and *Orius*.

### 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 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 (Candolfi et al., 2000)[11]. It is based on the hazard quotient (HQ) approach, assessing the risk to non-target arthropods in-field and off-field and considering either the $LR_{50}$ (lethal rate) or the $ER_{50}$ (effect rate) on reproduction

Based on the results of extended laboratory studies with *T. pyri*, *Aphidius*, *Chrysoperla* and *Orius* the $PER_{in\text{-}field}$ was below the rate with ≤ 50 % effect for all representative uses. Therefore, also off-field no risk to non-target arthropods is expected from the intended uses of 'CYMOXANIL 33% + ZOXAMIDE 33% WG'. The risk assessment for the off-field area is therefore covered by the risk assessment for the in-field area.

In addition, the aged residue test carried out with the product on *Typhlodromus pyri* (Colli M., 2006; report no. BT031/06) showed no adverse effects at the rate of 2.25 kg product/ha after 7 days, indicating an acceptable potential of re-colonisation/recovery within an ecologically relevant period.

It is therefore possible to conclude that the GAP uses of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' do not pose an unacceptable risk to non-target arthropods.

[11] Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods: from the ESCORT 2 Workshop (European standard characteristics of non-target arthropod regulatory testing): a joint BART, EPPO/CoE, OECD, and IOBC Workshop organised in conjunction with SETAC Europe and EC: held at Wageningen International Conference Centre, Wageningen, the Netherlands: 21-23 March 2000.

The product 'CYMOXANIL 33% + ZOXAMIDE 33% WG' shows in general a low toxicity to non-target arthropods.

Review Comments:

Based on the results of the conducted Tier 1 and 2 risk assessment it can be concluded that low risk for non-target arthropods is expected from the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' according to the proposed use pattern. No unacceptable effects on non-target arthropods are expected in in-field and off-field habitats.

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

### 9.8.1 Toxicity data

Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with zoxamide, cymoxanil and their relevant metabolites. Full details on these studies are provided in the respective EU DAR, EU RAR and related documents. In addition, new data on zoxamide and its soil metabolites submitted with this application are listed in Appendix 1 and are summarised in Appendix 2.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' were neither evaluated as part of the EU assessment of zoxamide nor cymoxanil. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

**Table 9.8-1: EU endpoints - effects of Cymoxanil on earthworms**

| Test species | Time scale | EU agreed endpoints (EFSA Scientific Report of Cymoxanil (2008)) |
|---|---|---|
| Earthworm | Acute | $LC_{50}$ > 1000 mg/kg dw soil |
| Earthworm | Chronic | NOEC = 6.6 mg/kg dw soil |

**Table 9.8-2: 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 |
|---|---|---|---|---|
| **Zoxamide and its metabolites** | | | | |
| *Eisenia fetida* | Zoxamide | 14 d acute<br>10 % peat content | $LC_{50}$ > 1070 mg a.s./kg soil dw<br>$LC_{50,corr}$ > 535 mg a.s./kg soil dw* | EFSA (2017) |
| *Eisenia andrei* | Zoxamide ** | 56 d chronic<br>5 % peat content | NOEC = 2.453 mg/kg dw (analysed)<br>$NOEC_{corr}$ = 1.227 mg a.s./kg dw* | Friedrich, S. (2020) / 17 48 TEC 0009 |
| *Eisenia fetida* | RH-127450 | 14 d acute<br>10 % peat content | $LC_{50}$ >1000 mg a.s./kg dw<br>$LC_{50,corr}$ > 500 mg s.s./kg dw* | EFSA (2017) |
| *Eisenia fetida* | RH-127450 | 56 d chronic<br>10 % peat content | NOEC = 10 mg a.s./kg dw<br>$NOEC_{corr}$ = 5 mg a.s./kg dw* | Gray, J. (2020) / 3202376 |

| Species | Substance | Exposure system | Results | Reference |
|---|---|---|---|---|
| *Eisenia fetida* | RH-141455 | 56 d chronic<br>10 % peat content | NOEC = 5 mg a.s./kg soil dw<br>$NOEC_{corr}$ = 2.5 mg a.s./kg dw* | EFSA (2017) |
| *Eisenia fetida* | RH-163353 | 56 d chronic<br>10 % peat content | NOEC = 10 mg a.s./kg dw<br>$NOEC_{corr}$ = 5 mg a.s./kg dw* | Gray, J. (2020) / 3202389 |
| *Eisenia fetida* | RH-24549 | 56 d chronic<br>10 % peat content | NOEC = 10 mg a.s./kg dw<br>$NOEC_{corr}$ = 5 mg a.s./kg dw*<br>$EC_{10}$ = 7.449 mg a.s./kg dw<br>$EC_{10;corr}$ = 3.724 mg a.s./kg dw* | Gray, J. (2020) / 3202395 |
| *Folsomia candida* | Zoxamide ** | 28 d chronic<br>5 % peat content | NOEC = 217 mg a.s./kg dw<br>$NOEC_{corr}$ = 108.5 mg a.s./kg dw* | Parsons, Ch. (2020) / GOW-17-13 |
| *Folsomia candida* | RH-141455 | 28 d chronic<br>5 % peat content | NOEC = 50 mg a.s./kg dw<br>$NOEC_{corr}$ = 25 mg met./kg dw* | xxx (2020) / 3202382<br>Study not valid |
| *Folsomia candida* | RH-163353 | 28 d chronic<br>5 % peat content | NOEC = 4.76 mg a.s./kg dw<br>$NOEC_{corr}$ = 2.38 mg met./kg dw* | xxx (2020) / 3202390<br>Study not valid |
| ~~*Hypoaspis aculeifer*~~ | ~~Zoxamide **~~ | ~~14 d, chronic<br>5 % peat content~~ | ~~NOEC = 217 mg a.s./kg dw<br>$NOEC_{corr}$ = 108.5 mg a.s./kg dw*~~ | ~~Parsons, Ch. (2020) / GOW-17-14~~ |
| *Hypoaspis aculeifer* | RH-141455 | 14 d, chronic<br>5 % peat content | NOEC = 50 mg a.s./kd dw<br>$NOEC_{corr}$ = 25 mg met./kg dw* | Gray, J. (2020) / 3202383 |
| *Hypoaspis aculeifer* | RH-163353 | 14 d chronic<br>5 % peat content | NOEC = 27.78 mg a.s./kg dw<br>$NOEC_{corr}$ = 13.89 mg met./kg dw* | Gray, J. (2020) / 3202391 |
| **~~Cymoxanil and its metabolites~~** | | | | |
| 'CYMOXANIL 33% + ZOXAMIDE 33% WG' | | | | |
| *Eisenia fetida* | 'CYMOXANIL 33% + ZOXAMIDE 33% WG' | 56 d, chronic<br>10 % peat content | $NOEC_{repro}$ = 4.55 mg prod./kg dw<br>$NOEC_{corr}$ = 2.275 mg prod./kg dw* | Friedrich, S. (2020) Report No. 17 48 TEC 0008 |
| *Folsomia candida* | 'CYMOXANIL 33% + ZOXAMIDE 33% WG' | 28 d, chronic<br>5 % peat content | NOEC > 1000 mg prod./kg dw<br>$NOEC_{corr}$ > 500 mg prod./kg dw* | Parsons, Ch. (2020) Report No. GOW-17-3 |
| *Hypoaspis aculeifer* | 'CYMOXANIL 33% + ZOXAMIDE 33% WG' | 14 d, chronic<br>5 % peat content | NOEC > 1000 mg prod./kg dw<br>$NOEC_{corr}$ > 500 mg prod./kg dw* | Parsons, Ch. (2020) Report No. GOW-17-4 |
| **Field studies** | | | | |
| ~~**Schulz L. 2020, Report No. 18 48 FEW 0001:** Potential effects of field populations of earthworms after spray application of Zoxium 240 (containing nominally 240 g/L zoxamide) to bare soil at a pattern of 5x 140 g a.s./ha (0.5833 L test item/ha) = total of 700 g a.s./ha/season, 5x 180 g a.s./ha (0.75 L test item/ha) = total of 900 g a.s./ha/season and 5x 280 g a.s./ha (1.1667 L test item/ha) = total of 1400 g a.s./ha/season with an interval of 7–8 days were investigated on a typical arable field in Saxony, Germany. As a result, the test item had no statistically significant adverse effects on single species, ecological groups and total earthworm abundance and biomass one year after the first application.~~ | | | | |

| Species | Substance | Exposure system | Results | Reference |
|---|---|---|---|---|
| **(Schulz L. 2020, Report No. 19 48 FEW 0003):** Potential effects of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' and potential recovery of field populations of earthworms after the application of the test item 'CYMOXANIL 33% + ZOXAMIDE 33% WG' were investigated in a field study on a typical arable land in Saxony/Germany over a duration of about one year. The test item (containing nominally 33 % w/w cymoxanil and 33 % w/w zoxamide) was applied five times onto the blank field with an interval of 7-9 days at application rates of 0.45 kg test item/ha (low rate) and 0.90 kg test item/ha (high rate), corresponding to 148.5 g cymoxanil/ha + 148.5 g zoxamide/ha (low rate) and 297 g cymoxanil/ha + 297 g zoxamide/ha (high rate). The surface monitoring on days 1 - 3 after each application showed that there were no acute primary effects on earthworms by Cymoxanil 33% + Zoxamide 33% WG. No alive, moribund or dead earthworms were found on the soil surface neither in the test item nor in the control monitoring areas. No statistically significant reductions in total earthworm abundance and biomass could be observed for the tested application rates of 5 x 0.45 kg test item/ha and 5 x 0.90 kg test item/ha about 1, 6 and 12 months after 1st application. Furthermore, no statistically significant reductions in abundance and biomass of the different earthworm species (*Aporrectodea caliginosa* and *Lumbricus terrestris*) and ecological groups (endogeic and anecic earthworms) could be observed for the tested application rates about 1, 6 and 12 months after 1st application. | | | | |
| **Litter bag test** | | | | |
| -- | | | | |

* Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002.
** Study performed with GWN-9790EU (Zoxium 240 SC)

Besides the parent compound zoxamide, the metabolites RH-127450, RH-24549, RH-163353 and RH-141455 have been identified as potentially relevant for the soil risk assessment in the EFSA (2017) Peer Review Conclusion for zoxamide. In soils at 20°C, levels of the three major metabolites occurring at > 10% AR peaked on days 3-7 after application of $^{14}$C-zoxamide. Maximum concentrations were 8.1-15.1% AR (RH-127450), 5.5-33.8% AR (RH-24549) and 7.9-15.0% AR (RH-163353). RH-141455 was detected at >5% AR on more than two occasions with 8% on day 28, 7.4% on day 56 and 5.8% AR on day 120 after application of $^{14}$C-zoxamide. However, further studies on soil metabolites have been performed since then.
In general, a lower toxicity of the zoxamide soil metabolites compared to the parent compound has been confirmed by all the available chronic earthworm studies. These are available for zoxamide and all four potentially relevant soil metabolites. This is not the case for the soil mites and springtails tested. Here, the available studies with the metabolites RH-141455 and RH-163353 show lower ecotoxicity endpoints compared to the parent compound zoxamide. *Folsomia* and *Hypoaspis* studies with the soil metabolites RH-24549 and RH-127450 are not available. However, these are peaking in the soil 3-7 days after application of zoxamide with $DT_{50}$ values of 5.2 days (RH-127450) and 6.84 days (RH-24549), and so it can be assumed that their toxicity has been intrinsically tested with the available zoxamide *Folsomia* and *Hypoaspis* studies running over 28 and 14 days, respectively.

The overall toxicity of zoxamide and its metabolites has been considered in a field earthworm study with Zoxium 240 SC.

The overall toxicity of zoxamide, cymoxanil and their metabolites has been considered in a field earthworm study with 'CYMOXANIL 33% + ZOXAMIDE 33% WG'.

### 9.8.1.1 Justification for new endpoints

New studies are available for 'CYMOXANIL 33% + ZOXAMIDE 33% WG' which are required to fulfil the data requirements for plant protection products in accordance with Regulation (EC) No 1107/2009. The study references are listed in Appendix 1 and detailed study summaries are presented in Appendix 2.

In addition, new information about the chronic toxicity of zoxamide and its metabolites are presented following the requests raised after the Peer Review and reported in EFSA (2017).

*Chronic endpoints for zoxamide and its metabolites on earthworms and other soil macro-organisms*

EFSA (2017) requested *"Further data for the chronic risk assessment to earthworm for the active substance and the metabolites RH-127450, RH-24549, RH-163353 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5)."* These studies were performed and are summarised in Appendix 2 to this document.
A chronic laboratory and a field earthworm study with zoxamide have been performed with a SC formulation containing 240 g/L zoxamide, the representative formulation during AIR. The composition of Zoxium 240 SC (GWN-9790EU) is included in Part C. The studies are regarded applicable to address the potential risk of zoxamide on earthworms.

According to EFSA (20017): *"Further data are needed to address the risk to soil macro-organisms other than earthworms for the metabolites RH-163353 and RH-141455 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5)."* These data were provided.

In addition to the further studies on soil macro-organisms (other than earthworms) requested by EFSA (2017), studies on *Folsomia* and *Hypoaspis* have been performed with the active substance zoxamide. These studies were conducted with the representative formulation during AIR, a 240 g/L zoxamide SC formulation. The composition of Zoxium 240 SC (GWN-9790 EU) is included in Part C. The studies are regarded applicable to address the potential risk for soil macro-organisms (besides earthworms) from the use of zoxamide.

In general, a lower toxicity of the zoxamide soil metabolites compared to the parent compound has been confirmed by the available chronic earthworm studies. These are available for all four potentially relevant soil metabolites. However, this assumption is not true for the soil mites and springtails tested. Here, the available studies with the metabolites RH-141455 and RH-163353 show lower ecotoxicity endpoints compared to the parent compound zoxamide. *Folsomia* and *Hypoaspis* studies with the soil metabolites RH-24549 and RH-127450 are not available. Nevertheless, these metabolites are peaking in the soil 3-7 days after application of zoxamide with $DT_{50}$ values of 5.2 days (RH-127450) and 6.84 days (RH-24549), and so it can be assumed that their toxicity has been intrinsically tested with the available zoxamide *Folsomia* and *Hypoaspis* studies running over 28 and 14 days, respectively. In parallel, a risk assessment for *Folsomia* and *Hypoaspis* has been performed for RH-24549 and RH-127450 based on the available ecotoxicity endpoints for zoxamide, considering an additional conservative safety factor of 100 and 10, respectively.

### 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.

According to the assessment of environmental fate data, soil accumulation of zoxamide and its metabolites RH-127450, RH-24549 and RH-163353 – even after multiple year's application on the same field – does not play a role and is not expected. Nevertheless, $PEC_{soil,accu}$ values were always considered in the following risk assessment for safety reasons.

Cymoxanil

For grape and potato, the $PEC_{soil}$ for the a.s. and its relevant soil metabolites were calculated with FOCUS modelling, as detailed in Section 8 of this Registration Report. Applications to grapes are covered by the higher use rate on potatoes. Consequently, they are considered relevant to assess the risk for 'CYMOXANIL 33% + ZOXAMIDE 33% WG' under a worst-case approach (risk envelope). The highest $PEC_{soil}$ for the a.s. and its metabolites, are summarised in the table below.

**Table 9.8-3: Maximum $PEC_{soil}$ for Cymoxanil and its metabolites**

| **$PEC_{soil}$ (mg/kg dw soil)** | | | | |
|---|---|---|---|---|
| **Scenario** | **Cymoxanil** | **IN U3204** | **IN W3595** | **IN JX915** |
| Potato | **0.247** | **0.000147** | **0.000039** | **0.000065** |

The maximum $PEC_{soil}$ values were then used in the risk assessment using the endpoints provided in Table above and the results presented below.

**Table 9.8-4: Risk assessment for Cymoxanil on earthworms based on worst-case assumptions**

| **Crop group scenario** | **Exposure scenario** | **Substance** | **Toxicity value (mg a.s./kg)** | **Maximum $PEC_{soil}$ (mg a.s./kg)** | **TER** |
|---|---|---|---|---|---|
| Vines, potatoes and tomatoes up to 6 x 0.1485 kg a.s./ha | Acute | Cymoxanil | $LC_{50}$ > 1000 mg/kg dw soil | 0.247 | 4049 |
| | Acute | CYMOXANIL 33% + ZOXAMIDE 33% WG' | >330 | 0.247 | 1336 |
| | Long-term | Cymoxanil | NOEC = 6.6 mg/kg dw soil | 0.247 | 27 |
| TER trigger values: Acute<10; Long-term<5. | | | | | |

The TER values are above trigger values of 10 (acute risk) and 5 (chronic risk), indicating acceptable risk to earthworms for the intended uses.

For the metabolites of Cymoxanil, reference is done to the EU review of this active ingredient, in agreement with the "risk envelope approach" (SANCO 11244/2011 ). Laboratory studies of the behaviour of cymoxanil under aerobic conditions in soil indicated rapid degradation. On this basis, exposure of earthworms to the metabolites of cymoxanil may be assumed to have occurred during the aerobic conditions that prevailed during the 14 day incubation of cymoxanil in artificial soil. The acute toxicity endpoint therefore takes into account any effects of exposure to degradation products.

**Table 9.8-5: 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 'CYMOXANIL 33% + ZOXAMIDE 33% WG' on wine grapes**

| **Intended use** | Wine grapes | | |
|---|---|---|---|
| **Acute effects on earthworms** | | | |
| **Substance** | **$LC_{50}$ (mg/kg dw)** | **$PEC_{soil}$ (mg/kg dw)*** | **$TER_a$ (criterion TER ≥ 10)** |
| Zoxamide | > 535 | 0.216 | > 2 476.9 |
| RH-127450 | > 500 | 0.024 | > 20 833 |
| **Chronic effects on earthworms** | | | |
| **Substance** | **NOEC/$EC_{10}$ (mg/kg dw)** | **$PEC_{soil}$ (mg/kg dw)*** | **$TER_{lt}$ (criterion TER ≥ 5)** |
| Zoxamide | 1.227 | 0.216 | 5.68 |
| RH-127450 | 5 | 0.024 | 208.3 |
| RH-24549 | ~~5~~ 3.724 | 0.036 | ~~138.9~~ 105.1 |
| RH-163353 | 5 | 0.032 | 156.3 |
| RH-141455 | 2.5 | 0.013 | 192.3 |
| 'CYMOXANIL 33% + ZOXAMIDE 33% WG' | 2.275 | 0.241 # | 9.44 |
| **Chronic effects on other soil macro- and mesofauna** | | | |
| **Substance** | **NOEC (mg/kg dw)** | **$PEC_{soil}$ (mg/kg dw)*** | **$TER_{lt}$ (criterion TER ≥ 5)** |
| Zoxamide (*Folsomia candida*) | 108.5 | 0.216 | 502.3 |
| RH-141455 (*Folsomia candida*) ** | ~~25~~ 10.85 | 0.013 | ~~1 923.1~~ 834.6 |
| RH-163353 (*Folsomia candida*) ** | ~~2.38~~ 10.85 | 0.032 | ~~74.4~~ 339.1 |
| RH-127450 (*Folsomia candida*) *** | 1.085 | 0.024 | 45.2 |
| RH-24549 (*Folsomia candida*) *** | 1.085 | 0.036 | 30.1 |
| ~~Zoxmide (*Hypoaspis aculeifer*)~~ | ~~108.5~~ | ~~0.216~~ | ~~502.3~~ |
| RH-141455 (*Hypoaspis aculeifer*) | 25 | 0.013 | 1 923.1 |
| RH-163353 (*Hypoaspis aculeifer*) | 13.89 | 0.032 | 434.1 |
| ~~RH-127450 (*Hypoaspis aculeifer*) **~~ | ~~10.85~~ | ~~0.024~~ | ~~448.9~~ |
| ~~RH-24549 (*Hypoaspis aculeifer*) **~~ | ~~10.85~~ | ~~0.036~~ | ~~301.4~~ |
| 'CYMOXANIL 33% + ZOXAMIDE 33% WG' (*Folsomia candida*) | > 500 | 0.241# | > 2 074.7 |
| 'CYMOXANIL 33% + ZOXAMIDE 33% WG' (*Hypoaspis aculeifer*) | > 500 | 0.241# | > 2 074.7 |

TER values shown in bold fall below the relevant trigger.

* worst-case $PEC_{soil,}$ taking into account actual single and multiple applications, as well as possible accumulation over the years

** Chronic toxicity endpoints used for metabolites assuming a 10-fold higher toxicity compared to the parent compound

*** Chronic toxicity endpoints used for metabolites assuming a 100-fold higher toxicity compared to the parent compound

# single application PEC values are considered relevant for the ecotoxicological risk assessment; multiple applications and long-term PECs are better described by active substance data

**Table 9.8-6: 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 'CYMOXANIL 33% + ZOXAMIDE 33% WG' on potatoes**

| **Intended use** | Wine grapes | | |
|---|---|---|---|
| **Acute effects on earthworms** | | | |
| **Substance** | **$LC_{50}$ (mg/kg dw)** | **$PEC_{soil}$ (mg/kg dw)*** | **$TER_a$ (criterion TER ≥ 10)** |
| Zoxamide | > 535 | 0.215 | > 2 488.4 |
| RH-127450 | > 500 | 0.024 | > 20 833 |
| **Chronic effects on earthworms** | | | |
| **Substance** | **NOEC/$EC_{10}$ (mg/kg dw)** | **$PEC_{soil}$ (mg/kg dw)*** | **$TER_{lt}$ (criterion TER ≥ 5)** |
| Zoxamide | 1.227 | 0.215 | 5.71 |
| RH-127450 | 5 | 0.024 | 208.3 |
| RH-24549 | ~~5~~ 3.724 | 0.036 | ~~138.9~~ 105.1 |
| RH-163353 | 5 | 0.032 | 156.3 |
| RH-141455 | 2.5 | 0.013 | 192.3 |
| 'CYMOXANIL 33% + ZOXAMIDE 33% WG' | 2.275 | 0.240 # | 9.48 |
| **Chronic effects on other soil macro- and mesofauna** | | | |
| **Substance** | **NOEC (mg/kg dw)** | **$PEC_{soil}$ (mg/kg dw)*** | **$TER_{lt}$ (criterion TER ≥ 5)** |
| Zoxamide (*Folsomia candida*) | 108.5 | 0.215 | 504.7 |
| RH-141455 (*Folsomia candida*) ** | ~~25~~ 10.85 | 0.013 | ~~1 923.1~~ 834.6 |
| RH-163353 (*Folsomia candida*) ** | ~~2.38~~ 10.85 | 0.032 | ~~74.4~~ 339.1 |
| RH-127450 (*Folsomia candida*) *** | 1.085 | 0.024 | 45.2 |
| RH-24549 (*Folsomia candida*) *** | 1.085 | 0.036 | 30.1 |
| ~~Zoxmide (*Hypoaspis aculeifer*)~~ | ~~108.5~~ | ~~0.215~~ | ~~504.7~~ |
| RH-141455 (*Hypoaspis aculeifer*) | 25 | 0.013 | 1 923.1 |
| RH-163353 (*Hypoaspis aculeifer*) | 13.89 | 0.032 | 434.1 |
| ~~RH-127450 (*Hypoaspis aculeifer*) **~~ | ~~10.85~~ | ~~0.024~~ | ~~448.9~~ |
| ~~RH-24549 (*Hypoaspis aculeifer*) **~~ | ~~10.85~~ | ~~0.036~~ | ~~301.4~~ |
| 'CYMOXANIL 33% + ZOXAMIDE 33% WG' (*Folsomia candida*) | > 500 | 0.240# | > 2 083.3 |
| 'CYMOXANIL 33% + ZOXAMIDE 33% WG' (*Hypoaspis aculeifer*) | > 500 | 0.240# | > 2 083.3 |

TER values shown in bold fall below the relevant trigger.

* worst-case $PEC_{soil,}$ taking into account actual single and multiple applications, as well as possible accumulation over the years

** Chronic toxicity endpoints used for metabolites assuming a 10-fold higher toxicity compared to the parent compound

*** Chronic toxicity endpoints used for metabolites assuming a 100-fold higher toxicity compared to the parent compound

# single application PEC values are considered relevant for the ecotoxicological risk assessment; multiple applications and long-term PECs are better described by active substance data

All acute and chronic TER values for earthworms and other non-target soil organisms (meso- and macrofauna) exposed to zoxamide and its relevant soil metabolites are greater than the Commission Regulation (EU) No. 546/2011 triggers of 10 and 5 – even if considering worst-case assumptions.

For 'CYMOXANIL 33% + ZOXAMIDE 33% WG', the TER values for the chronic earthworm, *Folsomia* and *Hypoaspis aculeifer* risk assessment are above the trigger of 5 for the grape and potato uses. For these calculations, $PEC_{soil,accu}$ values considering single applications were taken into account; multiple applications and long-term PECs are better described by active substance data.

In addition, the overall toxicity of ~~zoxamide and its metabolite and of~~ zoxamide, cymoxanil and their metabolites on earthworms has been assessed in field earthworm studies with ~~Zoxium 240 SC and~~ 'CYMOXANIL 33% + ZOXAMIDE 33% WG'~~, respectively~~. ~~In these studies,~~ potential effects of field populations of earthworms after spray application of ~~Zoxium 240 SC and~~ 'CYMOXANIL 33% + ZOXAMIDE 33% WG' to bare soils at exaggerated application pattern did not show any statistically significant effects on single species, ecological groups and total earthworm abundance or biomass one year after application (~~Schulz L. 2020, Report No. 18 48 FEW 0001 and~~ Schulz L. 2020, Report No. 19 48 FEW 0003).

### 9.8.2.2 Higher-tier risk assessment

All acute and chronic TER values for earthworms and other non-target soil organisms (meso- and macrofauna) exposed to zoxamide, cymoxanil and their relevant soil metabolites are greater than the Commission Regulation (EU) No. 546/2011 triggers of 10 and 5 – even if considering worst-case assumptions. This holds also true for 'CYMOXANIL 33% + ZOXAMIDE 33% WG' - the TER values for the chronic earthworm, *Folsomia* and *Hypoaspis aculeifer* risk assessment are above the trigger of 5 for the grape and potato uses.

In addition, the overall toxicity of ~~zoxamide and its metabolite and of~~ zoxamide, cymoxanil and their metabolites on earthworms has been assessed in field earthworm studies with ~~Zoxium 240 SC and~~ 'CYMOXANIL 33% + ZOXAMIDE 33% WG'~~, respectively~~. ~~In these studies,~~ potential effects of field populations of earthworms after spray application of ~~Zoxium 240 SC and~~ 'CYMOXANIL 33% + ZOXAMIDE 33% WG' to bare soils at exaggerated application pattern did not show any statistically significant effects on single species, ecological groups and total earthworm abundance or biomass one year after application (~~Schulz L. 2020, Report No. 18 48 FEW 0001 and~~ Schulz L. 2020, Report No. 19 48 FEW 0003).

### 9.8.3 Overall conclusions

The risk assessment was conducted according to the Guidance Document on Terrestrial Ecotoxicology (2002).

New data on zoxamide and its metabolites are provided within this submission in order to fulfill requests of EFSA (2017). Moreover, new studies with 'CYMOXANIL 33% + ZOXAMIDE 33% WG' have been performed in order to fulfil the data requirements for plant protection products in accordance with Regulation (EC) No 1107/2009.

All acute and chronic TER values for earthworms and other non-target soil organisms (meso- and macrofauna) exposed to zoxamide and its relevant soil metabolites are greater than the Commission Regulation (EU) No. 546/2011 triggers of 10 and 5 – even if considering worst-case assumptions.

For 'CYMOXANIL 33% + ZOXAMIDE 33% WG', the TER values for the chronic earthworm, *Folsomia* and *Hypoaspis aculeifer* risk assessment are above the trigger of 5 for the grape and potato uses. For these

calculations, $PEC_{soil,accu}$ values considering single applications were taken into account; multiple applications and long-term PECs are better described by active substance data.

In addition, the overall toxicity of ~~zoxamide and its metabolite and of~~ zoxamide, cymoxanil and their metabolites on earthworms has been assessed in field earthworm studies with ~~Zoxium 240 SC and~~ 'CYMOXANIL 33% + ZOXAMIDE 33% WG'~~, respectively~~. ~~In these studies,~~ potential effects of field populations of earthworms after spray application of ~~Zoxium 240 SC and~~ 'CYMOXANIL 33% + ZOXAMIDE 33% WG' to bare soils at exaggerated application pattern did not show any statistically significant effects on single species, ecological groups and total earthworm abundance or biomass one year after application (~~Schulz L. 2020, Report No. 18 48 FEW 0001 and~~ Schulz L. 2020, Report No. 19 48 FEW 0003).

Overall, the risk to earthworms and other non-target soil organisms (meso- and macrofauna) is acceptable following use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' according to the proposed use pattern.

Review Comments:

The long-term risks of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' to soil meso- and macro-organisms were assessed from toxicity exposure ratios between toxicity endpoints and maximum $PEC_{soil}$. The relevant predicted environmental concentration in soil ($PEC_{soil}$) for risk assessment covering the proposed use pattern was taken from Part B Section 8 (Environmental Fate).

Safe uses of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' were confirmed based on $TER_{LT}$ calculations for formulation, active substances and their metabolites.

## 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 zoxamide, cymoxanil and their relevant metabolites. Full details of these studies are provided in the respective EU DAR, EU RAR and related documents. In addition, new data on zoxamide metabolites submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Effects on soil microorganisms of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' were not evaluated as part of the EU assessment of zoxamide nor cymoxanil. Therefore, they are presented here.

The new data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

| Endpoint | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| **Zoxamide and its metabolites** | | | | |
| N-mineralisation | zoxamide | 42 d, aerobic | < 25% at 2 mg a.s./kg soil dw | EFSA (2017) |
| C-mineralisation | zoxamide | 28 d, aerobic | < 25% at 2 mg a.s./kg soil dw | EFSA (2017) |
| N-mineralisation | RH-141455 | 28 d, aerobic | < 25% at 0.2-1 mg | EFSA (2017) |

| Endpoint | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| | | | a.s./kg soil dw | |
| N-mineralisation | RH-127450 | 28 d, aerobic | < 25% at 0.195 mg a.s./kg soil dw | Jarrom, R. (2019); report no. 3202377 |
| N-mineralisation | RH-24549 | 28 d, aerobic | < 25% at 0.350 mg a.s./kg soil dw | Jarrom, R. (2019); Report no. 3202396 |
| N-mineralisation | RH-163353 | 28 d, aerobic | < 25% at 0.365 mg a.s./kg soil dw | Jarrom, R. (2019); report no. 3202392 |
| **'CYMOXANIL 33% + ZOXAMIDE 33% WG'** | | | | |
| N-mineralisation | 'CYMOXANIL 33% + ZOXAMIDE 33% WG' | 28 d, aerobic sandy loam | < 25% at 4 mg prod./kg soil dw | Schulz L. (2020) Report no. 17 48 SMO 0004 |

#### 9.9.1.1 Justification for new endpoints

New studies are available for 'CYMOXANIL 33% + ZOXAMIDE 33% WG' which are required to fulfil the data requirements for plant protection products in accordance with Regulation (EC) No 1107/2009. New data are listed in Appendix 1 and detailed study summaries are presented in Appendix 2.

*New data for zoxamide metabolites RH-127450, RH-24549, RH-163353*

According to EFSA (2017) *"Further data to address the risk to soil microorganisms for metabolites RH-127450, RH-24549, RH-163353 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5)."* These studies have been performed and are presented with this submission.

### 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 assessment covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.8). As a worst-case only PECs for the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' on grapes were taken into account. The risk assessment will cover the uses on potatoes (risk envelope approach).

**Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' on wine grapes**

| **Intended use** | Wine grapes | | |
|---|---|---|---|
| **N-mineralisation** | | | |
| **Product/active substance** | **Max. conc. with effects ≤ 25 % (mg/kg dw)** | **$PEC_{soil}$ * (mg/kg dw)** | **Risk acceptable? yes/no** |
| zoxamide | 2 | 0.216 | Yes |
| RH-127450 | 0.195 | 0.024 | Yes |

| | | | |
|---|---|---|---|
| RH-24549 | 0.350 | 0.036 | Yes |
| RH-163353 | 0.365 | 0.032 | Yes |
| RH-141455 | 1 | 0.013 | Yes |
| 'CYMOXANIL 33% + ZOXAMIDE 33% WG' | 4 | 0.241 # | Yes |
| **C-mineralisation** | | | |
| **Product/active substance** | **Max. conc. with effects ≤ 25 % (mg/kg dw)** | **$PEC_{soil}$ (mg/kg dw)** | **Risk acceptable? yes/no (max.conc./$PEC_{soil}$ ratio)** |
| zoxamide | 2 | 0.216 | Yes |

* worst-case $PEC_{soil}$, taking into account actual single and multiple applications, as well as possible accumulation over the years
\# single application PEC values are considered relevant for the ecotoxicological risk assessment; multiple applications and long-term PECs are better described by active substance data

Effects at expected soil concentrations for proposed uses of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' are below the Commission Regulation (EU) No. 546/2011 triggers of 25%, indicating that the risk to soil micro-organisms is acceptable following the use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG'.

### 9.9.3 Overall conclusions

The risk assessment was conducted according to the Guidance Document on Terrestrial Ecotoxicology (2002).

New data on zoxamide metabolites are provided in order to fulfill the requests of the EFSA (2017). Moreover, a soil nitrogen degradation study with 'CYMOXANIL 33% + ZOXAMIDE 33% WG' has been performed to comply with data requirements of Regulation (EC) No 1107/2009 for plant protection products.

The risk of 'CYMOXANIL 33% + ZOXAMIDE 33% WG', zoxamide and its metabolites to soil micro-organisms was evaluated by comparison of no-effect concentrations, derived from laboratory tests, with worst-case $PEC_{soil}$ values. As a result, the effects at the predicted soil concentrations for the uses of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' are below the Commission Regulation (EU) No. 546/2011 triggers of 25%, indicating no undue risks to soil micro-organisms.

Review Comments:

'CYMOXANIL 33% + ZOXAMIDE 33% WG' had no significant effect on soil micro-organisms at 4 mg product/kg dry soil. This is approximately 16 times higher than the maximum $PEC_{soil}$ of 0.241 mg product/kg dry soil following the worst-case application pattern. The risk assessment performed for zoxamide and its metabolites confirmed low risk to soil micro-organisms. This supports the conclusion that under field conditions, use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' at the proposed rates poses no unacceptable risk to non-target soil micro-organisms.

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

### 9.10.1 Toxicity data

Studies on the toxicity to non-target terrestrial plants have been carried out with the representative formulations of zoxamide and cymoxanil. Full details of these studies are provided in the respective EU DAR, EU RAR and related documents.

'CYMOXANIL 33% + ZOXAMIDE 33% WG'

Effects on non-target terrestrial plants of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' were not evaluated as part of the EU assessment of zoxamide. The data is submitted for the product authorisation, is listed in Appendix 1 and summarised in Appendix 2.

To assess the effects of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' on terrestrial plants, a vegetative vigour test on different species of non-target-plants was carried out.

This study showed that application of 1.35 kg formulation/ha (equivalent to 0.4455 kg a.s./ha) had no significant phytotoxic effects on two mono- and four dicotyledon species of plants. The only significant effect observed was a 16% decrease in dry biomass with *P. sativum*. The $EC_{50}$ was therefore assumed to be higher than 0.4455 kg a.s./ha.

**Table 9.10-1: Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants – CYMOXANIL 33% + ZOXAMIDE 33% WG'**

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Avena sativa*<br>*Oryza sativa*<br>*Cucumis sativa*<br>*Glycine max*<br>*Helianthus annus*<br>*Pisum sativum* | CYMOXANIL 33% + ZOXAMIDE 33% WG' | Vegetative vigour test<br>21 days | Plant survival:<br>$ER_{50}$ > 1.35 kg a.s./ha (for all species)<br><br>Fresh shoot weight<br>NOER: 1.35 kg a.s./ha (for all species)<br><br>Dry biomass:<br>*Pisum sativum*, $EC_{50}$ > 0.4455 kg a.s./ha | KCP 10.6.2/01<br>Colli M. (2007) |

#### 9.10.1.1 Justification for new endpoints

A vegetative vigour study with 'CYMOXANIL 33% + ZOXAMIDE 33% WG' has been conducted. The study is listed in Appendix 1 and a study summary is presented in Appendix 2.

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

Zoxamide

During EU review only screening data on terrestrial vascular plants were considered relevant and sufficient (see RAR zoxamide, 2017). No adverse effects were seen at dose rates up to 500 g a.s./ha. Therefore, no studies on seedling emergence and vegetative vigour were regarded to be required. Moreover, as the active substance is not a herbicide and/or plant growth regulator, Tier I studies examining the effects on seedling emergence and vegetative vigour were not requested.

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

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

To assess the effects of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' on terrestrial plants, a vegetative vigour test on different species of non-target-plants was carried out. This study showed that application of 1.35 kg formulation/ha (equivalent to 0.4455 kg a.s./ha) had no significant phytotoxic effects on two mono- and four dicotyledon species of plants. The only significant effect observed was a 16% decrease in dry biomass with *P. sativum*. The $EC_{50}$ was therefore assumed to be higher than 0.4455 kg a.s./ha.

**Exposure**

Effects on non-target plants are of concern in the off-field environment, where they may be exposed to spray drift. Following the provisions of the 'Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC' (Anonymous 2002), the amount of spray drift reaching off-crop habitats is calculated using the 90th percentile estimates derived by the BBA (2000) from the spray-drift predictions of Ganzelmeier & Rautmann (2000). Only a single application was considered, as factors such as plant growth will reduce residues per unit area between multiple applications. Following a worst-case approach, the highest single application rate of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' (0.45 kg product/ha, equivalent to 0.1485 kg a.s./ha) was used, and a drift of 8.02% was assumed, considering applications on late grapevine (default distance: 3 m from the edge of the crop). The resulting off-field predicted environmental rate ($PER_{off\text{-}field}$) was 0.0119 kg a.s./ha.

**Risk assessment**

No effects over 50% were observed at an application rate three times higher than the single rate proposed for 'CYMOXANIL 33% + ZOXAMIDE 33% WG'. The calculated maximum $PER_{off\text{-}field}$ of 0.0119 kg a.s./ha is considerably lower than the EC50 of 0.4455 kg a.s./ha obtained from the vegetative vigour test. It is therefore possible to conclude that 'CYMOXANIL 33% + ZOXAMIDE 33% WG' poses no unacceptable risk to terrestrial non-target plants in off-crop areas following the proposed uses.

Tier 2 TER calculations show that the risk to terrestrial non-target plants in off-crop areas is acceptable following use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' according to the proposed use pattern without consideration of mitigation measures.

### 9.10.1.4 Higher-tier risk assessment

Not relevant.

### 9.10.1.5 Risk mitigation measures

No risk mitigation needed.

### 9.10.2 Overall conclusions

The risk assessment was conducted according to the Guidance Document on Terrestrial Ecotoxicology (2002).

During AIR of zoxamide, only screening data on terrestrial vascular plants were considered relevant and sufficient (see RAR zoxamide, 2017). No adverse effects were seen at dose rates up to 500 g a.s./ha.

Therefore, no studies on seedling emergence and vegetative vigour were regarded to be required. Moreover, as the active substance is not an herbicide and/or plant growth regulator, Tier I studies examining the effects on seedling emergence and vegetative vigour were not requested.

To assess the effects of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' on terrestrial plants, a vegetative vigour test on different species of non-target-plants was carried out. This study showed that application of 1.35 kg formulation/ha (equivalent to 0.4455 kg a.s./ha) had no significant phytotoxic effects on two mono- and four dicotyledon species of plants. The only significant effect observed was a 16% decrease in dry biomass with *P. sativum*. The $EC_{50}$ was therefore assumed to be higher than 0.4455 kg a.s./ha. Thus, no effects over 50% were observed at an application rate three times higher than the single rate proposed for 'CYMOXANIL 33% + ZOXAMIDE 33% WG'. The calculated maximum $PER_{off\text{-}field}$ of 0.0119 kg a.s./ha is considerably lower than the EC50 of 0.4455 kg a.s./ha obtained from the vegetative vigour test. It is therefore possible to conclude that 'CYMOXANIL 33% + ZOXAMIDE 33% WG' poses no unacceptable risk to terrestrial non-target plants in off-crop areas following the proposed uses.

Review Comments:

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

Based on the risk assessment it can be concluded that the proposed use of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' poses no unacceptable risk to non-target plants, if applied according to the recommended use pattern. Particular precautions to reduce the environmental concentrations resulting from 'CYMOXANIL 33% + ZOXAMIDE 33% WG' applications are not required.

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

Tests on other non-target species are not required.

## 9.12 Monitoring data (KCP 10.8)

There are no other relevant data for the active substance zoxamide or the product on organisms in the environment generated from monitoring schemes.

## 9.13 Classification and Labelling

According to the criteria given in Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008, the following classification and labelling with regard to ecotoxicological data is proposed for the preparation:

**Table 9.13-1: Justified proposals for classification and labelling for GWN-10392 according to Regulation (EC) No 1272/2008 with regard to ecotox data**

| | |
|---|---|
| Hazard class(es), categories: | Aquatic Acute 1<br>Aquatic Chronic 1 |

| | |
|---|---|
| Hazard pictograms or Code(s) for hazard pictogram(s): |  GHS09 |
| Signal word: | Warning! |
| Hazard statement(s): | H400: Very toxic to aquatic life<br>H410: Very toxic to aquatic life with long lasting effects |
| Precautionary statement(s): | P237: Avoid release to the environment<br>P391: Collect spillage<br>P501: Dispose of contents/container to a licensed hazardous-waste disposal contractor or collection site except for empty clean containers which can be disposed of as non-hazardous waste. |
| Additional labelling phrases: | EUH401: To avoid risks to man and the environment, comply with the instructions for use. |

# 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.

The names of authors of vertebrate studies will be blanked in final version of RR.

**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<br>Y/N | Owner |
|---|---|---|---|---|---|
| ~~KCP 10.1.1.1~~<br><br>~~(KCA 8.~~ | ~~xxx~~ | ~~2006~~ | ~~CYMOXANIL 33% + ZOXAMIDE 33% WG (SIP 40936): an acute oral toxicity study with the northern bobwhite.~~<br>~~Oxon Italia SpA, Italy~~<br>~~xxx, Report No. 613-103~~<br>~~GLP~~<br>~~Not published~~ | ~~Y~~ | ~~GWI Sipcam Oxon S.p.A.~~ |
| KCP 10.1.2.2 | Romanini M. | 2011 | Magnitude of cymoxanil residues in grasses weeds in a non-crop field area after one spray application of CYMOXANIL 45% WG<br>CREG, Research Centre "E. Gagliardini" - SIPCAM S.p.A. - Salerano sul Lambro (LO) - Italy<br>Report no. CREG 2142<br>GLP, Unpublished<br><br>IIA study on the active substance | ~~Y~~ N | Sipcam Oxon S.p.A. |
| KCP 10.1.2.2 | Schabacker J. *et al.* | 2011 | Cymoxanil – higher tier vole risk assessments for cymoxanil uses in Europe<br>RIFCON GmbH - Heidelberg - Germany<br>Report no R11406<br>No GLP, Unpublished<br><br>IIA study on the active substance | ~~Y~~ N | Sipcam Oxon S.p.A. |

| 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 10.2.1<br><br>(KCA 8.2.1) | xxx | 2020 | RH-163353: Fish, acute toxicity test<br>Gowan Crop Protection Ltd., UK<br>xxx, Report No. 3202385<br>GLP<br>Not published | Y | GWI |
| KCP 10.2.1<br><br>(KCA 8.2.1) | xxx | 2020 | RH-141455: Fish, acute toxicity test<br>Gowan Crop Protection Ltd., UK<br>xxx, Report No. 3202716<br>GLP<br>Not published | Y | GWI |
| KCP 10.2.1<br><br>(KCA 8.2.1) | xxx. | 2020 | RH-127450: Fish, acute toxicity test<br>Gowan Crop Protection Ltd., UK<br>xxx, Report No. 3202373<br>GLP<br>Not published | Y | GWI |
| ~~KCP 10.2.1~~<br><br>~~(KCA 8.2.4.1)~~ | ~~xxx.~~ | ~~2020~~ | ~~RH-163353: Acute toxicity to *Daphnia magna*~~<br>~~Gowan Crop Protection Ltd., UK~~<br>~~xxx, Report No. 3202386~~<br>~~GLP~~<br>~~Not published~~ | ~~Y~~ | ~~GWI~~ |
| ~~KCP 10.2.1~~<br><br>~~(KCA 8.2.4.1)~~ | ~~xxx~~ | ~~2020~~ | ~~RH-141455: Acute toxicity to *Daphnia magna*~~<br>~~Gowan Crop Protection Ltd., UK~~<br>~~xxx, Report No. 3202380~~<br>~~GLP~~<br>~~Not published~~ | ~~Y~~ | ~~GWI~~ |
| KCP 10.2.1<br><br>(KCA 8.2.4.2) | Hugill, E. | 2020 | RH-127450: Mysid acute toxicity test<br>Gowan Crop Protection Ltd., UK<br>Smithers ERS Ltd., UK, Report No. 3202374<br>GLP<br>Not published | ~~Y~~ N | GWI |

| 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 10.2.1<br><br>(KCA 8.2.4.2) | Hugill, E. | 2020 | RH-24549: Mysid acute toxicity test<br>Gowan Crop Protection Ltd., UK<br>Smithers ERS Ltd., UK, Report No. 3202394<br>GLP<br>Not published | ~~Y~~ N | GWI |
| KCP 10.2.1<br><br>(KCA 8.2.4.2) | Hugill, E. | 2020 | RH-139432: Mysid acute toxicity test<br>Gowan Crop Protection Ltd., UK<br>Smithers ERS Ltd., UK, Report No. 3202398<br>GLP<br>Not published | ~~Y~~ N | GWI |
| KCP 10.2.1<br><br>(KCA 8.2.4.2) | Jarrom, R. | 2020 | RH-163353: Mysid acute toxicity test<br>Gowan Crop Protection Ltd., UK<br>Smithers ERS Ltd., UK, Report No. 3202387<br>GLP<br>Not published | ~~Y~~ N | GWI |
| KCP 10.2.1<br><br>(KCA 8.2.4.2) | Hugill, E. | 2020 | RH-141455: Mysid acute toxicity test<br>Gowan Crop Protection Ltd., UK<br>Smithers ERS Ltd., UK, Report No. 3202381<br>GLP<br>Not published | ~~Y~~ N | GWI |
| KCP 10.2.1<br><br>(KCA 8.2.6) | Hugill, E. | 2020 | RH-127450: Inhibition of growth on the alga *Raphidocelis subcapitata*<br>Gowan Crop Protection Ltd., UK<br>Smithers ERS Ltd., UK, Report No. 3202375<br>GLP<br>Not published | ~~Y~~ N | GWI |
| KCP 10.2.1<br><br>(KCA 8.2.6) | Jarrom, R. | 2020 | RH-163353: Inhibition of growth to the alga *Raphidocelis subcapitata*<br>Gowan Crop Protection Ltd., UK<br>Smithers ERS Ltd., UK, Report No. 3202388<br>GLP<br>Not published | ~~Y~~ N | GWI |

| 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 10.2.1<br><br>(KCA 8.2.7)~~ | ~~xxx~~ | ~~2020~~ | ~~Effects Zoxamide technical on *Lemna gibba* in a growth inhibition test under semi-static test conditions<br>Gowan Crop Protection Ltd., UK<br>xxxx, Report No. 18-48-ALE-0005<br>GLP<br>Not published~~ | ~~Y~~ | ~~GWI~~ |
| KCP 10.2.1<br><br>(KCA 8.2.1) | xxx | 2007 | Acute toxicity of Cymoxanil 33% + Zoxamide 33% WG to rainbow trout (*Oncorhynchus mykiss*), determined under flow-through conditions<br>Oxon Italia S.p.A., Italy<br>xxx, Report No. CH-E–023/2006<br>GLP<br>Not published | Y | GWI<br>Sipcam Oxon S.p.A. |
| KCP 10.2.1<br><br>(KCA 8.2.1) | Croce V. | 2007 | Acute toxicity of Cymoxanil 33% + Zoxamide 33 % WG to *Daphnia magna* in a 48-hour immobilization test under semi-static exposure-limit test<br>Oxon Italia Sp.A., Italy<br>ChemService S.r.l., Italy, Report No. CH-001/2007<br>GLP<br>Not published | ~~Y~~ N | GWI<br>Sipcam Oxon S.p.A. |
| KCP 10.2.1<br><br>(KCA 8.2.6) | Croce V. | 2007 | Toxicity of Cymoxanil 33% + Zoxamide 33% WG to green algae *Pseudokirchneriella subcapitata* determined in a growth inhibition study<br>Oxon Italia S.p.A., Italy<br>ChemService S.r.l. Italy, Report No. CH-002/2007<br>GLP<br>Not published | ~~Y~~ N | GWI<br>Sipcam Oxon S.p.A. |
| ~~KCP 10.2.2<br><br>(KCA 8.2.2.1)~~ | ~~xxxx~~ | ~~2020~~ | ~~Final report addendum for RH-117,281 technical: An early-life-stage toxicity test with the sheepshead minnow (*Cyprinodon variegatus*)<br>Gowan Crop Protection Ltd., UK<br>xxx, Report No. 129A-143A<br>GLP<br>Not published~~ | ~~Y~~ | ~~GWI~~ |
| ~~KCP 10.2.2~~ | ~~xxx~~ | ~~1998~~ | ~~RH-117,281 technical: An early-life-stage toxicity test with the sheepshead minnow (*Cyprinodon varie-~~ | Y | GWI |

| 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 |
|---|---|---|---|---|---|
| ~~(KCA 8.2.2.1)~~ | | | ~~*gatus*)~~<br>~~xxx, Report No. 97RC 0078~~<br>~~xxx, Report No. 129A-143A~~<br>~~GLP~~<br>~~Not published~~ | | |
| KCP 10.3.1.1 | Colli M. | 2006 | Effects, acute oral and acute contact toxicity of CYMOXANIL 33% + ZOXAMIDE 33% WG on the honeybee, *Apis mellifera*, L., in laboratory (Limit test).<br>Oxon Italia S.p.A., Italy<br>Biotecnologie BT S.r.l, Italy, Report No BT026/06<br>GLP<br>Not published | ~~Y~~ N | GWI Sipcam Oxon S.p.A. |
| KCP 10.3.1.1 | Amsel, K. | 2017 | Acute toxicity of Cymoxanil 33% + Zoxamide 33% to the bumblebee *Bombus terrestris* L. under laboratory conditions<br>Gowan Crop Protection Ltd., UK, Oxon Italia S.p.A, Italy<br>Biochem Agrar, Gerichshain, Germany, Report No. 17 48 BBA 0003<br>GLP<br>Not published | N | GWI Sipcam Oxon S.p.A. |
| KCP 10.3.1.2 | Ruhland, S. | 2018 | Chronic toxicity of Cymoxanil 33% + Zoxamide 33% WG to the honey bee *Apis mellifera* L. under laboratory conditions<br>Gowan Crop Protection Ltd., UK, Oxon Italia S.p.A, Italy<br>BioChem agrar, Germany, Report No. 17 48 BAC 0005<br>GLP<br>Not published | N | GWI Sipcam Oxon S.p.A. |
| ~~KCP 10.3.1.3~~<br><br>~~(KCA 8.3.1.3)~~ | ~~xxx~~ | ~~2018~~ | ~~Zoxamide: Honey bee (*Apis mellifera* L.) larval toxicity, repeated exposure~~<br>~~xxx, A Gowan Group Company, USA~~<br>~~xxx, Report No. 12791.6307~~<br>~~GLP~~<br>~~Not published~~ | ~~Y~~ | ~~GWI~~ |
| KCP 10.3.1.3 | Scheller, K. | 2019 | Reboot - Repeated exposure of honey bee (*Apis mellifera* L.) larvae under laboratory conditions (in vitro)<br>Gowan Crop Protection Ltd., UK, SIPCAM OXON S.p.A, Italy | N | GWI Sipcam Oxon |

| 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; Germany, Report No. 17 48 BLC 0005<br>GLP<br>Not published | | S.p.A. |
| KCP 10.3.1.6 | Schnurr, A. | 2020 | Effects of Cymoxanil 33% + Zoxamide 33% WG on the honeybee Apis mellifera L. under field conditions with additional assessments on colony and brood development<br>Gowan Crop Protection Ltd., UK, SIPCAM OXON S.p.A, Italy<br>BioChem agrar, Germany, Report No. 18 48 BFB 0001<br>GLP<br>Not published | N | GWI Sipcam Oxon S.p.A. |
| KCP 10.3.1.6 | Schnurr, A. | 2020 | Effects of Cymoxanil 33% + Zoxamide 33% WG on the honeybee *Apis mellifera* L. under field conditions in Spain (Southern zone) with additional assessments on colony and brood development<br>Gowan Crop Protection Ltd., UK, SIPCAM OXON S.p.A., Italy<br>BioChem agrar, Germany, Report No. 19 48 BFB 0001<br>GLP<br>Not published | N | GWI Sipcam Oxon S.p.A. |
| KCP 10.3.2.2 | Colli M. | 2006 | Effects of CYMOXANIL 33% ÷ ZOXAMIDE 33% WG on the Aphid Parasitoid, *Aphidius rhopalosiphi* De Stefani Perez (Hymenoptera, Braconidae) in Laboratory (Limit test).<br>Oxon Italia SpA, Italy<br>Biotecnologie BT S.r.l, Italy, Report No BT028/06<br>GLP<br>Not published | ~~Y~~ N | GWI Sipcam Oxon S.p.A. |
| KCP 10.3.2.2 | Colli M. | 2011 | Effects of CYMOXANIL 33% + ZOXAMIDE 33% WG on the Predatory Mite, *Typhlodromus pyri* Scheuten (Acari Phytoseiidae) under extended laboratory conditions (Rate response test).<br>Oxon Italia S.p.A., Italy<br>Biotecnologie BT S.r.l, Italy, Report No. BT031/06 (second edition)<br>GLP<br>Not published | ~~Y~~ N | GWI Sipcam Oxon S.p.A. |
| KCP 10.3.2.2 | Vinall, S. | 2018 | Cymoxanil 33% + Zoxamide 33% WG – A rate-response extended laboratory test to evaluate the effects of fresh residues on the predatory bug *Orius laevigatus* (Hemiptera; Anthocoridae)<br>Gowan Crop Protection Ltd., UK, Oxon Italia S.p.A, Italy | N | GWI Sipcam Oxon S.p.A. |

| 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 |
|---|---|---|---|---|---|
| | | | Mambo-Tox, Southampton, UK, Report No. GOW-17-2<br>GLP<br>Not published | | |
| KCP 10.3.2.2 | Vaughan, R. | 2017 | Reboot (GWN-9823) – A rate-response extended laboratory test to evaluate the effects of fresh residues on the green lacewing, Chrysoperla carnea (Neuroptera, Chrysopidae)<br>Gowan Crop Protection Ltd., UK, Oxon Italia S.p.A, Italy<br>Mambo-Tox, Southampton, UK, Report No. GOW-17-1<br>GLP<br>Not published | N | GWI<br>Sipcam Oxon S.p.A. |
| KCP 10.4.1.1<br><br>(KCA 8.4.1.) | Friedrich, S. | 2019 | Effects of Zoxium 240 SC on the reproduction of the earthworm *Eisenia andrei* in artificial soil with 5 % peat<br>Gowan Crop Protection Ltd., UK<br>BioChem agrar, Germany, Report No. 17 48 TEC 0009<br>GLP<br>Not published | ~~Y~~ N | GWI |
| KCP 10.4.1.1<br><br>(KCA 8.4.1) | Gray, J. | 2020 | RH-127450: Effect on reproduction in the earthworm *Eisenia fetida*<br>Gowan Crop Protection Ltd., UK<br>Smithers ERS Ltd., UK, Report No. 3202376<br>GLP<br>Not published | ~~Y~~ N | GWI |
| KCP 10.4.1.1<br><br>(KCA 8.4.1) | Gray, J. | 2020 | RH-24549: Effect on reproduction in the earthworm *Eisenia fetida*<br>Gowan Crop Protection Ltd., UK<br>Smithers ERS Ltd., UK, Report No. 3202395<br>GLP<br>Not published | ~~Y~~ N | GWI |
| KCP 10.4.1.1<br><br>(KCA 8.4.1) | Gray, J. | 2020 | RH-163353: Effect on reproduction in the earthworm *Eisenia fetida*<br>Gowan Crop Protection Ltd., UK<br>Smithers ERS Ltd., UK, Report No. 3202389<br>GLP<br>Not published | ~~Y~~ N | GWI |

| 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 10.4.1.1<br><br>(KCA 8.4.1.) | Colli M. | 2006 | Acute toxicity of CYMOXANIL 33% + ZOXAMIDE 33% WG on Earthworms, *Eisenia fetida*, using an artificial soil test.<br>Oxon Italia S.p.A., Italy<br>Biotecnologie BT S.r.l, Italy, Report No. BT027/06<br>GLP<br>Not published | ~~Y~~ N | GWI Sipcam Oxon S.p.A. |
| KCP 10.4.1.1 | Friedrich, S. | 2020 | Effects of Cymoxanil 33 % + Zoxamide 33 % WG on the reproduction of the earthworm *Eisenia andrei* in artificial soil with 5 % peat<br>Gowan Crop Protection Ltd., UK, SIPCAM OXON S.p.A, Italy<br>BioChem agrar, Germany, Report No. 17 48 TEC 0008<br>GLP<br>Not published | N | GWI Sipcam Oxon S.p.A. |
| ~~KCP 10.4.1.2<br><br>(KCA 8.4.1)~~ | ~~Schulz, L.~~ | ~~2020~~ | ~~Effects of Zoxium 240 SC on earthworms under field conditions<br>Gowan Crop Protection Ltd., UK<br>BioChem agrar, Germany, Report No. 18 48 FEW 0001<br>GLP<br>Not published~~ | ~~N~~ | ~~GWI~~ |
| KCP 10.4.1.2 | Schulz, L. | 2020 | Effects of Cymoxanil 33% + Zoxamide 33% on earthworms under field conditions<br>Gowan Crop Protection Ltd., UK, SIPCAM OXON S.p.A, Italy<br>BioChem agrar, Germany, Report No. 19 48 FEW 0003<br>GLP<br>Not published | N | GWI Sipcam Oxon S.p.A. |
| KCP 10.4.2<br><br>(KCA 8.4.2) | Parsons, Ch | 2020 | Zoxium 240 SC - A laboratory test to determine the effects of fresh residues on the springtail *Folsomia candida* (Collembola, Isotomidae) in artificial soil substrate<br>Gowan Crop Protection Ltd., UK<br>Mambo-Tox Ltd., UK, Report No. GOW-17-13<br>GLP<br>Not published | ~~Y~~ N | GWI |
| ~~KCP 10.4.2~~ | ~~xxx~~ | ~~2020~~ | ~~Zoxium 240 SC – A laboratory test to determine the effects of fresh residues on the predatory soil mite *Hypoaspis aculeifer* (Acari, Laelapidae) in an artificial soil substrate~~ | ~~Y~~ | ~~GWI~~ |

| 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 |
|---|---|---|---|---|---|
| ~~(KCA 8.4.2)~~ | | | ~~Gowan Crop Protection Ltd., UK~~<br>~~xxx, Report No. GOW 17 14~~<br>~~GLP~~<br>~~Not published~~ | | |
| ~~KCP 10.4.2~~<br><br>~~(KCA 8.4.2)~~ | ~~xxx~~ | ~~2020~~ | ~~RH 163353: Collembolan reproduction test in soil~~<br>~~Gowan Crop Protection Ltd., UK~~<br>~~xxx, Report No. 3202390~~<br>~~GLP~~<br>~~Not published~~ | ~~Y~~ | ~~GWI~~ |
| ~~KCP 10.4.2~~<br><br>~~(KCA 8.4.2)~~ | ~~xxx~~ | ~~2020~~ | ~~RH 141455: Collembolan reproduction study~~<br>~~Gowan Crop Protection Ltd., UK~~<br>~~xxx, Report No. 3202382~~<br>~~GLP~~<br>~~Not published~~ | ~~Y~~ | ~~GWI~~ |
| KCP 10.4.2<br><br>(KCA 8.4.2) | Gray, J. | 2020 | RH-163353: Effect on reproduction of *Hypoaspis* (Geolaelaps) *aculeifer*<br>Gowan Crop Protection Ltd., UK<br>Smithers ERS Ltd., UK, Report No. 3202391<br>GLP<br>Not published | ~~Y~~ N | GWI |
| KCP 10.4.2<br><br>(KCA 8.4.2) | Gray, J. | 2020 | RH-141455: Effect on reproduction of *Hypoaspis* (Geolaelaps) *aculeifer*<br>Gowan Crop Protection Ltd., UK<br>Smithers ERS Ltd., UK, Report No. 3202383<br>GLP<br>Not published | ~~Y~~ N | GWI |
| KCP 10.4.2 | Parsons, Ch.. | 2020 | Cymoxanil 33% + Zoxamide 33% WG – a laboratory test to determine the effects of fresh residues on the springtail *Folsomia candida* (Collembola, Isotomidae).<br>Gowan Crop Protection Ltd., UK, SIPCAM OXON S.p.A, Italy<br>Mambo-Tox Ltd., UK, Report No. GOW-17-3<br>GLP<br>Not published | N | GWI<br>Sipcam Oxon S.p.A. |

| 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 10.4.2 | Parsons, Ch.. | 2020 | Cymoxanil 33% + Zoxamide 33% – A laboratory test to determine the effects of fresh residues on the predatory mite *Hypoaspis aculeifer* (Acari: Laelapidae)<br>Gowan Crop Protection Ltd, UK, SIPCAM OXON S.p.A, Italy<br>Mambo-Tox Ltd., U.K., Report No. GOW-17-4<br>GLP<br>Not published | N | GWI<br>Sipcam Oxon S.p.A. |
| KCP 10.5<br><br>(KCA 8.5) | Jarrom, R. | 2019 | RH-127450: Soil nitrogen transformation test<br>Gowan Crop Protection Ltd., UK<br>Smithers ERS Ltd., UK, Report No. 3202377<br>GLP<br>Not published | ~~Y~~ N | GWI |
| KCP 10.5<br><br>(KCA 8.5) | Jarrom, R. | 2019 | RH-24549: Soil nitrogen transformation test<br>Gowan Crop Protection Ltd., UK<br>Smithers ERS Ltd., UK, Report No. 3202396<br>GLP<br>Not published | ~~Y~~ N | GWI |
| KCP 10.5<br><br>(KCA 8.5) | Jarrom, R. | 2020 | RH-163353: Soil nitrogen transformation test<br>Gowan Crop Protection Ltd., UK<br>Smithers ERS Ltd., UK, Report No.3202392<br>GLP<br>Not published | ~~Y~~ N | GWI |
| KCP 10.5 | Schulz, L. | 2017 | Effects of Cymoxanil 33% + Zoxamide 33% WG on the activity of soil microflora (Nitrogen transformation test)<br>Gowan Crop Protection Ltd., UK<br>Biochem Agrar, Germany, Report No. 17 48 SMO 0004<br>GLP<br>Not published | N | GWI |
| KCP 10.6.2 | Colli M. | 2007 | Vegetative vigour limit test for non-target plants following single rate application of CYMOXANIL 33% + ZOXAMIDE 33% WG<br>Oxon Italia S.p.A., Italy | ~~Y~~ N | GWI<br>Sipcam Oxon S.p.A. |

| 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 |
|---|---|---|---|---|---|
| | | | Biotecnologie BT S.r.l, Italy, Report No. BT034/06<br>GLP<br>Not published | | |

GWI = Gowan Crop Protection Ltd.
Oxon / Sipcam Oxon S.p.A. = Oxon Italia S.p.A.; now Sipcam Oxon S.P.A.

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

| Data point | Author(s) | Year | Title<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 |
|---|---|---|---|---|---|
| KCA 8.1.1.1 | xxx | 1997 | RH-117,281 technical: 14-day acute oral $LD_{50}$ study in bobwhite quail<br>xxx, Report No. 94RC-0240, April 9 1997, ER Ref No. 2.6<br>xxx, Inc., Report No. RH117BWLD-595<br>GLP<br>Not published | Y | GWI |
| KCA 8.1.1.2 | xxx | 1997 | RH-117,281 technical: 8-day acute dietary $LC_{50}$ study in bobwhite quail<br>xxx, Report No. 94RC-0242, April 10 1997, ER Ref No. 2.4<br>xxx., Report No. RH117BWLC-395<br>GLP<br>Not published | Y | GWI |
| KCA 8.1.1.2 | xxx | 1997 | RH-117,281 technical: 8-day acute dietary $LC_{50}$ study in mallard ducklings<br>xxx, Report No. 94RC-0241, April 10 1997 ER Ref No. 2.5<br>xxx., Report No. RH117MDLC-395<br>GLP<br>Not published | Y | GWI |

| 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 |
|---|---|---|---|---|---|
| KCA 8.1.1.3 | xxx | 1998 | Avian reproduction study of RH-117,281 technical with northern bobwhite<br>xxx, Report No. 97RC-0081, December 9 1998, ER Ref No. 28.2<br>xxx., Report No. RH7281BW-97-2<br>GLP<br>Not published | Y | GWI |
| KCA 8.1.1.3 | xxx | 1999 | RH-117,281 technical: A reproduction study with the mallard (*Anas platyrhynchos*)<br>xxx, Report No. 98RC-0166, April 5 1999, ER Ref No. 33.13<br>xxx, Report No. 129-164<br>GLP<br>Not published | Y | GWI |
| KCA 8.2.1 | xxx | 1995 | Acute flow-through toxicity of RH-117,281 technical to rainbow trout (*Oncorhynchus mykiss*)<br>xxx, Report No. 94RC-0078, July 25 1995, ER Ref No: 2.1<br>xxx., Report No. 41681<br>GLP<br>Not published | Y | GWI |
| KCA 8.2.1 | xxx | 1995 | Acute flow-through toxicity of RH-117,281 technical to bluegill (*Lepomis macrochirus*)<br>xxx, Report No. 94RC-0080, July 25 1995, ER Ref No. 4.10<br>xxx, Report No. 41682<br>GLP<br>Not published | Y | GWI |
| KCA 8.2.1 | xxx | 1998 | RH-117,281 Technical: A flow-through life-cycle toxicity test with the fathead minnow (*Pimephales promelas)*<br>xxx, Report No. 97RC-0079, September 29 1998, ER Ref No. 17.4<br>xxx., Report No. 129A-141<br>GLP<br>Not published | Y | GWI |
| KCA 8.2.1 | xxx | 1998 | RH-117,281 Technical: A 96-hour flow-through acute toxicity test with the zebra fish (*Brachydanio rerio*)<br>xxx, Report No. 97RC-0134, May 15 1998, ER Ref No. 12.11<br>xxx., Report No. 129A-150 | Y | GWI |

| 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 |
|---|---|---|---|---|---|
| | | | GLP<br>Not published | | |
| KCA 8.2.1 | xxx | 1997 | RH-117,281 Technical: a 96-hour flow-through acute toxicity test with the sheepshead minnow (*Cyprinodon variegatus*)<br>xxx, Report No. 95RC-0274, May 15 1997, ER Ref No. 8.1<br>xxx., Report No. 129A-135<br>GLP<br>Not published | Y | GWI |
| KCA 8.2.1 | xxx | 1998 | Acute toxicity of RH-127,450 to the rainbow trout (*Oncorhynchus mykiss)* in a range-finding test under static conditions<br>xxx, Report No. 98RC-0095, September 11 1998, ER Ref No. 17.5<br>xxx, Report No. 44667<br>GLP<br>Not published | Y | GWI |
| KCA 8.2.1 | xxx | 2002 | Zoxamide Metabolite RH-139,432 - acute toxicity to rainbow trout (*Oncorhynchus mykiss*) under static conditions<br>xxx, Report No. 021296, September 27 2002, ER Ref. 47.4<br>xxx, Report No. 12550.6290<br>GLP<br>Not published | Y | GWI |
| KCA 8.2.1 | xxx | 2010 | GOW 008: Acute toxicity to zebra fish (*Danio rerio*) in a 96-hour study under static exposure<br>xxx, Report No. CH-E-081/2010, BT104/10, January 10, 2011<br>GLP<br>Not published | Y | GWI |
| KCA 8.2.2 | xxx | 1996 | Early life-stage toxicity of RH-117,281 technical to rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions<br>xxx, Report No. 94RC-0239, July 311996, ER Ref No. 7.1<br>xxx., Report No. 42400<br>GLP | Y | GWI |

| 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 |
|---|---|---|---|---|---|
| | | | Not published | | |
| KCA 8.2.2 | xxx | 1998 | RH-117,281 Technical: A flow-through life-cycle toxicity test with the fathead minnow (*Pimephales promelas*)<br>xxx, Report No. 97RC-0079, Semptember 29 1998, ER Ref No. 17.4<br>xxx, Report No. 129A-141<br>GLP<br>Not published | Y | GWI |
| KCA 8.2.2.1 | xxx | 2014 | Zebrafish (*Danio rerio*), early life stage toxicity test, flow through conditions, test item: zoxamide<br>xxx, Report No. GOW-001/4-43/A, October 31 2014<br>GLP<br>Not published | Y | GWI |
| KCA 8.2.2.3 | xxx. | 1998 | Uptake, depuration, bioconcentration and metabolism of $^{14}$C-RH-117,281 in bluegill sunfish (*Lepomis macrochirus*) under flow through test conditions<br>xxx, Report No. 34-98-145, September 15 1998, ER Ref. No. 15.1<br>xxx., Report No. RPT00328<br>GLP<br>Not published | Y | GWI |
| KCA 8.2.3 | Tognucci, A. | 1998 | Determination of the partition coefficient (n-octanol/water) of RH-127450<br>Rohm and Haas, Report No. 34-98-165, October 12 1998, ER Ref. No. 18.3<br>RCC Ltd.<br>GLP<br>No Published. | N | GWI |
| KCA 8.2.3 | Tognucci, A. | 1998 | Determination of the partition coefficient (n-octanol/water) of RH-139432<br>Rohm and Haas, Report No. 34-98-53, ER Ref. No. 31.3<br>RCC Ltd<br>GLP<br>Not published | N | GWI |
| KCA 8.2.4.1 | Sword, M.C., Gardner, C. | 1995 | Acute flow-through toxicity of RH-117,281 Technical to *Daphnia magna*<br>Rohm and Haas, Report No. 94RC-0081, July 25 1995, ER Ref No. 5.1 | N | GWI |

| 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 |
|---|---|---|---|---|---|
| | | | ABC Laboratories, Report No. 41683<br>GLP<br>Not published | | |
| KCA 8.2.4.1 | Rhodes, J.E., Williams, S. | 1998 | Acute toxicity of RH-127,450 to *Daphnia magna* in a range-finding test under static conditions<br>Rohm and Haas, Report No. 98RC-0096, September 11 1998, ER Ref No. 16.4<br>ABC Laboratories, Report No. 44666<br>GLP<br>Not published | N | GWI |
| KCA 8.2.4.1 | Mantilacci S. | 2010 | Acute toxicity of product GOW008 on *Daphnia magna* in a 48-hour immobilization test under static exposure<br>Biotecnologie BT srl, Report No. BT103/10, November 18 2010<br>GLP<br>Not published | N | GWI |
| KCA 8.2.4.1 | Caferella, M. A | 2002 | Zoxamide metabolite RH-139,432 - acute toxicity to daphnids (*Daphnia magna*) under static conditions<br>Dow AgroSciences LLC, Report No. 021297, September 27 2002, ER Ref. No. 47.3<br>Springborn Smithers Laboratories, Report No. 1t2550.6289<br>GLP<br>Not published | N | GWI |
| KCA 8.2.4.2 | Roberts, C.A., Swigert, J.P | 1997 | RH-117,281 Technical: A 96-hour flow-through acute toxicity test with the saltwater mysid (*Mysidopsis bahia*)<br>Rohm and Haas, Report No. 95RC-0275, May 5 1999<br>Wildlife International Ltd., Report No. 129A-136<br>GLP<br>Not published | N | GWI |
| KCA 8.2.5.1 | Murrell, H., Rhodes, J.E., Stewart, S. | 1997 | Chronic toxicity of RH-117,281 technical to *Daphnia magna* under flow-through test conditions<br>Rohm and Haas, Report No. 95RC-0273, June 12 1997, ER Ref No. 6.11<br>ABC Laboratories, Report No. 43209<br>GLP<br>Not published | N | GWI |

| 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 |
|---|---|---|---|---|---|
| KCA 8.2.5.2 | Drottar, K.R.,<br>Krueger, H.O. | 1998 | RH-117,281 Technical: A flow-through life-cycle toxicity test with the saltwater mysid (*Mysidopsis bahia*)<br>Rohm and Haas, Report No. 97RC-0077, June 17 1998<br>Wildlife International Ltd., Report No. 129A-142<br>GLP<br>Not published | N | GWI |
| KCA 8.2.5.3 | van der Kolk, J. | 1998 | RH-117,281: Chronic effects on midge larvae (*Chironomus riparius*) in a water/sediment system.<br>Rohm and Haas, Report No. 97RC-0083, May 26 1998, ER Ref No. 13.3<br>Springborn Laboratories (Europe)AG, Report No. 97-063-1007<br>GLP<br>Not published | N | GWI |
| KCA 8.2.6.1 | Ziegler, T.A., Stewart, S. | 1996 | Acute toxicity of RH-117,281 Technical to S*elenastrum capricornutum* Printz.<br>Rohm and Haas, Report No. 94RC-0238, June 20 1996, ER Ref No. 1.1<br>ABC Laboratories, Report No. 42399<br>GLP<br>Not published | N | GWI |
| KCA 8.2.6.1 | Kuhl, R.,<br>Härtel, Ch. | 2015 | Toxicity of (R)-Zoxamide to *Desmodesmus subspicatus* in an algal growth inhiition test.<br>Ibacon GmbH, Report No. 93441210<br>GLP<br>Not published | N | GWI |
| KCA 8.2.6.1 | Kuhl, R.,<br>Härtel, Ch. | 2015 | Toxicity of (S)-Zoxamide to *Desmodesmus subspicatus* in an algal growth inhiition test.<br>Ibacon GmbH, Report No. 93431210<br>GLP<br>Not published | N | GWI |
| KCA 8.2.6.1 | Hengsberger, A.,<br>Härtel, Ch. | 2015 | RH-141455: Toxicity to *Pseudorichneriella subcapitata* in an algal growth inhibition test<br>Ibacon GmbH, Report No. 98661210, July 01, 2015<br>GLP<br>Not published | N | GWI |
| KCA 8.2.6.1 | Drottar, K.R., | 1998 | RH-117,281 Technical: A 96-hour toxicity test with the freshwater alga (*Anabaena flos-aquae*) | N | GWI |

| 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 |
|---|---|---|---|---|---|
| | Sutherland, C.A., Krueger, H.O. | | Rohm and Haas, Report No. 97RC-0130, August 7, 1998, ER Ref No. 13.2<br>Wildlife International, Ltd., Report No. 129A-154<br>GLP<br>Not published | | |
| KCA 8.2.6.1 | Drottar, K.R., Sutherland, C.A., Krueger, H.O. | 1998 | RH-117,281 Technical: A 96-hour toxicity test with the freshwater diatom (*Navicula pelliculosa*)<br>Rohm and Haas, Report No. 97RC-0131, ER Ref No. 13.5<br>Wildlife International, Ltd., Report No. 129A-153<br>GLP<br>Not published | N | GWI |
| KCA 8.2.6.1 | Drottar, K.R., Sutherland, C.A., Krueger, H.O. | 1998 | RH-117,281 Technical: A 96-hour toxicity test with the freshwater alga (*Scenedesmus subspicatus*)<br>Rohm and Haas, Report No. 97RC-0133, August 7, 1998, ER Ref No. 13.4<br>Wildlife International, Ltd., Report No. 129A-151<br>GLP<br>Not published | N | GWI |
| KCA 8.2.6.1 | Drottar, K.R., Krueger, H.O. | 1998 | RH-117,281 Technical: A 96-hour toxicity test with the marine diatom (*Skeletonema costatum*)<br>Rohm and Haas, Report No. 97RC-0132, July 21, 1998, ER Ref No. 12.10<br>Wildlife International, Ltd., Report No. 129A-152<br>GLP<br>Not published | N | GWI |
| KCA 8.2.6.1 | Ward, S.C., Murdock, C.W. | 1998 | Toxicity of RH-117,281 2F (240 SC) *to Selenastrum capricornutum* Printz<br>Rohm and Haas, Report No. 97RC-0094, September 23, 1998, ER Ref No. 14.6<br>ABC Laboratories, USA, Report No. 44196<br>GLP<br>Not published | N | GWI |
| KCA 8.2.6.1 | Rhodes, J.E., Williams, S. | 1998 | Acute toxicity of RH-127,450 to the green alga, S*elenastrum capricornutum* Printz<br>Rohm and Haas, Report No. 98RC-0097, October 30, 1998, ER Ref No. 28.3<br>ABC Laboratories Inc., Report No. 44665<br>GLP<br>Not published | N | GWI |

| 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 |
|---|---|---|---|---|---|
| KCA 8.2.6.1 | Rhodes, J.E., Williams, S. | 1999 | Acute toxicity of RH-163,353 to S*elenastrum capricornutum* Printz in a range-finding test under static conditions<br>Rohm and Haas, Report No. 99RC-0023, May 3, 1999, ER Ref No. 36.1<br>ABC Laboratories Inc., Report No. 45164<br>GLP<br>Not published | N | GWI |
| KCA 8.2.6.1 | Hoberg, J.R | 2002 | Zoxamide Metabolite RH-139,432 - toxicity to freshwater green algae, *Scenedesmus subspicatus*<br>Dow AgroSciences LLC, Report No. 021298, September 25, 2002, ER Ref. 47.5<br>Springborn Smithers Laboratories, Report No. 12550.6288<br>GLP<br>Not published | N | GWI |
| KCA 8.2.6.1 | Juckeland, D. | 2015 | Effects of RH-24549 on *Desmodesmus subspicatus* in an algal growth inhibition test<br>BioChem agrar, Report No. 15 10 48 026 W, June 25 2015<br>GLP<br>Not published | N | GWI |
| KCA 8.2.7 | Drottar, K.R., Krueger, H.O. | 1998 | RH-117,281 Technical: A 14-day static-renewal toxicity test with duckweed (*Lemna gibba* G3)<br>Rohm and Haas, Report No. 97RC-0080, June 24 1998, ER Ref No. 12.7<br>Wildlife International, Ltd., Report No. 129A-147<br>GLP<br>Not published | N | GWI |
| KCA 8.3.1.1.1 | Kirkland, R.L. | 1993 | Acute contact toxicity of RH-117,281 technical to honey bees<br>Rohm and Haas, Report No. 92RC-0235, August 9 1993, ER Ref No. 12.6<br>Bio Research, Report No. 109-93<br>GLP<br>Not published | N | GWI |
| KCA 8.3.1.1.2 | Engelhard, E.K. | 1998 | RH-117,281 2R (240 SC): Laboratory oral and contact test with the honeybee, *Apis mellifera*<br>Rohm and Haas, Report No. 97RC-0095, April 25 1998, ER Ref No. 11.6,<br>Springborn Laboratories (Europe) AG, Report No. 97-066-1007<br>GLP<br>Not published | N | GWI |

| 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 |
|---|---|---|---|---|---|
| KCA 8.3.1.2 | Schmitzer, S., Ehmke, A. | 2014 | Chronic oral toxicity test of Zoxium 240 SC on the honey bee (*Apis mellifera* L.) in the laboratory<br>IBACON GmbH, Report No. 80052136, February 3 2014<br>GLP<br>Not published | N | GWI |
| KCA 8.3.1.3 | Schmitzer, S. | 2014 | Study on the effects of Zoxium 240 SC on honey bee brood (*Apis mellifera* L.) – brood feeding test<br>BioChem agrar, Report No. 80051031, March 27 2014<br>GLP<br>Not published | N | GWI |
| KCA 8.3.1.4 | Schmitzer, S. | 2014 | Study on the effects of Zoxium 240 SC on honey bee brood (*Apis mellifera* L.) – brood feeding test<br>BioChem agrar, Report No. 80051031, March 27 2014<br>GLP<br>Not published | N | GWI |
| KCA 8.3.2.1 | Engelhard, E.K. | 1998 | RH-117,281 2R (240 SC): Laboratory acute toxicity test with the parasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera: Braconidae)<br>Rohm and Haas, Report No.: 97RC-0106, March 3 1998, ER Ref No. 11.8<br>Springborn Laboratories (Europe) AG, Report No. 97-062-1007<br>GLP<br>Not published | N | GWI |
| KCA 8.3.2.2 | Engelhard, E.K. | 1998 | RH-117,281 2R (240 SC): Laboratory toxicity test with the predacious mite, *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae)<br>Rohm and Haas, Report No. 97RC-0105, March 3 1998, ER Ref No. 11.3<br>Springborn Laboratories (Europe) AG, Report No. 97-070-1007<br>GLP<br>Not published | N | GWI |
| KCA 8.3.2.2 | Engelhard, E.K. | 1998 | RH-117,281 2R (240 SC): Laboratory toxicity test with the predacious mite, *Amblyseius andersoni* Chant (Acari: Phytoseiidae)<br>Rohm and Haas, Report No. 97RC-0111, March 3 1998, ER Ref No. 11.7<br>Springborn Laboratories (Europe) AG, Report No. 97-075-1007 | N | GWI |

| 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 |
|---|---|---|---|---|---|
| | | | GLP<br>Not published | | |
| KCA 8.3.2.2 | Engelhard, E.K. | 1998 | RH-117,281 2R (240 SC): Laboratory toxicity test with the spiders, *Pardosa sp*. (Araneae: Lycosidae)<br>Rohm and Haas, Report No. 97RC-0107, March 3, 1998, ER Ref No. 11.9<br>Springborn Laboratories (Europe) AG, Report No. 97-059-1007<br>GLP<br>Not published | N | GWI |
| KCA 8.3.2.2 | Engelhard, E.K. | 1998 | RH-117,281 2R (240 SC): Laboratory toxicity test with the green lacewing *Chrysoperla carnea* Steph. (Neuroptera: Chrysopidae)<br>Rohm and Haas, Report No. 97RC-0109, March 3 1998, ER Ref No. 11.11<br>Springborn Laboratories (Europe) AG, Report No. 97-068-1007<br>GLP<br>Not published | N | GWI |
| KCA 8.3.2.2 | Engelhard, E.K. | 1998 | RH-117,281 2R (240 SC): Laboratory contact toxicity test with the predator, *Orius insidiosus* (Heteroptera: Anthocoridae)<br>Rohm and Haas, Report No. 97RC-0110, March 3 1998, ER Ref No. 11.10<br>Springborn Laboratories (Europe) AG, Report No. 97-077-1007<br>GLP<br>Not published | N | GWI |
| KCA 8.3.2.2 | Engelhard, E.K. | 1998 | RH-117,281 2R (240 SC): Laboratory acute toxicity test with the ground beetle, *Poecilus cupreus L*. (Coleoptera: Carabidae)<br>Rohm and Haas, Report No. 97RC-0108, March 3 1998, ER Ref No. 11.2<br>Springborn Laboratories (Europe) AG, Report No. 97-064-1007<br>GLP<br>Not published | N | GWI |
| KCA 8.4.1 | Ganssmann, M. | 2015 | RH-141455: Effects on reproduction and growth of earthworms *Eisenia fetida* in artificial soil<br>IBACON GmbH, Report No. 98661022, June 1 2005<br>GLP<br>Not published | N | GWI |

| 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 |
|---|---|---|---|---|---|
| KCA 8.4.1 | Downing, J. | 1995 | RH-117,281 technical: toxicity to earthworm (*Eisenia fetida*)<br>Rohm and Haas, Report No. 94RC-0237, July 19 1995, ER Ref No. 5.2<br>ABC Laboratories Inc., Report No. 42398<br>GLP<br>Not published | N | GWI |
| KCA 8.4.1 | Bryan, R.L., Porch, J.R., Krueger, H.O. | 2000 | RH-127,450 technical: an acute toxicity study with the earthworm in an artificial soil substrate.<br>Rohm and Haas, Report No. 99RC-0282, March 7 2000, ER Ref No. 41.5<br>Wildlife International, Ltd., Easton, MD, USA, Report No. 129-173<br>GLP<br>Not published | N | GWI |
| KCA 8.4.1 | Nienstedt, K. | 1999 | A chronic toxicity and reproduction test exposing the earthworm *Eisenia fetida* to RH-117,281 Technical material in OECD artificial soil, based on the BBA-guideline VI, 2-2 (1994) and the ISO-draft (ISO/DIS 11268-2)<br>Rohm and Haas, Report No. 98RC-0181, April 14 1999, ER Ref No. 34.1<br>Springborn Laboratories (Europe) AG, Report No. 99-092-1007<br>GLP<br>Not published | N | GWI |
| KCA 8.4.1 | Nienstedt, K. | 2001 | Effects of RH-7281 technical applied on natural soil on the cocoon and juvenile production of the earthworm *Eisenia fetida*<br>Rohm and Haas, Report No. 00RC-0209, February 22 2001, ER Ref No. 45.2<br>Springborn Laboratories (Europe) AG, Report No. 1007.070.631<br>GLP<br>Not published | N | GWI |
| KCA 8.4.2 | Young, D.H. | 2000 | Evaluation of the biological activity of the RH-117281 metabolites RH-24549, 127450 and 163353<br>Rohm and Haas, Report No. DIS-00-281, ER Ref No. 44.1<br>GLP<br>Not published | N | GWI |
| KCA 8.5 | Hammesfahr, U. | 2015 | RH-141455: Effects on the activity of soil microflora in the laboratory (nitrogen transformation)<br>IBACON GmbH, Report No. 98661080, May 1, 2015 | N | GWI |

| 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 |
|---|---|---|---|---|---|
| | | | GLP<br>Not published | | |
| KCA 8.5 | van der Kolk, J. | 1998 | RH-117,281 Technical: Determination of the effects on soil microflora activity<br>Rohm and Haas, Report No. 97RC-0084, September 7 1998, ER Ref No. 14.1<br>Springborn Laboratories (Europe) AG, Report No. 97-060-1007<br>GLP<br>Not published | N | GWI |
| KCA 8.5 | van der Kolk, J. | 2000 | RH-117,281 Technical: Determination of the effects on nitrogen transformation by microflora in soil<br>Rohm and Haas, Report No. 00RC-0085, ER Ref No. 44.12<br>Springborn Laboratories (Europe) AG, Report No. 1007.070.747<br>GLP<br>Not published | N | GWI |
| KCA 8.6 | Nunez, M.V. | 1998 | Greenhouse phytotoxicity tests with RH-7281 2F<br>Rohm and Haas, Report No. 98R-1092, August 31 1998, ER Ref No. 28.4,<br>GLP<br>Not published | N | GWI |
| KCA 8.6 | Nunez, M.V. | 1998 | Greenhouse crop phytotoxicity tests with RH-117281 2F<br>Rohm and Haas, Report No. 98R-1114, November 23 1998, ER Ref No. 28.5<br>GLP<br>Not published | N | GWI |
| KCA 8.6 | Sames, B.A. | 1998 | Insecticidal screening report - pre-screen insecticidal activity with RH-117,281; Primary screening activity per RH-117281<br>Rohm and Haas, Report No. 98R-1113, November 5 1998, ER Ref No. 28.6<br>GLP<br>Not published | N | GWI |
| KCA 8.8 | Heim, D.,<br>Heim, L. | 2002 | Activated sludge, respiration inhibition test of Zoxamide<br>Dow AgroSciences, Report No. GH-C 5421, February 7 2002, CA 3, ER Ref. No. 47.2<br>GLP | N | GWI |

| 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 |
|---|---|---|---|---|---|
| | | | Not published | | |

GWI – Gowan Crop Protection Ltd.

For cymoxanil it is referred to the data available in the DAR (2005) and its addenda via Letter of Access of the company Sipcam Oxon.

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 10.1.1.1<br><br>(KCA 8. | xxx | 2006 | CYMOXANIL 33% + ZOXAMIDE 33% WG (SIP 40936): an acute oral toxicity study with the northern bobwhite.<br>Oxon Italia SpA, Italy<br>xxx, Report No. 613-103<br>GLP<br>Not published | Y | GWI<br>Sipcam<br>Oxon S.p.A. |
| KCP 10.2.1<br><br>(KCA 8.2.4.1) | xxx | 2020 | RH-163353: Acute toxicity to Daphnia magna<br>Gowan Crop Protection Ltd., UK<br>xxx, Report No. 3202386<br>GLP<br>Not published | N | GWI |
| KCP 10.2.1<br><br>(KCA 8.2.4.1) | Hugill, E. | 2020 | RH-141455: Acute toxicity to Daphnia magna<br>Gowan Crop Protection Ltd., UK<br>Smithers ERS Ltd., UK, Report No. 3202380<br>GLP<br>Not published | N | GWI |
| KCP 10.2.1<br><br>(KCA 8.2.7) | Juckeland, D. | 2020 | Effects Zoxamide technical on Lemna gibba in a growth inhibition test under semi-static test conditions<br>Gowan Crop Protection Ltd., UK<br>BioChem agrar, Germany, Report No. 18 48 ALE 0005<br>GLP<br>Not published | N | GWI |
| KCP 10.2.2<br><br>(KCA 8.2.2.1) | xxx | 2020 | Final report addendum for RH-117,281 technical: An early life-stage toxicity test with the sheepshead minnow (Cyprinodon variegatus)<br>Gowan Crop Protection Ltd., UK | N | GWI |

| 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 |
|---|---|---|---|---|---|
| | | | xxx, USA, Report No. 129A-143A<br>GLP<br>Not published | | |
| KCP 10.2.2<br><br>(KCA 8.2.2.1) | xxx | 1998 | RH-117,281 technical: An early life-stage toxicity test with the sheepshead minnow (Cyprinodon variegatus)<br>xxx, Report No. 97RC-0078<br>xxx, Report No. 129A-143A<br>GLP<br>Not published | Y | GWI |
| KCP 10.3.1.3<br><br>(KCA 8.3.1.3) | xxxx | 2018 | Zoxamide: Honey bee (Apis mellifera L.) larval toxicity, repeated exposure<br>xxx, A Gowan Group Company, USA<br>xxx, Report No. 12791.6307<br>GLP<br>Not published | N | GWI |
| KCP 10.4.1.2<br><br>(KCA 8.4.1) | Schulz, L. | 2020 | Effects of Zoxium 240 SC on earthworms under field conditions<br>Gowan Crop Protection Ltd., UK<br>BioChem agrar, Germany, Report No. 18 48 FEW 0001<br>GLP<br>Not published | N | GWI |
| KCP 10.4.2<br><br>(KCA 8.4.2) | xxx | 2020 | Zoxium 240 SC – A laboratory test to determine the effects of fresh residues on the predatory soil mite Hypoaspis aculeifer (Acari, Laelapidae) in an artificial soil substrate<br>Gowan Crop Protection Ltd., UK<br>xxx, Report No. GOW-17-14<br>GLP<br>Not published | N | GWI |
| KCP 10.4.2<br><br>(KCA 8.4.2) | xxx | 2020 | RH-163353: Collembolan reproduction test in soil<br>Gowan Crop Protection Ltd., UK<br>Smithers ERS Ltd., UK, Report No. 3202390<br>GLP<br>Not published | N | GWI |

| 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 10.4.2<br><br>(KCA 8.4.2) | xxx | 2020 | RH-141455: Collembolan reproduction study<br>Gowan Crop Protection Ltd., UK<br>xxx, Report No. 3202382<br>GLP<br>Not published | N | GWI |

**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 |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |

# Appendix 2 Detailed evaluation of the new studies

Review Comment:

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

As a lot of data gaps were identified during EU peer review (please refer to EFSA Journal 2017;15(9):4980) the critical for the assessment studies from Annex II were evaluated too.

During evaluation period, many studies reports were amended. The latest versions of those summaries are presented in CYMOXANIL 33% + ZOXAMIDE 33% WG / Reboot dRR. Here are presented overall conclusions of the studies assessment.

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

## A 2.1.1 KCP 10.1.1 Effects on birds

## A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

### A 2.1.1.1.1 Study 1 - CYMOXANIL 33% + ZOXAMIDE 33% WG: acute oral toxicity

| | |
|---|---|
| Comments of zRMS: | The study was not crucial for finalization of the risk assessment. |

| | |
|---|---|
| **Reference:** | **KCP 10.1.1.1/01** |
| Report | xxx., 2006: CYMOXANIL 33% + ZOXAMIDE 33% WG (SIP 40936): an acute oral toxicity study with the northern bobwhite<br>Oxon Italia S.p.A., Italy<br>xxx, Report No. 613-103, GLP, Not published |
| Guideline(s): | US-EPA Series 850 -Ecological Effects Test Guidelines, OPPTS N° 850.2100<br>FIFRA E Section 71-1<br>National Research Council (1996) Guide for the Care and Use of Laboratory Animals |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | No |

**Executive Summary**

The acute oral toxicity of 'Cymoxanil 33% + Zoxamide 33% WG' to birds was determined by exposing northern bobwhite to a single oral dose of the product.
One test concentration (2250 mg/kg) was chosen based upon the toxicity information known and a test on a control group was also performed. Five males and five females were assigned at each testing group.
A single dose of test substance was orally intubated into the crop or proventriculus of each bird.
No mortality in the control neither in test group was noticed, birds were normal in appearance and behaviour throughout the test. In the treated birds a small decrease in the weight and in feed consumption was observed in the 3 days after the treatment; later they presented an increasing increase of these parameters, reaching values comparable with the control.
The acute oral LC50 value for northern bobwhite exposed to 'Cymoxanil 33% + Zoxamide 33% WG' as a single oral dose was determined to be greater than 2250 mg/kg.

## I. MATERIALS AND METHODS

### A. MATERIALS

**1.Test Material:**

| | |
|---|---|
| **Description:** | Granular |
| **Lot/Batch #:** | BPL212 |
| **Purity:** | Nominal: Cymoxanil 33%, Zoxamide 33% WG<br>Declared according to CoA: Cymoxanil 32.29%, Zoxamide 33.18% |
| **Stability of test compound** | June 2008 (expiry date) |
| **2. Vehicle and/or positive control** | Distilled water |
| **3. Test animals** | Northern bobwhite quail |
| **Species:** | *Colinus virginianus* |
| **Strain** | Not relevant |
| **Age:** | 23 weeks |
| **Weight at dosing:** | 170 - 212 g. |
| **Source:** | K & L Quail, Oroville, CA 95965 |
| **Acclimation period** | 5 weeks |
| **Diet:** | Game bird ration formulated to Wildlife International, Ltd's specifications |
| **Water:** | Tap water, *ad libitum* |
| **Housing:** | Housed 5 animals by sex per pen 78 x 51 cm, h and sloping from 20 to 25 cm |
| **4. Environmental conditions** | |
| **Temperature:** | 23.8 ± 0.3 °C |
| **Humidity:** | 72 ± 6 % |
| **Air changes:** | Not available |
| **Photoperiod:** | 8 hours light (117 lux) |

### B. STUDY DESIGN AND METHODS

| | |
|---|---|
| **1. In life dates** | 30 June – 14 July 2006 |

**2. Animal assignment and treatment**

Ten northern bobwhite, five females and five males, were assigned to the treated and to the control group. 2.250 g/kg of 'Cymoxanil 33% + Zoxamide 33% WG' dispersed in deionised water were intubated into the crop or proventriculus of each bird present in the test group. A syringe and stainless steel cannula were used for this purpose. All birds received a constant dosage volume of 5 ml/kg of bodyweight; an equal volume of only diluent was given to the control.
Birds were observed at least daily during the acclimation and twice daily during the test. Bodyweights were measured at the beginning of the test and on days 3, 7 and 14. Average feed consumption was recorded over days 0-3, 4-7, 8-14.

**3. Statistics**

No statistical analysis was applied.

## II. RESULTS AND DISCUSSION

### A. MORTALITY

No mortality in the control and in the treated group was observed after 14 days. Appearance and behaviour of tested birds resulted normal during all the test.

### B. BODY WEIGHT

Reduction of bodyweight was noted in all birds, sometimes accompanied by a reduced food consumption. After day 3 there was an increase in mean bodyweight as well as a recovery in food consumption.

**Table IIIA 10.1.5-1 Mean body weights (g) and feed consumption of the northern bobwhites tested.**

| Experimental Group (mg/kg) | Sex | Mean body weights (g) | | | | Estimated mean feed consumption (g/bird/day) | | |
|---|---|---|---|---|---|---|---|---|
| | | *Day 0* | *Day 3* | *Day 7* | *Day 14* | *Days 0-3* | *Days 4-7* | *Days 8-14* |
| Control | M | 196 | 199 | 198 | 198 | 29 | 25 | 21 |
| | F | 192 | 200 | 198 | 200 | 26 | 20 | 18 |
| 2250 | M | 200 | 183 | 191 | 200 | 17 | 27 | 23 |
| | F | 184 | 176 | 180 | 186 | 19 | 28 | 24 |

### C. NECROPSY

The results of gross necropsies performed on all birds were not remarkable.

## III. CONCLUSIONS

The acute oral $LD_{50}$ value for the northern bobwhite exposed to 'Cymoxanil 33% + Zoxamide 33% WG' as a single oral dose was determined to be higher than 2250 mg/kg. The no-mortality level was 2250 mg/kg.

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

**A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds**

**A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals**

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

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

### A 2.2 KCP 10.2 Effects on aquatic organisms

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

### A 2.2.1.1 Study 1 - RH-163353: Fish, acute toxicity test

EFSA (2017) requested in its Peer Review Conclusion: *"Further data or refinement (exposure and/or effects) for the acute risk assessment of fish for the metabolites RH-127450, RH-163353 and RH-141455 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Sections 4 and 5)."* To close this data gap, a study on the acute toxicity of RH-163353 is presented.

The following study has been provided to Latvia as RMS for zoxamide and cMSs for interzonal evaluation in July 2020.

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

| | |
|---|---|
| **Reference:** | **KCP 10.2.1/01** |
| Report | xxx., 2020: RH-163353: Fish, acute toxicity test<br>Gowan Crop Protection Ltd., UK<br>xxx, Report No. 3202385, GLP, Unpublished |
| Guideline(s): | OECD 203 (adopted 18 June 2019) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | No |

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | RH-163353 (HHGCP001-00-2) |
| **Purity** | 98.97 % (w/w)<br>(re-certified under GLP: 99.48 % (w/w); enantiomeric ratio 48.6:51.4 (racemate)) |
| **Species** | *Oncorhynchus mykiss* (rainbow trout) |
| Age: | young fish of the same age (4.4-6.0 cm length) |

| | |
|---|---|
| Number of animals: | 7 (per treatment group and control) |
| Weight: | 0.62-1.90 g |
| Source: | Hebden, Skipton, North Yorkshire, United Kingdom |
| Acclimation period: | at least 9 days prior to testing |
| Feeding: | The fish were fed with a proprietary fish food, which was added to the holding tank in quantities dictated by the size of the fish. On a fortnightly basis during the holding period, a sub sample of 10 fish was weighed and a mean wet weight calculated. The wet weight was used to determine the quantity of food required for the number of fish remaining in the holding tank. The amount of food required per holding tank was weighed and stored in labelled feed containers placed in the fish holding room. The feed was distributed to the holding tank over two feeding intervals during the day. Uneaten food and debris were siphoned or cleaned from the tanks as required. |
| Housing: | 20 L glass aquaria, each fitted with an appropriate lid, containing 12 L of media, placed in a temperature-controlled room under artificial light and under continuous water renewal (flow-through) conditions |
| **Environmental conditions** | |
| Temperature: | 11.5 – 12.9 °C |
| Photoperiod: | 16 hours light, 8 hours dark |
| Test medium: | mains water, filtered (particulate filter and activated carbon filter) and UV sterilised |
| pH: | 6.77 – 7.48 |
| Dissolved oxygen: | 93.6 – 101.4 % air saturation |
| **Application rate(s)** | Range- finding test:<br>Nominally 10 and 100 mg/L (static conditions)<br>Definitive test:<br>Nominally 100 mg/L (static conditions) |
| **Post exposure observation period** | 96 hours |
| **Remarks** | None |

Based on the results of a range finder, the acute toxicity of the zoxamide metabolite RH-163353 to *Oncorhynchus mykiss* was tested at an application rate of nominally 100 mg/L (limit test) over 96-hours in a static test design. An untreated group served as control.

Concentrations of RH-163353 were determined by treating samples with acetonitrile containing 0.5% formic acid, then diluting further with acetonitrile/treated mains water (1:4 v:v) containing 0.1% formic acid as required to bring the response within the calibration range. Samples were analysed by liquid chromatography-time of flight mass spectrometry (LC-TOF/MS). The method has been validated according to SANCO 3029/99 rev. 4 (SMV 3202385-01V and updated by SMV 3202385-02V to include storage stability data). The limit of quantification (LOQ) was 0.001 mg/L.

RH-163353 is a racemate. The analytical method validation for the enantiomeric ratio analysis for RH-163353 was conducted under Smithers ERS Study Number 3202586 (established analytical procedure,

SMV 3202586-01V). A combination of the analytical procedures SMV 3202385-01V and SMV 3202586-01V was used to assess the enantiomeric ratio of the test substance in the test medium and calibration standard solution.

## Results and discussion

Environmental parameters remained within acceptable limits throughout the duration of the study (pH 6.77-7.43, temperature 11-12.9°C, Dissolved oxygen 93.6 – 101.4 % air saturation). The water hardness was 77 mg $CaCO_3$ /L, the chlorine content amounted to 0.01 mg $Cl_2$/L.

Analysis of the freshly prepared media at 0 hours and the corresponding old media at 96 hours showed measured concentrations of 108 and 102 mg/L, respectively. Given that measured concentrations were within the 80% to 120% of nominal acceptance range, the study results were based on nominal test item concentrations.

**Table A 1: Test item concentrations [mg/L]**

| | Measured concentration (mg/L) | | % nominal | |
|---|---|---|---|---|
| **Nominal test item concentration (mg/L)** | **0 hours (new media)** | **96 hours (old media)** | **0 hours (new media)** | **96 hours (old media)** |
| Control | < LOQ | 0.0037* | -- | -- |
| 100 | 108 | 102 | 108 | 102 |

LOQ = 0.001 mg/L

* Result was in excess of the LOQ probably due to an unintended carryover of residues. Re-analysis of the sample showed a measured concentration of 0.0039 mg/L therefore the 'back-up' samples were analysed. The results from the 'back-up- samples showed measured concentrations of 0.0047 and 0.0038 mg/L.

RH-163353 is a racemate. The ratio of its two enantiomers has been checked in a 96-hour test sample and in a freshly prepared calibration standard solution. As a result, the isomer ratio of the 96-hour 100 mg/L sample was within ±1.5% of the calibration standard and the ratio from the certificate of analysis for the test item batch. Thus, the enantiomeric ratio is deemed to be stable in the fish water during the course of the study.

No mortality of fish occurred during the course of the study. For completeness, the results of the range finder and of the definitive test are given.

**Table A 2: Cumulative mortality of fish**

| Nominal test item concentration (mg/L) | No. of fish exposed | Cumulative Mortality | | | | |
|---|---|---|---|---|---|---|
| | | **ca.2 hours** | **24 hours** | **48 hours** | **72 hours** | **96 hours** |
| Range-finding test | | | | | | |
| Control | 3 | 0 | 0 | 0 | 0 | 0 |
| 10 | 3 | 0 | 0 | 0 | 0 | 0 |
| 100 | 3 | 0 | 0 | 0 | 0 | 0 |
| Definitive test | | | | | | |
| Control | 7 | 0 | 0 | 0 | 0 | Control |
| 100 | 7 | 0 | 0 | 0 | 0 | 100 |

The fish were showing no sublethal effects.

Based on nominal concentrations, the 96-hour $LC_{50}$ value was determined to be >100 mg/L. The corresponding No Observed Effect Concentration (NOEC) is 100 mg/L.

Given that no effects were observed throughout the test, the results were determined empirically.

The validity criteria for control mortality (≤ 10 %) and Dissolved oxygen (≥ 60 % air saturation) were met. Therefore, the test was considered valid.

**Table A 3: Acute toxicity of RH-163353 on fish – study endpoints**

| Parameter | Test item concentration (mg/L) | | | |
|---|---|---|---|---|
| | 24 hours | 48 hours | 72 hours | 96 hours |
| $LC_{50}$ | >100 | >100 | >100 | >100 |
| NOEC | 100 | 100 | 100 | 100 |

## Conclusion

The acute toxicity of the zoxamide metabolite RH-163353 to *Oncorhynchus mykiss* was tested at an application rate of nominally 100 mg/L (limit test) over 96-hours in a static test design.

Based on nominal concentrations, the 96-hour LC50 value was determined to be >100 mg/L. The corresponding No Observed Effect Concentration (NOEC) is 100 mg/L.

(xxx. 2020)

### A 2.2.1.2 Study 2 - RH-141455: Fish, acute toxicity test

EFSA (2017) requested in its Peer Review Conclusion: *"Further data or refinement (exposure and/or effects) for the acute risk assessment of fish for the metabolites RH-127450, RH-163353 and RH-141455 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Sections 4 and 5)."* To close this data gap, a study on the acute toxicity of RH-163353 is presented.

The following study has been provided to Latvia as RMS for zoxamide and cMSs for interzonal evaluation in July 2020.

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

| | |
|---|---|
| **Reference:** | **KCP 10.2.1/02** |
| Report | xxx., 2020: RH-141455: Fish, acute toxicity test<br>Gowan Crop Protection Ltd., UK<br>xxx, Report No. 3202716, GLP, Unpublished |
| Guideline(s): | OECD 203 (adopted 18 June 2019) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication | No |

(if vertebrate study)

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | RH-141455 (HHGCP017-00-1) |
| **Purity** | 99.6 % (w/w) |
| **Species** | *Oncorhynchus mykiss* (rainbow trout) |
| Age: | young fish of the same age (3.8-4.3 cm length) |
| Number of animals: | 7 (per treatment group and control) |
| Weight: | 0.40 - 56 g |
| Source: | Northern Trout, Hebden, Skipton, United Kingdom |
| Acclimation period: | at least 9 days prior to testing |
| Feeding: | The fish were fed with a proprietary fish food, which was added to the holding tank in quantities dictated by the size of the fish. On a fortnightly basis during the holding period, a sub sample of 10 fish was weighed and a mean wet weight calculated. The wet weight was used to determine the quantity of food required for the number of fish remaining in the holding tank. The amount of food required per holding tank was weighed and stored in labelled feed containers placed in the fish holding room. The feed was distributed to the holding tank over two feeding intervals during the day. Uneaten food and debris was siphoned or cleaned from the tanks as required. |
| Housing: | 15 L glass aquaria, each fitted with an appropriate lid, containing 10 L of media, placed in a temperature-controlled room under artificial light and under continuous water renewal (flow-through) conditions |
| **Environmental conditions** | |
| Temperature: | 12.1– 12.4°C |
| Photoperiod: | 16 hours light, 8 hours dark |
| Test medium: | mains water, filtered (particulate filter and activated carbon filter) and UV sterilised |
| pH: | 6.15 – 7.77 |
| Dissolved oxygen: | 94.2 – 104.7 % air saturation |
| **Application rate(s)** | Nominally 4.1, 9.1, 20, 45 and 100 mg/L (static conditions) |
| **Post exposure observation period** | 96 hours |
| **Remarks** | A first definitve test (limit test) was performed, but terminated due mortalities observed at the 100 mg/L test concentration. Therefore, the test was repeated using a range of concentrations. In addition, fresh test item was used for the final test. |

The acute toxicity of the zoxamide metabolite RH-141455 to *Oncorhynchus mykiss* was tested at application rates of nominally 4.1, 9.1, 20, 45 and 100 mg/L over 96-hours in a static test design. An untreated group served as control.

Concentrations of RH-141455 were determined by treating samples with acetonitrile containing 0.2% formic acid, then diluting further with acetonitrile/treated mains water (1:1 v/v) containing 0.1% formic acid as required to bring the response within the calibration range. Samples were analysed by injection onto a liquid chromatography-time of flight mass spectrometry (LC-TOF/MS) system. The method has been validated according to SANCO 3029/99 rev. 4 (SMV 3202716-01V, updated by SMV 3202716-02V to include extended storage stability data). The limit of quantification (LOQ) was 0.1 mg/L.

## Results and discussion

Environmental parameters remained within acceptable limits throughout the duration of the study (pH 6.15-7.77, temperature 12.1– 12.4°C, Dissolved oxygen 94.2 – 104.7 % air saturation). The water hardness was 87 mg $CaCO_3$/L, the chlorine content amounted to 0.01 mg $Cl_2$/L.

Analysis of 0-hour samples showed measured concentrations ranging from 96-113 % of nominal, the 96-hour samples showed measured concentrations ranging from 120-126% of nominal. Analysis of the 0-hour and 96-hour 'back-up' samples showed measured concentrations ranging from 112-125 % of nominal and 112-120 % of nominal, respectively. The results confirmed that the test substance was stable in the test media over the period of the test. Although some measured concentrations were slightly above 120 % of nominal, the study results were based on nominal test item concentrations.

**Table A 4: Test item concentrations [mg/L]**

Values given for initial A sample/back-up B/back-up C samples

| | **Measured concentration (mg/L)** | | **% nominal** | |
|---|---|---|---|---|
| **Nominal test item concentration (mg/L)** | **0 hours (new media)** | **96 hours (old media)** | **0 hours (new media)** | **96 hours (old media)** |
| Control | all < LOQ | all < LOQ | -- | -- |
| 4.1 | 4.62/4.93/5.13 | 4.98/4.74/4.79 | 113/120/125 | 121/116/117 |
| 9.1 | 9.61/10.7/11.3 | 11.1/10.5/10.6 | 106/118/124 | 122/115/116 |
| 20 | 19.5/23.2/23.8 | 25.1/23.8/24.0 | 98/116/119 | 126/119/120 |
| 45 | 43.2/54.1/51.9 | 54.1/52.1/50.5 | 96/120/115 | 120/116/112 |
| 100 | 101/112/117 | 125/117/120 | 101/112/117 | 125/117/120 |

LOQ = 0.1 mg/L

No mortality of fish occurred during the course of the study.

**Table A 5: Cumulative mortality of fish**

| **Nominal test item concentration (mg/L)** | **No. of fish exposed** | **Cumulative Mortality** | | | |
|---|---|---|---|---|---|
| | | **24 hours** | **48 hours** | **72 hours** | **96 hours** |
| Control | 7 | 0 | 0 | 0 | 0 |
| 4.1 | 7 | 0 | 0 | 0 | 2* |
| 9.1 | 7 | 0 | 0 | 0 | 0 |
| 20 | 7 | 0 | 0 | 0 | 0 |
| 45 | 7 | 0 | 0 | 0 | 0 |
| 100 | 7 | 0 | 0 | 0 | 0 |

*Mortalities not considered to be test item related, but due to aggressive fish

The fish were showing mild sublethal effects in the higher test rates (swimming normally but abnormal bottom distribution/behaviour, lethargy, coughing), 1-2 fish also at the two lowest test concentrations (abnormal bottom distribution/behaviour, lethargic with dark pigmentation, coughing).

Based on nominal concentrations, the 96-hour $LC_{50}$ value was considered to be >100 mg/L. The corresponding No Observed Effect Concentration (NOEC) was considered to be 100 mg/L.

Given that no effects were observed throughout the test, the results were determined empirically.

The validity criteria for control mortality (≤ 10 %) and Dissolved oxygen (≥ 60 % air saturation) were both satisfied. Therefore, the test is valid.

**Table A 6: Acute toxicity of RH-141455 on fish – study endpoints**

| Parameter | Test item concentration (mg/L) | | | |
|---|---|---|---|---|
| | **24 hours** | **48 hours** | **72 hours** | **96 hours** |
| $LC_{50}$ | >100 | >100 | >100 | >100 |
| NOEC | 100 | 100 | 100 | 100 |

## Conclusion

The acute toxicity of the zoxamide metabolite RH-141455 to *Oncorhynchus mykiss* was tested at application rates of nominally 4.1, 9.1, 20, 45 and 100 mg/L over 96-hours in a static test design.

Based on nominal concentrations, the 96-hour $LC_{50}$ value was determined to be >100 mg/L. The corresponding No Observed Effect Concentration (NOEC) is 100 mg/L.

(xxx. 2020)

### A 2.2.1.3 Study 3 - RH-127450: Fish, acute toxicity test

EFSA (2017) requested in its Peer Review Conclusion: *"Further data or refinement (exposure and/or effects) for the acute risk assessment of fish for the metabolites RH-127450, RH-163353 and RH-141455 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Sections 4 and 5)."* To close this data gap, a study on the acute toxicity of RH-127450 is presented in the following.

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

| | |
|---|---|
| **Reference:** | **KCP 10.2.1/03** |
| Report | xxx, 2020: RH-127450: Fish, Acute toxicity test<br>Gowan Crop Protection Ltd., UK<br>xxx, Report No. 3202373, GLP, Unpublished |
| Guideline(s): | OECD 203 (adopted 18 June 2019) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

Duplication No
(if vertebrate study)

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | RH-127450 (116286) |
| **Purity** | 94.29 % (w/w); enantiomeric ratio 49.57 : 50.43 |
| **Species** | *Oncorhynchus mykiss* (rainbow trout) |
| Age: | young fish of the same age (3.9-4.9 cm length) |
| Number of animals: | 7 (per treatment group and control) |
| Weight: | 0.49 – 0.84 g |
| Source: | Northern Trout, Hebden, Skipton, United Kingdom |
| Acclimation period: | at least 9 days prior to testing |
| Feeding: | The fish were fed with a proprietary fish food, which was added to the holding tank in quantities dictated by the size of the fish. On a fortnightly basis during the holding period, a sub sample of 10 fish was weighed and a mean wet weight calculated. The wet weight was used to determine the quantity of food required for the number of fish remaining in the holding tank. The amount of food required per holding tank was weighed and stored in labelled feed containers placed in the fish holding room. The feed was distributed to the holding tank over two feeding intervals during the day. Uneaten food and debris was siphoned or cleaned from the tanks as required. |
| Housing: | 10 L glass aquaria, each fitted with an appropriate lid, containing 7 L of media, placed in a temperature-controlled room under artificial light and with daily renewal of the test media |
| **Environmental conditions** | |
| Temperature: | 12.0 – 13.8°C |
| Photoperiod: | 16 hours light, 8 hours dark |
| Test medium: | mains water, filtered (particulate filter and activated carbon filter) and UV sterilised |
| pH: | 7.34 – 7.69 |
| Dissolved oxygen: | 95.6 -104.5 % air saturation |
| **Application rate(s)** | Rage-finding test:<br>Nominally 1, 10 and 100 % saturated solution (static conditions)<br>Definitive test:<br>Nominally 10, 18, 32, 56 and 100 % saturated solution (semi-static conditions) |
| **Post exposure observation period** | 96 hours |
| **Remarks** | None |

Based on the results of a range-finder, the acute toxicity of the zoxamide metabolite RH-127450 to *Oncorhynchus mykiss* was tested at application rates of nominally 10, 18, 32, 56 and 100 % saturated solution over 96-hours in a semi-static test design. An untreated group served as control.

Concentrations of RH-127450 were determined by mixing aqueous samples with acetonitrile containing 0.5% formic acid, then diluting further with treated mains water/acetonitrile (4:1 v/v) containing 0.1% formic acid as required to bring the response within the calibration range. Samples are analysed by injection onto a liquid chromatography-time of flight mass spectrometry (LC-TOF/MS) system or a liquid chromatography triple quadrupole mass spectrometry (LC-TQMS) system. The method has been validated according to SANCO/3029/99 rev. 4 (SMV 3202373-01V, updated by SMV 3202373-02V to include storage stability and method validation data of LC-TQMS). The limit of quantification (LOQ) was 0.001 mg/L.

RH-127450 is a racemate. The enantiomeric ratio of the RH-127450 isomers has been determined in 96-hour test samples with method SMV 3202373-01V.E. The test solutions were extracted with acetonitrile, then diluted with acetonitrile/HPLC grade water (1:4 v/v) to bring the response within the calibration range. Samples were thereafter analysed by injection onto a liquid chromatography-time of flight mass spectrometry (LC-TOF/MS) system.

## Results and discussion

Environmental parameters remained within acceptable limits throughout the duration of the study (pH 7.34-7.69, temperature 12.0– 13.8°C, Dissolved oxygen 95.6 – 104.5% air saturation). The water hardness was 65-96 mg $CaCO_3$/L, the chlorine content amounted to 0.0-0.08 mg $Cl_2$/L.

Samples of test media were analysed at 0 (fresh media), 24 (old media), 72 (fresh media) and 96 hours (old media). The results obtained are presented below.

**Table A 7: Test item concentrations [mg/L]**

| Nominal test item concentration (% saturated solution) | Measured concentration (mg/L) | | | | Time-weighted mean measured concentration (mg/L) |
|---|---|---|---|---|---|
| | 0 hours (fresh media) | 24 hours (old media) | 72 hours (fresh media)* | 96 hours (old media) | |
| Control | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| 10 | 0.699 | 0.663 | 0.601 | 0.576 | 0.635 |
| 18 | 1.30 | 1.02 | 1.09 | 0.947 | 1.09 |
| 32 | 2.20 | 1.80 | 1.94 | 1.90 | 1.96 |
| 56 | 3.69 | 3.11 | 3.59 | 3.59 | 3.49 |
| 100 | 6.20 | 5.57 | 5.89 | 5.65 | 5.82 |

LOQ = 0.001 mg/L
* Initial batch failed analytical acceptance criteria therefore one set of the 'back-up' samples was analysed alongside the 96-hour samples

The test item concentrations decreased slightly over each 24-hour dosing period. As such, it was considered appropriate to base the results on the time-weighted mean measured test concentrations. These were calculated to be 0.635, 1.09, 1.96, 3.49 and 5.82 mg/L for the 10, 18, 32, 56 and 100% saturated solution test concentrations, respectively.

The measured ratio of the two enantiomers of RH-127450 in the 96-hour test samples was within ±1.1% of the ratio in the CoA of the test item and has therefore not changed during the lifetime of the study.

The fish showed a test item related mortality at 56 and 100 % saturated RH-127450 solution.

**Table A 8: Cumulative mortality of fish**

| Nominal test item concentration (% saturated solution) | No. of fish exposed | Cumulative Mortality | | | |
|---|---|---|---|---|---|
| | | 24 hours | 48 hours | 72 hours | 96 hours |
| Control | 7 | 0 | 0 | 0 | 0 |
| 10 | 7 | 0 | 0 | 0 | 0 |
| 18 | 7 | 0 | 0 | 0 | 0 |
| 32 | 7 | 0 | 0 | 0 | 0 |
| 56 | 7 | 0 | 1 | 1 | 1 |
| 100 | 7 | 3 | 6 | 6 | 7 |

The fish were showing mild to severe toxic effects at 56 and 100% saturated RH-127450 solution, starting with effects like hyperventilation, pigment changes, different swimming positions, showing thereafter abnormal swimming or lying on the bottom of the tank, resulting in dead fish mainly at the top concentration.

Based on time-weighted mean measured substance concentration, the 96-hour $LC_{50}$ value was determined to be 4.17 mg/L. The corresponding No Observed Effect Concentration (NOEC) was determined to be 1.96 mg/L.

The validity criteria for control mortality (≤ 10 %) and dissolved oxygen (≥ 60 % air saturation) were both satisfied. Therefore, the test is valid.

**Table A 9: Acute toxicity of RH-127450 on fish – study endpoints**

| Parameter | Test item concentration (mg/L) Bracketed values present the 95% confidence limits | | | |
|---|---|---|---|---|
| | 24 hours | 48 hours | 72 hours | 96 hours |
| $LC_{50}$ | >5.82 | 4.51 (3.94 – 5.15) | 4.51 (3.94 – 5.15) | 4.17 (3.61 – 4.82) |
| NOEC | 3.49 | 1.96 | 1.96 | 1.96 |

## Conclusion

The acute toxicity of the zoxamide metabolite RH-127450 to *Oncorhynchus mykiss* was tested at application rates of nominally 10, 18, 32, 56 and 100 % saturated solution (measured time-weighted mean test item concentrations of 0.635, 1.09, 1.96, 3.49 and 5.82 mg/L) over 96-hours in a semi-static test design.

Based on time-weighted mean measured substance concentrations, the 96-hour $LC_{50}$ value was determined to be 4.17 mg/L. The corresponding No Observed Effect Concentration (NOEC) is 1.96 mg/L.

(xxx. 2020)

### A 2.2.1.4 Study 4 - RH-163353: Acute toxicity to *Daphnia magna*

EFSA (2017) requested in its Peer Review Conclusion: "*Further data or refinement on aquatic invertebrates (Mysidopsis bahia) are needed to cover the risk for the metabolites RH-127450, RH-24549, RH-163353, RH-141455 and RH-139432 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5).*" To close this data gap, a study on the acute toxicity of RH-163353 on daphnids and mysids is presented.

The following study has been provided to Latvia as RMS for zoxamide and cMSs for interzonal evaluation in July 2020.

| | |
|---|---|
| Comments of zRMS: | The study was not crucial for finalization of the risk assessment, thus was not evaluated by zRMS. |

**Reference:** **KCP 10.2.1/04**

Report: xxx., 2020: RH-163353 Acute toxicity to *Daphnia magna*
Gowan Crop Protection Ltd., UK
xxx, Report No. 3202386, GLP, Unpublished

Guideline(s): OECD 202 (adopted April 13 2004)

Deviations: A temperature deviation was noted during the range finding and definitive test because the continuous temperature vessel variation was >2°C (range finding test = 2.3°C; definitive test = 2.1°C). This slight deviation (protocol specification = within 2°C) was not considered to have had an impact on the integrity of the study.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): No

## Materials and methods

| | |
|---|---|
| **Test material (Batch No.)** | RH-163353 (HHGCP01-00-2) |
| **Purity** | 99.48 % (w/w); enantiomeric ratio 48.6:51.4 (racemate) |
| **Species** | *Daphnia magna* |
| Age: | juveniles, < 24 hours old |
| Number of animals: | 5 / 4 replicates (20) |
| Weight: | not relevant |
| Source: | MicroBioTests Inc., Belgium |
| Acclimation period: | not relevant |
| Feeding: | *Daphnia magna* were not fed during the test. |
| Test vessels: | 100 mL glass beakers, filled with 50 mL test or control medium, in which the test organisms were suspended. |
| **Environmental conditions** | |
| Temperature: | 18.6 – 20.7°C (water) |
| Photoperiod: | 16-hour light, 8-hour dark, with a 30-minute dawn/dusk cycle |
| Test medium: | Elendt M4 |
| pH: | 7.26 – 7.81 |
| Dissolved oxygen: | 103.9 – 106.2% air saturation |

| | |
|---|---|
| **Application rate(s)** | Range-finding test:<br>Nominally 0.10, 1.0, 10 and 100 mg/L (static conditions)<br>Definitive test:<br>Nominally 100 mg/L (static conditions) |
| **Post exposure observation period** | 48 hours |
| **Remarks** | None |

Based on the results of a range finder, the acute toxicity of the zoxamide metabolite RH-163353 to groups of 5 first instar *Daphnia magna* (4 replicates per test item and control) was tested at an application rate of nominally 100 mg/L (limit test) over 48-hours in a static test design. After 24 and 48 hours, the *Daphnia magna* in each test vessel were observed for evidence of immobility. Untreated groups of the freshwater planktonic crustacean (in test medium without test item) served as control.

Concentrations of RH-163353 were determined by mixing the aqueous samples with acetonitrile containing 0.5% formic acid, then diluting further with Elendt: acetonitrile (4:1 v/v) containing 0.1% formic acid to bring the response within the calibration range. Samples were analysed by liquid chromatography-time of flight mass spectrometry (LC-TOF/MS). The method has been validated according to SANCO 3029/99 rev. 4 (SMV 3202386-01V and updated SMV 3202386-02V to include storage stability data). The limit of quantification (LOQ) was 0.001 mg/L.

RH-163353 is a racemate. The analytical method validation for the enantiomeric ratio analysis for RH-163353 was conducted under Smithers ERS Study Number 3202586 (established analytical procedure, SMV 3202586-01V). A combination of the analytical procedures SMV 3202385-01V and SMV 3202586-01V was used to assess the enantiomeric ratio of the test substance in the test medium and calibration standard solution.

## Results and discussion

Environmental parameters remained within acceptable limits throughout the duration of the study (pH 7.26-7.81, temperature 18.6-20.7°C, Dissolved oxygen 103.9–106.2% air saturation).

Analysis of the freshly prepared media at 0 hours and the corresponding old media at 48 hours showed measured concentrations of 104 and 69.8 mg/L, respectively. Given that measured concentrations were within the 80% to 120% of nominal acceptance range, the study results are based on nominal test item concentrations.

**Table A 10: Test item concentrations**

| | **Measured concentration (mg/L)** | | **% nominal** | |
|---|---|---|---|---|
| **Nominal test item concentration (mg/L)** | **0 hours (new media)** | **48 hours (old media)** | **0 hours (new media)** | **48 hours (old media)** |
| Control | < LOQ | < LOQ | -- | -- |
| 100 | 104 | 96.8 | 104 | 93.1 |

LOQ = 0.1 mg/L

RH-163353 is a racemate. The ratio of its two enantiomers has been checked in a 96-hour test sample and in a freshly prepared calibration standard solution. As a result, the isomer ratio of the 96-hour 100 mg/L sample was within ±1.5% of the ratio from the certificate of analysis for the test item batch. Thus, the enantiomeric ratio is deemed to be stable in the *Daphnia* media during the course of the study.

There was no immobilisation of the daphnids in the control and 100 mg/L treatment during the test.

**Table A 11: Toxicity to *Daphnia magna***

| Nominal test item concentration (mg/L) | No. of *Daphnia magna* Exposed | Immobility at 24-hours (%) | Immobility at 48-hours (%) |
|---|---|---|---|
| Control | 20 | 0 | 0 |
| 100 | 20 | 0 | 0 |

Based on nominal concentrations, the 48-hour $EC_{50}$ value was determined to be > 100 mg/L. The corresponding no observed effect concentration (NOEC) is 100 mg/L.

Given that no effects were observed throughout the test, the results were determined empirically.

The validity criteria for control immobility (≤ 10 %) and Dissolved oxygen (≥ 3 mg/L) were met. The test is therefore considered valid.

**Table A 12: Acute toxicity of RH-163353 on *Daphnia* – study endpoints**

| Parameter | Test item concentration (mg/L) | |
|---|---|---|
| | 24 hours | 48 hours |
| $LC_{50}$ | >100 | >100 |
| NOEC | 100 | 100 |

## Conclusion

The acute toxicity of the zoxamide metabolite RH-163353 to *Daphnia magna* was tested at an application rate of nominally 100 mg/L (limit test) over 48-hours in a static test design.

Based on nominal concentrations, the 48-hour $EC_{50}$ value was determined to be > 100 mg/L, respectively. The corresponding no observed effect concentrations (NOEC) is 100 mg/L.

All validity criteria were met. Therefore, the test was considered valid.

(Jarrom R. 2020)

### A 2.2.1.5 Study 5 - RH-141455: Acute toxicity to *Daphnia magna*

EFSA (2017) requested in its Peer Review Conclusion: "*Further data or refinement on aquatic invertebrates (Mysidopsis bahia) are needed to cover the risk for the metabolites RH-127450, RH-24549, RH-163353, RH-141455 and RH-139432 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5).*" To close this data gap, a study on the acute toxicity of RH-141455 on daphnids and mysids is presented.

The following study has been provided to Latvia as RMS for zoxamide and cMSs for interzonal evaluation in July 2020.

| | |
|---|---|
| Comments of zRMS: | The study was not crucial for finalization of the risk assessment, thus was not evaluated by zRMS. |

**Reference:** **KCP 10.2.1/05**

Report xxx, 2020: RH-141455: Acute toxicity to *Daphnia magna*

Gowan Crop Protection Ltd., UK
xxx, Report No. 3202380, GLP, Unpublished

Guideline(s): OECD 202 (adopted April 13 2004)

Deviations: There was no confirmation in the range finding data that the vitamin stock was added to the Elendt M4 media by error. This protocol deviation has no impact on the integrity of the study because the main test was performed without deviation and the immobilisation at all treatments was ≤ 10% - indicating that there was no impact on *Daphnia* if the vitamin stock was not added.
A temperature deviation was noted during the test because the 100 mg/L treatment vessel temperature deviated by 2.2°C from the initial vessel temperature. This slight deviation (protocol specification = within 2°C) was not considered to have had an impact on the integrity of the study, as there was not test substance immobilisation during the test.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) No

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | RH-141455 (A19X08291) |
| **Purity** | 92.77 % (w/w) |
| **Species** | *Daphnia magna* |
| Age: | juveniles, < 24 hours old |
| Number of animals: | 5 / 4 replicates (20) |
| Weight: | not relevant |
| Source: | MicroBioTests Inc., Belgium |
| Acclimation period: | not relevant |
| Feeding: | *Daphnia magna* were not fed during the test |
| Test vessels: | 100 mL glass beakers, filled with 50 mL test or control medium, in which the test organisms were suspended. |
| **Environmental conditions** | |
| Temperature: | 19.6 - 20.2°C (water) |
| Photoperiod: | 16-hour light, 8-hour dark, with a 30-minute dawn/dusk cycle |
| Test medium: | Elendt M4 medium |
| pH: | 6.00 – 7.80 |
| Dissolved oxygen: | 98.8 – 101.5% air saturation |
| **Application rate(s)** | Range-finding test:<br>Nominally 0.10, 1.0, 10 and 100 mg/L (static conditions)<br>Definitive test: |

| | |
|---|---|
| | Nominally 100 mg/L (static conditions) |
| **Post exposure observation period** | 48 hours |
| **Remarks** | None |

Based on the results of a range finder, the acute toxicity of the zoxamide metabolite RH-141455 to groups of 5 first instar *Daphnia magna* (4 replicates per test item and control) was tested at an application rate of nominally 100 mg/L (limit test) over 48-hours in a static test design. After 24 and 48 hours, the *Daphnia magna* in each test vessel were observed for evidence of immobility. Untreated groups of the freshwater planktonic crustacean (in test medium without test item) served as control.

Concentrations of RH-141455 were determined by mixing the aqueous samples with acetonitrile containing formic acid, then diluting further with Elendt: acetonitrile (4:1 v/v) containing 0.1% formic acid to bring the response within the calibration range. Samples were analysed by liquid chromatography-time of flight mass spectrometry (LC-TOF/MS). The method has been validated according to SANCO 3029/99 rev. 4 (SMV 3202380-03V). The limit of quantification (LOQ) was 0.1 mg/L.

## Results and discussion

Environmental parameters remained within acceptable limits throughout the duration of the study (pH 6.00-7.80, temperature 19.6°-20.2°C, dissolved oxygen 98.8-101.5% air saturation).

The analytical results confirm the correct test substance dosing, as the 100 mg/L 0-hour result was within 80 - 120% nominal range (i.e. 93.1%). The test item remained stable throughout the course of the study with a recovery of 110 % of the initially measured concentration. Given that measured concentrations were within the 80% to 120% of nominal acceptance range, the study results are based on nominal test item concentrations.

**Table A 13: Test item concentrations**

| | **Measured concentration (mg/L)** | | **% of initial measured** | |
|---|---|---|---|---|
| **Nominal test item concentration (mg/L)** | **0 hours (new media)** | **48 hours (old media)** | **0 hours (new media)** | **48 hours (old media)** |
| Control | < LOQ | < LOQ | -- | -- |
| 100 | 93.1 | 102 | 93.1 | 110 |

LOQ = 0.1 mg/L

There was no immobilisation of the daphnids in the control and 100 mg/L treatment during the test.

**Table A 14: Toxicity to *Daphnia magna***

| **Nominal test item concentration (mg/L)** | **No. of *Daphnia magna* Exposed** | **Immobility at 24-hours (%)** | **Immobility at 48-hours (%)** |
|---|---|---|---|
| Control | 20 | 0 | 0 |
| 100 | 20 | 0 | 0 |

Based on nominal concentrations, the 48-hour $EC_{50}$ value was determined to be > 100 mg/L. The corresponding no observed effect concentration (NOEC) is 100 mg/L.

Given that no effects were observed throughout the test, the results were determined empirically.

The validity criteria for control immobility (≤ 10 %) and Dissolved oxygen (≥ 3 mg/L) were met. The test is therefore considered valid.

**Table A 15: Acute toxicity of RH-141455 on *Daphnia* – study endpoints**

| Parameter | Test item concentration (mg/L) | |
|---|---|---|
| | 24 hours | 48 hours |
| $LC_{50}$ | >100 | >100 |
| NOEC | 100 | 100 |

## Conclusion

The acute toxicity of the zoxamide metabolite RH-141455 to *Daphnia magna* was tested at an application rate of nominally 100 mg/L (limit test) over 48-hours in a static test design.

Based on nominal concentrations, the 48-hour $EC_{50}$ value was determined to be > 100 mg/L, respectively. The corresponding no observed effect concentrations (NOEC) is 100 mg/L.

All validity criteria were met. Therefore, the test was considered valid.

(Hugill E. 2020)

### A 2.2.1.6 Study 06 - RH-127450: Mysid acute toxicity test

EFSA (2017) requested in its Peer Review Conclusion; "*Further data or refinement on aquatic invertebrates (Mysidopsis bahia) are needed to cover the risk for the metabolites RH-127450, RH-24549, RH-163353, RH-141455 and RH-139432 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5).*" To close this data gap, a study on the acute toxicity of RH-127450 on mysids is presented.

The following study has been provided to Latvia as RMS for zoxamide and cMSs for interzonal evaluation in July 2020.

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OCSPP 850.1035 (2016) and according to the principles of GLP. In the definitive test all validity criteria were met: the control mortality during the test was 5% and the dissolved oxygen range during the test was between 81.9 and 102.7% ASV. The study is considered to be reliable and suitable for the risk assessment. |

**Reference:** **KCP 10.2.1/06**

Report: Hugill, E., 2020: RH-127450: Mysid acute toxicity test
Gowan Crop Protection Ltd., UK
Smithers ERS Ltd., UK, Report No. 3202374, GLP, Unpublished

Guideline(s): OCSPP 850.1035 (2016).

Deviations: During the range finding test, the salinity of the control and highest concentration (100% saturated solution) exceed the ± 1 ppt protocol requirement, as these were measured to be 22 and 18 ppt, respectively at the start of the test. This was not identified at the time in error, however, has no impact as the protocol requirement was tighter than the guideline (± 2 ppt).

Additional Dissolved oxygen concentrations were taken from the treatment vessels where 100% mortality was noted at the Study Director's direction. These were taken to confirm that the high mortality was not related to low oxygen levels, as mysids are very sensitive to oxygen levels. However, this protocol deviation has no impact on the integrity of the study.
The protocol only required statistical analysis to be conducted on the 48 and 96-hour results, but should have included the conduct of statistical analysis on the 24 and 72-hour data, as this is a requirement of the OCSPP guideline.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) No

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | RH-127450 (HHGCP-002-00-1) |
| **Purity** | 99.22 % (w/w)<br>(re-certiffied under GLP: 99.51 % (w/w)) |
| **Species** | *Americamysis bahia* |
| Age: | juveniles, < 24 hours old |
| Number of animals: | 5 / 4 replicates (20) |
| Weight: | not relevant |
| Source: | in-house breeding of the test facility |
| Acclimation period: | not relevant |
| Feeding: | 24-hour old *Artemia sp. Nauplii* |
| Test vessels: | 600 mL glass beakers, filled with 400 mL test or control medium, in which the test organisms were suspended |
| **Environmental conditions** | |
| Temperature: | 25.1-25.6°C (water) |
| Photoperiod: | 16-hour light, 8-hour dark, with a 30-minute dawn/dusk cycle |
| Test medium: | artificial (brackish) seawater<br>The media was prepared by dissolving artificial marine salts (e.g. Tropic Marin®) in reverse osmosis water to provide a salinity of 20 ± 1 ppt (‰). |
| pH: | 7.79 – 8.16 |
| Dissolved oxygen: | 81.9 – 102.7% air saturation |
| Salinity: | 20 – 21 (‰/ppt) |
| **Application rate(s)** | nominally 6.25, 12.5, 25, 50 and 100% saturated solution (static conditions) |
| **Post exposure observation period** | 96 hours |

| | |
|---|---|
| **Remarks** | None |

Based on the results of a range finder, the acute toxicity of the zoxamide metabolite RH-127450 to groups of 5 juvenile *Americamysis bahia,* less than 24 hours old (4 replicates per test item and control) was tested at nominal concentrations of 6.25, 12.5, 25, 50 and 100% saturated solution over 96 hours in a static test design. A control group was also included. After 24, 48, 72 and 96-hours the marine crustacean *Americamysis bahia* was observed for survival.

Analysis of the test media samples was conducted at 0 and 48-hours from freshly prepared media at each treatment and at 48 and 96 hours from pooled old media test vessels at each treatment.

Concentrations of RH-127450 were determined by treating brackish water samples with acetonitrile containing formic acid, then diluting further with brackish water: acetonitrile (4:1, v:v) containing 0.1% formic acid to bring the response within the calibration range. Samples were analysed by liquid chromatography-time of flight mass spectrometry (LC-TOF/MS). The method has been validated according to SANCO 3029/99 rev. 4 (SMV 3202374-01V (later updated SMV 3202374-02V to add the stability information). The limit of quantification (LOQ) was 0.01 mg/L.

## Results and discussion

Environmental parameters remained within acceptable limits throughout the duration of the study (pH 7.79-8.16, temperature 25.1°-25.6°C, Dissolved oxygen 81.9 – 102.7% air saturation).

The analytical results demonstrated that the final definitive test was dosed at the following concentrations: 0.462, 0.859, 1.77, 3.82 and 7.46 mg/L for the 6.25, 12.5, 25, 50 and 100% saturated solution concentrations, respectively. The test item remained stable throughout the course of the study with a recovery of 99.1 - 114% of the initial measured concentrations. As the test solutions were prepared by saturated solution, the study results are based on arithmetic mean measured concentrations (0.460, 0.910, 1.86, 3.99 and 7.71 mg/L for the 6.25, 12.5, 25, 50 and 100% saturated solution concentrations, respectively).

The control and test item solutions were observed to be colourless throughout the during the duration of the test.

**Table A 16: Test item concentrations**

| Nominal concentration (% saturated solution) | Sample analysed | Mean measured concentration (mg/L) | | Arithmetic mean measured concentration (mg/L) |
|---|---|---|---|---|
| | | 0 hours (new media) | 96 hours (old media) | |
| Control | A | < LOD | < LOQ | < LOQ |
| 6.25 | A | 0.462 | 0.458 | 0.460 |
| 12.5 | A | 0.713 | 0.954 | 0.910 |
| | B1* | 0.855 | 0.932 | |
| | B2* | 1.010 | 0.994 | |
| | Mean | 0.859 | 0.960 | |
| 25 | A | 1.77 | 1.94 | 1.86 |
| 50 | A | 3.82 | 4.15 | 3.99 |
| 100 | A | 7.46 | 7.96 | 7.71 |

LOQ = 0.01 mg/L, LOD = 0.0001 mg/L

* The 0 and 96-hour "B" samples were analysed to confirm the test concentrations at this treatment, as the initial "A" sample results for 0 and 96-hour were quite different (0-hour results were *ca* 25% lower than the 96-hour results). As all the three results were relatively close, the mean measured concentration has been calculated using all three results at each time point.

The toxicity results are summarised in the following table.

**Table A 17: Percentage mortality**

| Nominal concentration (% saturated solution) | Mean measured concentration (mg/L) | Percent mortality (%) | | | |
|---|---|---|---|---|---|
| | | 24-hours | 48-hours | 72-hours | 96-hours |
| Control | Control | 5 | 5 | 5 | 5 |
| 6.25 | 0.460 | 5 | 5 | 5 | 5 |
| 12.5 | 0.910 | 10 | 10 | 15 | 15 |
| 25 | 1.86 | 0 | 10 | 10 | 10 |
| 50 | 3.99 | 35[1] | 75[3] | 75 | 75 |
| 100 | 7.71 | 40[2] | 100 | 100 | 100 |

[1] Two surviving mysids at this treatment appeared confused and were swimming very fast in circles
[2] Twelve surviving mysids at this treatment were observed to be lethargic
[3] Two surviving mysids at this treatment were observed to be lethargic

Statistical analysis was performed using the CETIS program v 1.8.6.8. The 24-hour $LC_{50}$ values were calculated using a Linear Interpolation (ICPIN). The 48, 72 and 96-hour $LC_{50}$ values were calculated using untrimmed Spearman Kärber. Where possible the 95% confidence limits were calculated. The 24, 48, 72 and 96-hour no observed effect concentration (NOEC) was calculated using the Cochran-Armitage Trend Step-Down test. Where applicable, outliers according to Grubbs were considered.

**Table A 18: Acute toxicity of RH-127450 on *Americamysis bahia* – study endpoints**

| Parameter | Mean measured concentration (mg/L) Bracketed values present the 95% confidence limits | | | |
|---|---|---|---|---|
| | 24-hour | 48-hour | 72-hour | 96-hour |
| $LC_{50}$ | >7.71 (NC) | 3.05 (2.56 – 3.62) | 2.93 (2.43 – 3.53) | 2.93 (2.43 – 3.53) |
| NOEC | 1.86 | 1.86 | 1.86 | 1.86 |

The validity criteria for control mortality (≤10%) and Dissolved oxygen (60 – 105% air saturation value) were both met. The test is therefore considered valid

## Conclusion

The acute toxicity of the zoxamide metabolite RH-127450 to *Americamysis bahia* was tested at nominal concentrations of 6.25, 12.5, 25, 50 and 100% saturated solution over 96 hours in a static test design.

The test substance remained stable throughout the course of the study. However, since the test solutions were prepared by saturated solution, the study endpoints are based on arithmetic mean measured concentrations.

Based on mean measured concentrations, the 96-hour $LC_{50}$ value was determined at 2.93 mg/L, the 96-hour NOEC at 1.86 mg/L.

All validity criteria were met. Therefore, the test was considered valid.

(Hugill E. 2020)

### A 2.2.1.7 Study 07 - RH-24549: Mysid acute toxicity test

EFSA (2017) requested in its Peer Review Conclusion "*Further data or refinement on aquatic invertebrates (Mysidopsis bahia) are needed to cover the risk for the metabolites RH-127450, RH-24549, RH-163353, RH-141455 and RH-139432 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5).*" To close this data gap, a study on the acute toxicity with RH-24549 on mysids is presented.

The following study has been provided to Latvia as RMS for zoxamide and cMSs for interzonal evaluation in July 2020.

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OCSPP 850.1035 (2016) and according to the principles of GLP. In the definitive test all validity criteria were met: the control mortality during the test was 0% and the dissolved oxygen range during the test was between 70 and 99% ASV. The study is considered to be reliable and suitable for the risk assessment. |

**Reference:** **KCP 10.2.1/07**

Report: Hugill, E., 2020: RH-24549: Mysid acute toxicity test
Gowan Crop Protection Ltd., UK
Smithers ERS Ltd., UK, Report No. 3202394, GLP, Unpublished

Guideline(s): OCSPP 850.1035: Mysid Acute Toxicity Test

Deviations: The test media was prepared using reverse osmosis water rather than deionised water as per the definitive protocol. This protocol deviation has no impact on the integrity of the study because reverse osmosis water is acceptable according to OCSPP 850.1035.
The salinity in the highest concentration dropped to 18 ppt (protocol requirement = 20±1 ppt during the test). This protocol deviation has no impact on the integrity of the study because salinity at 20±2 ppt is acceptable according to OCSPP 850.1035.
The protocol required statistical analysis to be conducted on the 48 and 96-hour results. However, it should have included additional statistical analysis on the 24 and 72-hour data. This protocol deviation has no impact on the integrity of the study since the necessary calculations have been performed.
A protocol deviation occurred at the highest concentration, because at 0-hours the pH in one test vessel was below pH 7.5 (allowed range: pH 7.5-8.5). This pH deviation was considered to have not an impact on the integrity of the study, because the 50% lethal effects concentration was between 25-50 mg/L where the pH measurements were within the acceptable range.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): No

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | RH-24549 (FCC25806) |
| **Purity** | 99.59 % (w/w) |

| | |
|---|---|
| **Species** | *Americamysis bahia* |
| Age: | Juveniles, < 24 hours old |
| Number of animals: | 5 / 4 replicates (20) |
| Weight: | not relevant |
| Source: | in-house breeding of the test facility |
| Acclimation period: | not relevant |
| Feeding: | 24-hour old *Artemia sp. Nauplii* |
| Test vessels: | 600 mL glass beakers, filled with 400 mL test or control medium, in which the test organisms were suspended |
| **Environmental conditions** | |
| Temperature: | 24.6-25.6°C (water) |
| Photoperiod: | 16-hour light, 8-hour dark, with a 30-minute dawn/dusk cycle |
| Test medium: | artificial (brackish) seawater<br>The media was prepared by dissolving artificial marine salts (e.g. Tropic Marin®) in reverse osmosis water to provide a salinity of 20 ± 1 ppt (‰). |
| pH: | 7.25 – 8.09 |
| Dissolved oxygen: | 70.5 – 99.0% air saturation |
| Salinity: | 18 – 21 (‰/ppt) |
| **Application rate(s)** | nominally 6.25, 12.5, 25, 50 and 100 mg/L (static conditions) |
| **Post exposure observation period** | 96 hours |
| **Remarks** | None |

Based on the results of a range finder, the acute toxicity of the zoxamide metabolite RH-24549 to groups of 5 juvenile *Americamysis bahia*, less than 24 hours old (4 replicates per test item and control) was tested at nominal concentrations of 6.25, 12.5, 25, 50 and 100 mg/L. A control group was also included. After 24, 48, 72 and 96-hours the marine crustacean *Americamysis bahia* was observed for survival.

Analysis of the test media samples was conducted at 0 and 48-hours from freshly prepared media at each treatment and at 48 and 96 hours from pooled old media test vessels at each treatment.

Concentrations of RH-24549 were determined by treating brackish water samples with acetonitrile containing formic acid, then diluting further with brackish water: acetonitrile (4:1, v/v) containing 0.1% formic acid to bring the response within the calibration range. Samples were analysed by liquid chromatography-time of flight mass spectrometry (LC-TOF/MS) or triple quad mass spectrometry (LC-TQMS/MS). The method has been validated according to SANCO 3029/99 rev. 4 (SMV 3202394-02V, later updated to SMV 3202394-05V to include stability data) The limit of quantification (LOQ) was 0.1 mg/L.

## Results and discussion

Environmental parameters remained within acceptable limits throughout the duration of the study (pH 7.25 to 8.09, temperature 24.6 - 25.6°C, Dissolved oxygen 70.5 to ~~1~~99.0% air saturation).

The analytical results demonstrated that the test substance was dosed correctly because with exception to the 25 mg/L treatment (77.2% of nominal) the initial measured concentrations (0-hour) were within the 80 – 120% nominal range (86.7 - 94.8%). The test item remained stable throughout the course of the study with a recovery of 91.0 - 109% of initial measured concentrations. As the 25 mg/L treatment was not within the 80-120% nominal range, the study results are based on arithmetic mean measured concentrations.

**Table A 19: Test item concentrations**

| Nominal test item concentration (mg/L) | Measured concentration (mg/L) | | | Recovery | |
|---|---|---|---|---|---|
| | 0 hours (new media) | 96 hours (old media) | Arithmetic Mean | % of nominal concentration | % of initial measured concentration |
| Control | <LOQ | <LOQ | <LOQ | -- | -- |
| 6.25 | 5.42 | 5.92 | 5.67 | 86.7 | 109 |
| 12.5 | 11.1 | 11.4 | 11.3 | 88.8 | 103 |
| 25 | 19.3 | 21.1 | 20.2 | 77.2 | 109 |
| 50 | 47.4 | 44.8 | 46.1 | 94.8 | 94.5 |
| 100 | 92.8 | 84.4 | 88.6 | 92.8 | 91.0 |

LOQ = 0.1 mg/L

The toxicity results are summarised in the following table.

**Table A 20: Percentage mortality**

| Nominal concentration (mg/L) | Mean measured concentration (mg/L) | Percent mortality (%) | | | |
|---|---|---|---|---|---|
| | | 24-hours | 48-hours | 72-hours | 96-hours |
| Control | Control | 0 | 0 | 0 | 0 |
| 6.25 | 5.67 | 0 | 0 | 0 | 0 |
| 12.5 | 11.3 | 0 | 5 | 5 | 10 |
| 25 | 20.2 | 0 | 10 | 35 | 35 |
| 50 | 46.1 | 20 | 40 | 95 | 100 |
| 100 | 88.6 | 100 | 100 | 100 | 100 |

Statistical analysis was performed using the CETIS program v 1.8.6.8. The 24, 48, 72 and 96-hour $LC_{50}$ values were calculated using untrimmed Spearman Kärber. Where possible the 95% confidence limits were calculated. The 24, 48, 72 and 96-hour no observed effect concentration (NOEC) was calculated using the Cochran-Armitage Trend Step-Down test.

**Table A 21: Acute toxicity of RH-24549 on *Americamysis bahia* – study endpoints**

| Parameter | Mean measured concentration (mg/L) Bracketed values present the 95% confidence limits | | | |
|---|---|---|---|---|
| | 24-hour | 48-hour | 72-hour | 96-hour |
| $LC_{50}$ | 55.1 (48.3 – 62.9) | 42.9 (35.3 – 52.3) | 24.0 (20.1 – 28.6) | 23.2 (19.3 – 28.0) |
| NOEC | 20.2 | 11.3 | 11.3 | 5.67 |

The validity criteria for control mortality (≤10%) and Dissolved oxygen (60 – 105% air saturation value) were both met. The test is therefore considered valid

## Conclusion

The acute toxicity of the zoxamide metabolite RH-24549 to *Americamysis bahia* was tested at nominal concentrations of 6.25, 12.5, 25, 50 and 100 mg/L over 96 hours in a static test design.

The test substance remained stable throughout the course of the study. However, since one application rate was not within the 80-120% nominal range, the study results are based on arithmetic mean measured concentrations.

Based on mean measured concentrations, the 96-hour $LC_{50}$ value was determined at 23.2 mg/L, the corresponding 96-hour NOEC values at 5.67.

All validity criteria were met. Therefore, the test was considered valid.

(Hugill E. 2020)

### A 2.2.1.8 Study 08 - RH-139432: Mysid acute toxicity test

EFSA (2017) requested in its Peer Review Conclusion: *"Further data or refinement on aquatic invertebrates (Mysidopsis bahia) are needed to cover the risk for the metabolites RH-127450, RH-24549, RH-163353, RH-141455 and RH-139432 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5)."* To close this data gap, a study on the acute toxicity of RH-139432 on mysids is presented.

The following study has been provided to Latvia as RMS for zoxamide and cMSs for interzonal evaluation in July 2020.

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OCSPP 850.1035 (2016) and according to the principles of GLP. In the definitive test all validity criteria were met: the control mortality during the test was 10% and the dissolved oxygen range during the test was between 87.1 and 102.5% ASV. The study is considered to be reliable and suitable for the risk assessment. |

| | |
|---|---|
| **Reference:** | **KCP 10.2.1/08** |
| Report | Hugill, E., 2020: RH-139432: Mysid acute toxicity test<br>Gowan Crop Protection Ltd., UK<br>Smithers ERS Ltd., UK, Report No. 3202398, GLP, Unpublished |
| Guideline(s): | OCSPP 850.1035 (2016) |
| Deviations: | The test media was prepared using reverse osmosis water rather than deionised water as per the definitive protocol. This protocol deviation has no impact on the integrity of the study because reverse osmosis water is acceptable according to OCSPP 850.1035.<br>The study protocol requested statistical analysis only for the 48 and 96-hour results. However, according to OCSPP the protocol should also have been mentioned statistical evaluation of the 24 and 72-hour data, what has finally been performed and reported. |
| GLP: | Yes |

Acceptability: Yes

Duplication (if vertebrate study) No

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | RH-139432 (HHGCP005-00-1) |
| **Purity** | 99.01 % (w/w)<br>(re-certified under GLP: 99.35 % (w/w)) |
| **Species** | *Americamysis bahia* |
| Age: | juveniles, < 24 hours old |
| Number of animals: | 5 / 4 replicates (20) |
| Weight: | not relevant |
| Source: | in-house breeding of the test facility |
| Acclimation period: | not relevant |
| Feeding: | 24-hour old *Artemia sp. Nauplii* |
| Test vessels: | 600 mL glass beakers, filled with 400 mL test or control medium, in which the test organisms were suspended |
| **Environmental conditions** | |
| Temperature: | 24.9-25.5°C |
| Photoperiod: | 16-hour light, 8-hour dark, with a 30-minute dawn/dusk cycle |
| Test medium: | artificial (brackish) seawater<br>The media was prepared by dissolving artificial marine salts (e.g. Tropic Marin®) in reverse osmosis water to provide a salinity of 20 ± 1 ppt (‰). |
| pH: | 7.95 – 8.19 |
| Dissolved oxygen: | 87.2 – 102.5% air saturation |
| Salinity: | 19 – 21 (‰/ppt) |
| **Application rate(s)** | nominally 1.56, 3.125, 6.25, 12.5, 25 and 50% saturated solution (static conditions) |
| **Post exposure observation period** | 96 hours |
| **Remarks** | The mortality results for the initial definitive test suggested that the 96 hour $LC_{50}$ value would be between the 6.25 and 12.5% saturated solution and that the NOEC below 6.25 % saturated solution. The mortality results of an initial definitive test (with several protocol deviations) suggested that the 96-hour $LC_{50}$ value would be between the 6.25 and 12.5% saturated solution and the NOEC below 6.25 % saturated solution (the lowest concentration tested). Therefore, a second and final definitive test has been performed at refined application rates. |

Based on the results of the range-finding and initial definitive test, the acute toxicity of the zoxamide metabolite RH-139432 to groups of 5 juvenile *Americamysis bahia,* less than 24 hours old (4 replicates per test item and control) was tested at final nominal concentrations of 1.56, 3.125, 6.25, 12.5, 25 and 50% saturated solution over 96 hours in a static test design. A control group was also included. After 24, 48, 72 and 96-hours the marine crustacean *Americamysis bahia* was observed for survival.

Analysis of the test media samples was conducted at 0 and 48-hours from freshly prepared media at each treatment and at 48 and 96 hours from pooled old media test vessels at each treatment.

Concentrations of RH-139432 were determined by treating brackish water samples with acetonitrile containing 0.5% formic acid, then diluting further with brackish water: acetonitrile (4:1, v:v) containing 0.1% formic acid as required to bring the response within the calibration range. Samples were analysed by liquid chromatography-time of flight mass spectrometry (LC-TOF/MS) or triple quadrupole mass spectrometry (LC-TQMS). The method has been validated according to SANCO 3029/99 rev. 4 (SMV 3202398-02V). The limit of quantification (LOQ) was 0.1 mg/L.

## Results and discussion

Environmental parameters remained within acceptable limits throughout the duration of the study (pH 7.95-8.19, temperature 24.6-25.5°C, Dissolved oxygen 87.2-102.5% air saturation).

The analytical results demonstrated that the final definitive test was dosed at the following concentrations: 0.909, 1.78, 3.63, 7.24, 14.4 and 29.8 mg/L for the 1.56, 3.13, 6.25, 12.5, 25 and 50% saturated solution concentrations, respectively. The test item remained stable throughout the course of the study with a recovery of 95 - 101% of the initial measured concentrations. As the test solutions were prepared by saturated solution, the study results have been based on arithmetic mean measured concentrations.

The control and test item solutions were observed to be colourless throughout the during the duration of the test.

**Table A 22: Test item concentrations**

| Nominal concentration (% saturated solution) | Mean measured concentration (mg/L) | | Arithmetic mean measured concentration (mg/L) | % of initial measured concentration |
|---|---|---|---|---|
| | 0 hours (new media) | 96 hours (old media) | | |
| Control | < LOD | < LOQ | < LOQ | -- |
| 1.56 | 0.909 | 0.862 | 0.886 | 94.8 |
| 3.125 | 1.78 | 1.690 | 1.74 | 94.9 |
| 6.25 | 3.63 | 3.55 | 3.59 | 97.8 |
| 12.5 | 7.24 | 7.16 | 7.20 | 98.9 |
| 25 | 14.4 | 14.5 | 14.5 | 101 |
| 50 | 29.8 | 28.7 | 29.3 | 96.3 |

LOQ = 0.1 mg/L

The toxicity results are summarised in the following tables. For completeness, the results of the first and of the final definitive test are given.

**Table A 23: Percentage mortality of the <u>initial definitive test</u>**

| Nominal concentration (% saturated solution) | Percent mortality (%) | | | |
|---|---|---|---|---|
| | 24-hours | 48-hours | 72-hours | 96-hours |
| Control | 0 | 5 | 5 | 5 |

| | | | | |
|---|---|---|---|---|
| 6.25 | 10 | 30 | 35 | 35 |
| 12.5 | 20 | 50 | 55 | 55 |
| 25 | 20 | 75 | 95 | 100 |
| 50 | 85 | 100 | 100 | 100 |
| 100 | 100 | 100 | 100 | 100 |

**Table A 24: Percentage mortality of the final definitive test**

| Nominal concentration (% saturated solution) | Mean measured concentration (mg/L) | Number of *Americamysis bahia* exposed | Percent mortality (%) | | | |
|---|---|---|---|---|---|---|
| | | | 24-hours | 48-hours | 72-hours | 96-hours |
| Control | Control | 20 | 10 | 10 | 10 | 10 |
| 1.56 | 0.886 | 20 | 0 | 0 | 0 | 0 |
| 3.125 | 1.74 | 20 | 0 | 0 | 0 | 0 |
| 6.25 | 3.59 | 20 | 5 | 5 | 5 | 5 |
| 12.5 | 7.20 | 20 | 5 | 45 | 45 | 55 |
| 25 | 14.5 | 20 | 30 | 80 | 100 | 100 |
| 50 | 29.3 | 20 | 100 | 100 | 100 | 100 |

Statistical analysis was performed using the CETIS program v 1.8.6.8. The 24, 48, 72 and 96-hour $LC_{50}$ values were calculated using untrimmed Spearman Kärber. Where possible the 95% confidence limits were calculated. The 24, 48, 72 and 96-hour no observed effect concentration (NOEC) was calculated using the Cochran-Armitage Trend Step-Down test.

**Table A 25: Acute toxicity of RH-139432 on *Americamysis bahia* – study endpoints**

| Parameter | Mean measured concentration (mg/L)<br>Bracketed values present the 95% confidence limits | | | |
|---|---|---|---|---|
| | 24-hour | 48-hour | 72-hour | 96-hour |
| $LC_{50}$ | 16.6<br>(14.2 – 19.3) | 8.64<br>(7.04 – 10.6) | 7.47<br>(6.37 – 8.77) | 6.95<br>(5.92 – 8.17) |
| NOEC | 7.20 | 3.59 | 3.59 | 3.59 |

The validity criteria for control mortality (≤10%) and Dissolved oxygen (60 – 105% air saturation value) were both met. The test is therefore considered valid

## Conclusion

The acute toxicity of the zoxamide metabolite RH-139432 to *Americamysis bahia* was tested at nominal concentrations of 1.56, 3.125, 6.25, 12.5, 25 and 50% saturated solution over 96 hours in a static test design.

The test substance remained stable throughout the course of the study. However, since the test solutions were prepared by saturated solution, the study endpoints are based on arithmetic mean measured concentrations.

Based on mean measured concentrations, the 96-hour $LC_{50}$ value was determined at 6.95 mg/L, the 96-hour NOEC at 3.59 mg/L.

All validity criteria were met. Therefore, the test was considered valid.

(Hugill E. 2020)

### A 2.2.1.9 Study 09 - RH-163353: Mysid acute toxicity test

EFSA Peer Review Conclusion (2017) requested "*Further data or refinement on aquatic invertebrates (Mysidopsis bahia) are needed to cover the risk for the metabolites RH-127450, RH-24549, RH-163353, RH-141455 and RH-139432 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5).*" To close this data gap, a study on the acute toxicity of RH-163353 on daphnids and mysids is presented.

The following study has been provided to Latvia as RMS for zoxamide and cMSs for interzonal evaluation in July 2020.

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OCSPP 850.1035 (2016) and according to the principles of GLP. In the definitive test all validity criteria were met: the control mortality during the test was 0% and the dissolved oxygen range during the test was between 85.4 and 101% ASV. The study is considered to be reliable and suitable for the risk assessment. |

**Reference:** **KCP 10.2.1/09**

Report: Jarrom, R., 2020: RH-163353: Mysid acute toxicity test
Gowan Crop Protection Ltd., UK
Smithers ERS Ltd., UK, Report No. 3202387, GLP, Unpublished

Guideline(s): OCSPP 850.1035 (2016)

Deviations: The measured pH of the 100 mg/L solution at test initiation was 7.37 (0.13 outside of the specified range of 7.5 – 8.5), pH adjustment was not made. This protocol deviation has no impact on the integrity of the study as no mortality was seen in the 100 mg/L replicates, demonstrating no negative effects on the test organisms.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): No

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | RH-163353 (HHGCP001-00-2) |
| **Purity** | 99.48 % (w/w); enantiomeric ratio 48.6:51.4 (racemate) |
| **Species** | *Americamysis bahia* |
| Age: | juveniles, < 24 hours old |
| Number of animals: | 5 / 4 replicates (20) |
| Weight: | not relevant |
| Source: | in-house breeding of the test facility |
| Acclimation period: | not relevant |

| | |
|---|---|
| Feeding: | 24-hour old *Artemia sp. Nauplii* |
| Test vessels: | 600 mL glass beakers, filled with 400 mL test or control medium, in which the test organisms were suspended |
| **Environmental conditions** | |
| Temperature: | 24.3-25.8°C (water) |
| Photoperiod: | 16-hour light, 8-hour dark, with a 30-minute dawn/dusk cycle |
| Test medium: | artificial (brackish) seawater<br>The media was prepared by dissolving artificial marine salts (e.g. Tropic Marin®) in reverse osmosis water to provide a salinity of 20 ± 1 ppt (‰). |
| pH: | 7.37 – 7.97 |
| Dissolved oxygen: | 85.4 – 101% air saturation |
| Salinity: | 20 – 21 (‰/ppt) |
| **Application rate(s)** | nominally 6.25, 12.5, 25, 50 and 100 mg/L (static test conditions) |
| **Post exposure observation period** | 96 hours |
| **Remarks** | None |

Based on the results of a range finder, the acute toxicity of the zoxamide metabolite RH-163353 to groups of 5 juvenile *Americamysis bahia,* less than 24 hours old (4 replicates per test item and control) was tested at nominal concentrations of 6.25, 12.5, 25, 50 and 100 mg/L. A control group was also included. After 24, 48, 72 and 96-hours the marine crustacean *Americamysis bahia* was observed for survival.

Analysis of the test media samples was conducted at 0 and 48-hours from freshly prepared media at each treatment and at 48 and 96 hours from pooled old media test vessels at each treatment.

Concentrations of RH-163353 were determined by treating brackish water samples with acetonitrile containing 0.5% formic acid, then diluting further with brackish water: acetonitrile (4:1, v/v) containing 0.1% formic acid to bring the response within the calibration range. Samples were analysed by liquid chromatography-time of flight mass spectrometry (LC-TOF/MS). The method has been validated according to SANCO 3029/99 rev. 4 (SMV 3202387-01V, later updated SMV 3202387-02V to add stability information). The limit of quantification (LOQ) was 0.1 mg/L.

RH-163353 is a racemate. The 50:50 ratio of its enantiomers in the test media has been analytically verified using method SMV 3202387-01V.

## Results and discussion

Environmental parameters remained within acceptable limits throughout the duration of the study (pH 7.37-7.97, temperature 24.3°-25.8°C, Dissolved oxygen 85.4 to 101% air saturation).

The analytical results demonstrated that the test substance was dosed correctly because the initial measured concentrations (0-hour) were within the 80 – 120% nominal range (i.e. 93.5 - 100%). The test item remained stable throughout the course of the study with a recovery of 95.5 – 99.1% of initial measured concentrations. Since the analysed test item concentrations in the new and old media samples stayed within their nominal ranges (80 - 120%) throughout the test, the study results are based on nominal concentrations.

RH-163353 is a racemate. The 50:50 ratio of its enantiomers in the test media has been analytically verified in old media samples and compared to the validated enantiomeric ratio provided in the certificate of analysis for the test substance. As a result, the measured ratio of the 96-hours samples were within 2% (i.e. ≤1.13%) of the validated enantiomeric ratio and so was not deemed to have changed during the course of the study.

**Table A 26: Test item concentrations**

| Nominal test item concentration (mg/L) | Measured concentration (mg/L) | | Recovery | |
|---|---|---|---|---|
| | 0 hours (new media) | 96 hours (old media) | % of nominal concentration | % of initial measured concentration |
| Control | <LOQ | <LOQ | -- | -- |
| 6.25 | 5.84 | 5.79 | 93.5 | 99.1 |
| 12.5 | 12.5 | 12.0 | 100 | 95.5 |
| 25 | 24.3 | 23.9 | 97.0 | 98.6 |
| 50 | 47.8 | 46.2 | 95.5 | 96.8 |
| 100 | 97.7 | 96.2 | 97.7 | 98.5 |

LOQ = 0.1 mg/L

The toxicity results are summarised in the following table.

**Table A 27: Percentage mortality**

| Nominal concentration (mg/L) | Percent mortality (%) | | | |
|---|---|---|---|---|
| | 24-hours | 48-hours | 72-hours | 96-hours |
| Control | 0 | 0 | 0 | 0 |
| 6.25 | 0 | 0 | 0 | 0 |
| 12.5 | 0 | 0 | 0 | 0 |
| 25 | 5 | 5 | 5 | 5 |
| 50 | 0 | 0 | 0 | 0 |
| 100 | 0 | 0 | 0 | 0 |

As there was no mortality up to and including the test item application rate of 100 mg/L the $LC_{50}$ is reported as being greater than the highest test concentration. The 24, 48, 72 and 96-hour no observed effect concentration (NOEC) was determined empirically.

**Table A 28: Acute toxicity of RH-163353 on *Americamysis bahia* – study endpoints**

| Parameter | Mean measured concentration (mg/L) | | | |
|---|---|---|---|---|
| | 24-hour | 48-hour | 72-hour | 96-hour |
| $LC_{50}$ | >100 | >100 | >100 | >100 |
| NOEC | 100 | 100 | 100 | 100 |

The validity criteria for control mortality (≤10%) and Dissolved oxygen (60 – 105% air saturation value) were both met. The test is therefore considered valid

## Conclusion

The acute toxicity of the zoxamide metabolite RH-163353 to *Americamysis bahia* was tested at nominal concentrations of 6.25, 12.5, 25, 50 and 100 mg/L over 96 hours in a static test design.

Since the analysed test item concentrations in the new and old media samples stayed within their nominal ranges (80 - 120%) throughout the test, the study results were based on nominal concentrations. The 96-hour $LC_{50}$ value was determined at >100 mg/L, the corresponding 96-hour NOEC values at 100 mg/L.

RH-163353 is a racemate. Its 50:50 ratio during the course of the study in the test media has been analytically verified.

All validity criteria were met. Therefore, the test was considered valid.

(Jarrom R. 2020)

### A 2.2.1.10 Study 10 - RH-141455: Mysid acute toxicity test

EFSA (2017) requested in its Peer Review Conclusion: "*Further data or refinement on aquatic invertebrates (Mysidopsis bahia) are needed to cover the risk for the metabolites RH-127450, RH-24549, RH-163353, RH-141455 and RH-139432 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5).*" To close this data gap, a study on the acute toxicity of RH-141455 on daphnids and mysids is presented.

The following study has been provided to Latvia as RMS for zoxamide and cMSs for interzonal evaluation in July 2020.

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OCSPP 850.1035 (2016) and according to the principles of GLP. In the definitive test all validity criteria were met: the control mortality during the test was 10% and the dissolved oxygen range during the test was between 84.6 and 102.5% ASV. The study is considered to be reliable and suitable for the risk assessment. |

| | |
|---|---|
| **Reference:** | **KCP 10.2.1/10** |
| Report | Hugill, E., 2020: RH-141455: Mysid acute toxicity test<br>Gowan Crop Protection Ltd., UK<br>Smithers ERS Limited, UK, Report No. 3202381, GLP, Unpublished |
| Guideline(s): | OSPP 850.1035: Mysid Acute Toxicity Test |
| Deviations: | Only some minor deviations during the range-finder, which are considered to be not relevant for the integrity of the (main) study results. |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication<br>(if vertebrate study) | No |

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | RH-141455 (A19X08291) |
| **Purity** | 92.77 % (w/w) |

| | |
|---|---|
| **Species:** | *Americamysis bahia* |
| Age: | juveniles, < 24 hours old |
| Number of animals: | 5 / 4 replicates (20) |
| Weight: | not relevant |
| Source: | in-house breeding of the test facility |
| Acclimation period: | not relevant |
| Feeding: | 24-hour old *Artemia sp. Nauplii* |
| Test vessels: | 600 mL glass beakers, filled with 400 mL test or control medium, in which the test organisms were suspended |
| **Environmental conditions** | |
| Temperature: | 24.1–25.2°C |
| Photoperiod: | 16-hour light, 8-hour dark, with a 30-minute dawn/dusk cycle |
| Test medium: | artificial (brackish) seawater<br>The media was prepared by dissolving artificial marine salts (e.g. Tropic Marin®) in reverse osmosis water to provide a salinity of 20 ± 1 ppt (‰). |
| pH: | 6.53–7.97 |
| Dissolved oxygen: | 87.4–102.5 air saturation |
| Salinity: | 20-22 ‰/ppt |
| **Application rate(s)** | Range-finding test:<br>Nominally 0.10, 1.0, 10 and 100 mg/L (static conditions)<br>Definitive test:<br>Nominally 100 mg/L (limit test; semi-static conditions) |
| **Post exposure observation period** | 96 hours |
| **Remarks** | Since the test substance was found to have dropped the pH to below pH 7.5 at the 100 mg/L application rate, the range-finding test has been performed with and without pH adjustment at the 100 mg/L application rate. However, pH adjustment was finally regarded to be not necessary, and therefore the main test was performed without pH adjustment.<br>An initial definitive/limit test had to be stopped after 24-hours due to high control mortality (>10%). |

Based on the results of a range finder, the acute toxicity of the zoxamide metabolite RH-141455 to groups of 5 juvenile *Americamysis bahia*, less than 24 hours old (4 replicates per test item and control) was tested under semi-static conditions (one renewal at 48-hours) at a nominal concentration of 100 mg/L (limit test). A control group was also included. After 24, 48, 72 and 96-hours the marine crustacean *Americamysis bahia* was observed for survival.

Analysis of the test media samples was conducted at 0 and 48-hours from freshly prepared media at each treatment and at 48 and 96 hours from pooled old media test vessels at each treatment.

Concentrations of RH-141455 were determined by treating brackish water samples with Milli-Q water/acetonitrile (8:2, v:v) containing 0.1% formic acid, then diluting with brackish water/Milli-Q water/acetonitrile (1:8:2, v:v) containing 0.1% formic acid) as required to bring the response within the calibration range. Samples were analysed by liquid chromatography-time of flight mass spectrometry (LC-TOF/MS). The method has been validated according to SANCO 3029/99 rev. 4 (SMV 3202381-01V, later updated to SMV 3202381-03V to add stability data and update the gradient program). The limit of quantification (LOQ) was 0.25 mg/L.

The results were statistically evaluated using the CETIS program v 1.8.6.8. The 24, 48, 72 and 96-hour no observed effect concentration (NOEC) was estimated using the Equal Variance t Two-Sample test. Outliers were excluded using Grubbs test – in case applicable.

## Results and discussion

Environmental parameters remained within acceptable limits throughout the duration of the study (pH 6.53-7.46, temperature 24.1–25.2°C, Dissolved oxygen 84.6 to 102.3% air saturation).

The analytical results demonstrated that the definitive/limit test was dosed correctly, as the measured concentrations for the fresh 0 and 48-hour samples were 100 and 111% of nominal, respectively. The test item remained stable over the 48-hour renewal period because the measured concentrations in the old media samples at 48 and 96-hour were 102 and 94.6% of the initial measured concentrations, respectively. As the measured concentrations for the new and old media maintained within the nominal range (80 - 120%) throughout the test, the study results were based on nominal test item concentrations.

**Table A 29: Test item concentrations**

| Nominal test item conc. (mg/L) | Measured concentration (mg/L) | | | | | Recovery | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | % of nominal | | % of initial measured | | Mean measured |
| | 0 h (new media) | 48 h (old media) | 48 h (new media) | 96 h (old media) | Mean | 0 h (new media) | 48 h (new media) | 48 h (old media) | 48 h (old media) | % of nominal |
| Control | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | -- | -- | -- | -- | -- |
| 100 | 100 | 102 | 111 | 105 | 105 | 100 | 111 | 102 | 94.6 | 105 |

LOQ = 0.25 mg/L

The toxicity results are summarised in the following table.

**Table A 30: Percentage mortality**

| Nominal concentration (mg/L) | Percent mortality (%) | | | |
|---|---|---|---|---|
| | 24-hours | 48-hours | 72-hours | 96-hours |
| Control | 5 | 10 | 10 | 10 |
| 100 | 5 | 10 | 15 | 15 |

The mortality at the 100 mg/L treatment was shown to be statistically not significant. Therefore, the $LC_{50}$ is reported as being greater than the highest test concentration. The 24, 48, 72 and 96-hour no observed effect concentration (NOEC) was determined empirically.

**Table A 31: Acute toxicity of RH-141455 on *Americamysis bahia* – study endpoints**

| Parameter | Nominal concentration (mg/L) | | | |
|---|---|---|---|---|
| | 24-hour | 48-hour | 72-hour | 96-hour |
| $LC_{50}$ | >100 | >100 | >100 | >100 |
| NOEC | 100 | 100 | 100 | 100 |

The validity criteria for control mortality (≤10%) and Dissolved oxygen (60 – 105% air saturation value) were both met. The test is therefore considered valid

## Conclusion

Based on the results of a range-finder, the acute toxicity of the zoxamide metabolite RH-141455 to *Americamysis bahia* was tested at a nominal concentration of 100 mg/L (limit test) over 96 hours under semi-static conditions (one renewal at 48-hours).

Since the analysed test item concentrations in the new and old media samples stayed within their nominal ranges (80 - 120%) throughout the test, the study results were based on nominal concentrations.

The 96-hour $LC_{50}$ value was determined at >100 mg/L, the corresponding 96-hour NOEC values at 100 mg/L.

All validity criteria were met. Therefore, the test was considered valid.

(Hugill E. 2020)

### A 2.2.1.11 Study 11 - RH-127450: Inhibition of growth to the alga *Raphidocelis subcapitata*

EFSA (2017) requested in its Peer Review Conclusion: "*Further algae studies following the latest OECD 201 guideline are needed or further detailed information on all validity criteria requested by the latest OECD 201 guideline from the studies provided in the RAR for zoxamide, RH-127450 and RH-163353 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5).*" To close this data gap, new studies on the inhibition of alga growth by the zoxamide metabolites RH-127450 and RH-163353 are presented.

With regard to the EFSA request on alga studies with the parent compound zoxamide: The alga studies with zoxamide which are available in the RAR for zoxamide (2017) have been re-evaluated at a later stage during AIR based on additionally provided information. The results of the re-evaluation were included in the RAR, the alga studies were regarded valid. This was confirmed by Latvia as RMS for zoxamide. As such, the endpoints from the alga studies with the active substance zoxamide available in the RAR (2017) are valid and applicable for the aquatic risk assessment.

The following study has been provided to Latvia as RMS for zoxamide and cMSs for interzonal evaluation in July 2020.

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

**Reference: KCP 10.2.1/11**

| | |
|---|---|
| Report | Hugill, E., 2020: RH-127450: Inhibition of growth to the alga *Raphidocelis subcapitata*<br>Gowan Crop Protection Ltd., UK<br>Smithers ERS Ltd., Report No. 3202375, GLP, Unpublished |
| Guideline(s): | OECD 201 (adopted 23 March 2006, corrected 28 July 2011) |
| Deviations: | An initial definitive test was conducted at nominal concentrations of 1, 3.2, 10, 32 and 100% saturated solution. However, it was necessary to repeat the main test due to an equipment failure (lux meter) and since, by error, 24- and 48-hour samples were not taken for analysis.<br>The appearance of the test item in the test media was only observed at the start and at the end of the test. This deviation has no impact on the integrity of the study as the non-inoculated media observation at 72-hours showed the test substance to be in solution. |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | No |

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | RH-127450 (HHGCP-002-00-1) |
| **Purity** | 99.22 % (w/w)<br>(re-certiffied under GLP: 99.51 % (w/w)) |
| **Species** | Alga *Raphidocelis subcapitata* (strain 278/4; formerly known as *Pseudokirchneriella subcapitata)* |
| Age: | not applicable |
| Weight: | not applicable |
| Source: | Culture Collection of Algae and Protozoa (CCAP), SAMS Research Service Ltd., Oban, UK |
| Acclimation period: | 72 hours |
| Test vessel: | 250 mL Erlenmeyer (conical) glass flasks, filled with 100 mL of test or control medium, capped with foam bungs, continuously agitated at ~100 rpm |
| **Environmental conditions** | |
| Test medium: | OECD alga nutrient medium<br>(according to Annex 3 of the OECD TG 201) |
| Temperature: | 22.2 – 23.1°C |
| pH: | 7.95 – 8.21 |
| Light: | Continuous light at 4.66 – 5.53 kLux |
| **Application rate(s)** | 1, 3.2, 10, 32 and 100 % saturated solution under static test conditions |

| | |
|---|---|
| **Post exposure observation period** | 72 hours |
| **Remarks** | None |

Based on the results of a range-finder, three replicate cultures of the green alga *Raphidocelis subcapitata* (strain 278/4; alga formerly known as *Pseudokirchneriella subcapitata*) in an exponential growth phase were exposed to RH-127450 at nominal concentrations of 1, 3.2, 10, 32 and 100 % saturated solution under static conditions over 72 h. A control treatment (6 replicate cultures) was also included in the test. A toxic reference test with potassium dichromate was separately performed in January 2019.

Each test vessel was inoculated with $1 \times 10^4$ algae cells/mL. Algae cell counts were conducted at 24-hour intervals. The test item concentrations were analytically verified at 0, 24, 48 and 72-hours.

Concentrations of RH-127450 were determined by treating OECD medium samples with acetonitrile containing formic acid, then diluting further with OECD medium: acetonitrile (4:1, v/v) containing 0.1% formic acid as required to bring the response within the calibration range. Samples were analysed by injection onto a liquid chromatography-time of flight mass spectrometry (LC-TOF/MS). The method has been validated according to SANCO 3029/99 rev. 4 (SMV 3202375-01V, later updated to SMV 3202337-02V to add stability data). The limit of quantification (LOQ) was 0.01 mg/L.

The results were statistically evaluated using the CETIS program v 1.8.6.8. The no observed effect concentrations for yield and growth rate at all timepoints were determined using a Dunnett multiple comparison test because the data showed a non-monotonic but parametric trend. Linear interpolation analysis was performed in order to estimate $EC_{10}$, $EC_{20}$ and $EC_{50}$ values for the 72-hour final yield and the 0-24, 0-48, 0-72 hours average specific growth rate (μ) time intervals. Where possible, 95% confidence limits were calculated for the $EC_{10}$, $EC_{20}$ and $EC_{50}$ values. Outliers were assessed using Grubbs extreme value – in case applicable.

## Results and discussion

Environmental parameters remained within acceptable limits throughout the duration of the study (pH 7.95–8.21, temperature 22.2 – 23.1°C, light intensity range of 4.66 – 5.53 kLux).

The non-inoculated test media remained colourless throughout the course of the study, the inoculated changed to pale green besides the solution of the highest test item concentration, which was also colourless at the end of the incubation period.

No morphological abnormalities were observed in any of the tested concentrations.

The measured concentrations at the start of the test were 0.0826, 0.269, 0.816, 2.67 and 8.19 mg/L, respectively for the 1, 3.2, 10, 32 and 100% saturated solution concentrations. The test item remained relatively stable over the test period, as with exception of the highest concentration (100% saturated solution) all measured test substance concentrations between 24 and 72-hours were within the 80-120% nominal range (101 – 119% of initial measured). As the test concentrations were saturated solutions and the measured concentrations were not maintained at the 100% saturated solution concentration, the study results were based on geometric mean measured concentrations.

**Table A 32: Test item concentrations**

| **Nominal test item conc. (% saturated solution)** | **Measured concentration (mg/L)** | **Recovery (% of initial measured) Inoculated** | | | **Recovery (% of initial measured) non inoculated** | **Geometric mean measured concentration (mg/L)** |
|---|---|---|---|---|---|---|
| | **at 0 hours** | **at 24 hours** | **at 48 hours** | **at 72 hours** | **at 72 hours** | **0-72 hours** |
| Control | < LOQ | -- | - | - | - | < LOQ |

| | | | | | | |
|---|---|---|---|---|---|---|
| 1 | 0.0826 | 103 | 105 | 101 | 105 | 0.0842 |
| 3.2 | 0.269 | 119 | 108 | 104 | 110 | 0.289 |
| 10 | 0.816 | 108 | 103 | 105 | 116 | 0.849 |
| 32 | 2.67 | 107 | 104 | 105 | 112 | 2.77 |
| 100 | 8.19 | 79.8 | 69.8 | 75.6 | 86.9 | 6.60 |

LOQ = 0.01 mg/L

When compared with the control, substantial growth inhibition (>50% based on yield) was only observed at the highest test concentration.

**Table A 33: Effects of RH-127450 on *Raphidocelis subcapitata* growth rate and yield 72 hours after exposure**

| Geometric mean measured concentration (mg/L) | % inhibition of growth rate relative to control | % inhibition of yield relative to control |
|---|---|---|
| Control | -- | -- |
| 0.0842 | -0.767 | -2.419 |
| 0.289 | -4.850 | -16.360 |
| 0.849 | -1.806 | -5.667 |
| 2.77 | 0.916 | 2.764 |
| 6.60 | 24.375 | 53.020 |

Negative values show an increase relative to control

**Table A 34: 72-hour yield ($E_yC_x$) and growth rate ($E_rC_x$) toxicity and no observed effect concentration (NOEC)**

| Parameter | Toxicity values based on geometric mean measured concentrations (mg/L) Bracketed values present the 95% confidence limits | | | |
|---|---|---|---|---|
| | Yield | Average Specific Growth Rate | | |
| | 0-72 hour | 0-24 hour | 0-48 hour | 0-72 hour |
| $EC_{10}$ | 2.86 (0.436-3.52) | 3.23 (0.570-3.68) | 3.40 (1.19-4.13) | 3.70 (2.98-4.53) |
| $EC_{20}$ | 3.47 (2.79-4.24) | 4.00 (2.43-4.50) | 4.69 (3.03-6.32) | 5.38 (3.88-7.84) |
| $EC_{50}$ | 5.98 (4.37-NC) | >6.60 (NC) | >6.60 (NC) | >6.60 (NC) |
| NOEC | 2.77 | 2.77 | 2.77 | 2.77 |

NC = not calculated

All validity criteria were met:

| Parameter | Criterion | Observed value |
|---|---|---|
| Control cell density increase | Increase by a factor of at least 16 | 17 |
| Coefficient of variation of average specific growth rates at 72h | ≤ 7% | 1.38 |
| Mean coefficient of variation for specific growth rates (Individual replicates – 0-24, 24-48, 48-72 hours) | ≤ 35% | 24.1 |

## Conclusion

Based on the results of a range-finder, three replicate cultures of the green alga *Raphidocelis subcapitata* (strain 278/4; alga formerly known as *Pseudokirchneriella subcapitata*) in an exponential growth phase were exposed to RH-127450 at nominal concentrations of 1, 3.2, 10, 32 and 100 % saturated solution under static conditions over 72 h.

The study results were based on geometric mean measured concentrations.

The $EC_{50}$ for 72-hour yield and average specific growth rate were 5.98 and >6.60 mg/L, respectively. The NOEC for 72-hour yield and average specific growth rate were both 2.77 mg/L.

All validity criteria were met. Therefore, the test was considered valid.

(Hugill E. 2020)

### A 2.2.1.12 Study 12 - RH-163353: Inhibition of growth to the alga *Raphidocelis subcapitata*

EFSA (2017) requested in its Peer Review Conclusion: "*Further algae studies following the latest OECD 201 guideline are needed or further detailed information on all validity criteria requested by the latest OECD 201 guideline from the studies provided in the RAR for zoxamide, RH-127450 and RH-163353 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5).*" To close this data gap, new studies on the inhibition of alga growth by the zoxamide metabolites RH-127450 and RH-163353 are presented.

With regard to the EFSA request on alga studies with the parent compound zoxamide: The alga studies with zoxamide which are available in the RAR for zoxamide (2017) have been re-evaluated at a later stage during AIR based on additionally provided information. The results of the re-evaluation were included in the RAR, the alga studies were regarded valid. This was confirmed by Latvia as RMS for zoxamide. As such, the endpoints from the alga studies with the active substance zoxamide available in the RAR (2017) are valid and applicable for the aquatic risk assessment.

The following study has been provided to Latvia as RMS for zoxamide and cMSs for interzonal evaluation in July 2020.

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

| | |
|---|---|
| **Reference:** | **KCP 10.2.1/12** |
| Report | Jarrom, R., 2020: RH-163353: Inhibition of growth to the alga *Raphidocelis subcapitata*<br>Gowan Crop Protection Ltd., UK<br>Smithers ERS Ltd., UK, Report No. 3202388, GLP, Unpublished |
| Guideline(s): | OECD 201 (adopted 23 March 2006, corrected 28 July 2011) |
| Deviations: | Additional light measurements were taken at 24 and 48-hours to ensure the light remained within the correct ranges.<br>The maximum temperature recorded during the test was 24.6°C and therefore, this exceeded the protocol range (21 - 24°C). This temperature deviation occurred within the first 45-hour of the test. As the test temperatures were measured at |

approximately 24-hour intervals it is not possible to confirm the exact length of the out of specification period, however, the temperature was out of specification (actual temperature = 24.4°C) when recorded at ca 21-hours but was back in specification (actual temperature = 23.4°C) when recorded at 45-hours. As the temperature remained within 2°C, the algae growth was good and achieved all the control validity criteria, this protocol deviation was not considered to have an impact on the integrity of the study.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) No

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | RH-163353 (HHGCP001-00-2) |
| **Purity** | 99.48 % (w/w); enantiomeric ratio 48.6:51.4 (racemate) |
| **Species** | Alga *Raphidocelis subcapitata* (strain 278/4; formerly known as *Pseudokirchneriella subcapitata)* |
| Age: | not applicable |
| Weight: | not applicable |
| Source: | Culture Collection of Algae and Protozoa (CCAP), SAMS Research Service Ltd., Oban, UK |
| Acclimation period: | 72 hours |
| Test vessel: | 250 mL Erlenmeyer (conical) glass flasks, filled with 100 mL of test or control medium, capped with foam bungs, continuously agitated at ~100 rpm |
| **Environmental conditions** | |
| Test medium: | OECD alga nutrient medium<br>(according to Annex 3 of the OECD TG 201) |
| Temperature: | 22.7 – 24.6 °C |
| pH: | 7.94 – 9.79 |
| Light: | Continuous light at 4.61 – 8.72 kLux |
| **Application rate(s)** | Range- finding test:<br>0.10, 1.0, 10 and 100 mg/L under static test conditions<br>Definitive test:<br>100 mg/L under static test conditions (limit test) |
| **Post exposure observation period** | 72 hours |
| **Remarks** | None |

Based on the results of a range-finder, six replicate cultures of the green alga *Raphidocelis subcapitata* (strain 278/4; alga formerly known as *Pseudokirchneriella subcapitata*) in an exponential growth phase were exposed to RH-163353 at a nominal concentration of 100 g/L (limit test) under static conditions

over 72 h. A control treatment (6 replicate cultures) was also included in the test. A toxic reference test with potassium dichromate was separately performed in December 2019.

Each test vessel was inoculated with $1 \times 10^4$ algae cells/mL. Algae cell counts were conducted at 24-hour intervals. The test item concentrations were analytically verified at 0 and 72-hours. The 0-hour samples were taken from bulk preparations prior to the addition of algae. The 72-hour inoculated samples were taken from pooled inoculated vessels at each treatment.

Concentrations of RH-163353 were determined by treating OECD medium samples with acetonitrile containing 0.5% formic acid, then diluting further with OECD medium: acetonitrile (4:1, v/v) containing 0.1% formic acid as required to bring the response within the calibration range. Samples were analysed by liquid chromatography-time of flight mass spectrometry (LC-TOF/MS). The method has been validated according to SANCO 3029/99 rev. 4 (Smithers ERS study number 3202388, established analytical procedure SMV 3202388-01V). The limit of quantification (LOQ) was 0.1 mg/L.

RH-163353 is a racemate. The ratio of the two enantiomers was determined in each of the samples and compared with the validated certificate of analysis enantiomeric ratio. A combination of the analytical procedures SMV 3202388-01V and SMV 3202586-01V were used to assess the enantiomeric ratio.

No statistical analysis of the results was performed, as there was no growth inhibition noted compared to the control up to and including the highest test item concentration tested. The reported values have been derived empirically.

## Results and discussion

Environmental parameters (pH and temperature) remained within acceptable limits throughout the study. The pH values ranged from 7.94 to 9.79, room temperature ranged from 22.7 to 24.6°C and the light intensity ranged from 4.61 to 8.73 kLux during of the study.

The non-inoculated test media remained colourless throughout the course of the study, the inoculated changed to pale green for both the control and the test item concentration.

No morphological abnormalities were observed.

The analytical results confirmed test substance dosing, as the 0-hour results was within the 80 - 120% nominal range (92.5%). The test item was stable over the test period with a mean measured test substance concentration at the end of the study within the 80 - 120% nominal range (i.e. 105%). Moreover, all the measured test substance concentrations were within the 80 - 120% nominal range. The study results were therefore based on nominal test item concentrations.

The ratio of the two enantiomers of RH-163353 in the 72-hours 100 mg/L sample was within 1.5% (0.3%) of the enantiomeric ratio provided for the test substance in the certificate of analysis.

**Table A 35: Test item concentrations**

| Nominal test item concentration (mg/L) | Measured concentration (mg/L) | | Recovery (% of initial measured) |
|---|---|---|---|
| | at 0 hours | at 72 hours | at 72 hours |
| Control | < LOQ | < LOQ | - |
| 100 | 92.5 | 96.7 | 105 |

LOQ = 0.1 mg/L

When compared with the control, there was no growth inhibition observed at the 100 mg/L treatment. The 72-hour growth rate ($ErC_{50}$) and yield ($EyC_{50}$) as well as the corresponding NOEC values were derived empirically.

**Table A 36: Effects of RH-163353 on *Raphidocelis subcapitata* growth rate and yield 72 hours after exposure**

| Nominal test item concentration (mg/L) | % inhibition of growth rate relative to control | % inhibition of yield relative to control |
|---|---|---|
| Control | -- | -- |
| 100 | -2.596 | -13.655 |

Negative values show an increase relative to control

**Table A 37: 72-hour yield ($E_yC_x$) and growth rate ($E_rC_x$) toxicity and no observed effect concentration (NOEC)**

| Parameter | | Toxicity values based on nominal test item concentrations (mg/L) |
|---|---|---|
| | | 72 hours |
| Yield | $E_yC_{50}$ | >100 |
| | NOEC | 100 |
| Average Specific Growth Rate | $E_rC_{50}$ | >100 |
| | NOEC | 100 |

All validity criteria were met:

| Parameter | Criterion | Observed value |
|---|---|---|
| Control cell density increase | Increase by a factor of at least 16 | 114 |
| Coefficient of variation of average specific growth rates at 72h | ≤ 7 % | 2.09 |
| Mean coefficient of variation for specific growth rates (Individual replicates – 0-24, 24-48, 48-72 hours) | ≤ 35 % | 8.76 |

## Conclusion

Based on the results of a range-finder, six replicate cultures of the green alga *Raphidocelis subcapitata* (strain 278/4; alga formerly known as *Pseudokirchneriella subcapitata*) in an exponential growth phase were exposed to RH-163353 at a nominal concentration of 100 g/L (limit test) under static conditions over 72 h.

The 72-hour $E_yC_{50}$ and $E_rC_{50}$ values based on nominal concentrations were both >100 mg/L. The 72-hour NOEC values for yield and growth rate were both estimated to be 100 mg/L.

All validity criteria were met; therefore, the test was considered valid.

(Jarrom R. 2020)

### A 2.2.1.13 Study 13 – Zoxamide tech.: Effects on *Lemna gibba*

The endpoints from a *Lemna* study with zoxamide (xxx, 1998b) were re-evaluated during AIR. The 7-days $EC_{50}$ endpoint (here: 7-days $IC_{50}$ value) of 18 µg a.i./L was regarded as not valid any more due to lack of information and changed to the NOEC of 9.0 µg a.i./L for growth rate. Therefore, a new *Lemna*

study with zoxamide technical has been performed and is presented hereafter.

The following study has been provided to Latvia as RMS for zoxamide and cMSs for interzonal evaluation in July 2020.

| | |
|---|---|
| Comments of zRMS: | The study was not crucial for finalization of the risk assessment, thus was not evaluated by zRMS. |

**Reference:** **KCP 10.2.1/13**

Report: xxx., 2020: Effects of Zoxamide technical on *Lemna gibba* in a growth inhibition test under semi-static test conditions
Gowan Crop Protection Ltd., UK
xxx, Report No. 18 48 ALE 0005, GLP, Not published

Guideline(s): OECD 221 (2006)

Deviations: No

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): No

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | Zoxamide technical (2016051601) |
| **Purity** | 97.62 % (w/w)<br>ratio of R- and S-isomer of zoxamide: 50.65 : 49.35 (racemate) |
| **Species** | Duckweed - *Lemna gibba* L. |
| Age: | initial colonies consisted of 2 - 4 fronds having a similar size; free from other organisms (algae and protozoa) |
| Weight: | not applicable |
| Source: | Purchased from "Institut für allgemeine Botanik" at the University of Jena, Germany, in June 2007 and since then cultivated in the test facility |
| Acclimation period: | 7 days |
| Test vessel: | glass beaker with a glass lid (volume: 150 mL), filled with 100 mL of test or control medium; 3 fronts/colony or 9 fronts/vessel inserted at study initiation |
| **Environmental conditions** | |
| Test medium: | 20X AAP growth medium (according to OECD 221 guideline) |
| Temperature: | 22.6 – 23.2°C (recorded in the water bath) |
| pH: | 7.39 - 7.95 |
| Light: | continuous illumination (on average 111 µE $*m^{-2}*s^{-1}$) |

| | |
|---|---|
| **Application rate(s)** | 0.33, 1.00, 3.01, 9.01, 27.03, 81.05, 243.02 µg/L test item (nominal) under semi-static conditions |
| Vehicle: | Dimethylformamide (DMF) |
| **Post exposure observation period** | 7 days |
| **Remarks** | |
| **Remarks** | None |

Three replicate cultures of *Lemna gibba* L. were exposed under semi-static conditions to zoxamide technical (batch no.: 2016051601, analysed purity of 97.62 ± 2.02% w/w or 967.2 ± 20.2 g/kg) at nominal concentrations of 0.33, 1.00, 3.01, 9.01, 27.03, 81.05, 243.02 µg test item/L over a period of 7 days. Dimethylformamide saved as solvent for the active substance. An untreated control and a solvent control were included in the study, with 6 replicate each. A toxic reference test with 3,5-dichlorophenol confirmed the sensitivity of the test system.

Each test vessel was inoculated with 3 fronts/colony or 9 fronts/vessel. During the course of the study frond numbers per vessel and any changes in plant development were observed on days 0, 3, 5 and 7, the dry weight of plants per test vessel was determined on days 0 and 7 of the study.

Specimens were stabilised using methanol (1:1, v/v) and stored deep frozen (≤ -18 °C) for a maximum of 27 days until analysis.

The test item concentrations were analytically verified at test start, at each renewal, and at the end of the test in fresh and spent solutions. The test item concentrations were measured by reversed phase high performance liquid chromatography (RP-HPLC) and mass spectrometric determination with a method validated according to SANCO 3029/99 rev. 4 at an LOQ of 0.168 µg/L zoxamide.

After homogenisation of the specimens, the aquatic medium was diluted 1: 1 (v/v) with methanol and analysed.

The two untreated controls (with and without solvent (vehicle)) were compared by STUDENT's t-test. As a result, there were no statistically significant differences between control and solvent control. Therefore, the solvent control was used for comparison with the treated samples. Regression analysis was performed using individual replicate responses, not treatment group means. From average specific growth rates and yield, recorded in a series of test solutions, effect concentrations of $ErC_{10}$, $ErC_{20}$ and $ErC_{50}$, (average specific growth rate) and $EyC_{10}$, $EyC_{20}$ and $EyC_{50}$ (yield) was determined using concentrations-response modelling (non-linear regression, 3 or 4 parameters Normal CDF = cumulative distribution function). To determine a LOEC and to derive a NOEC for effects on growth rate, it was necessary to compare treatment means using analysis of variance (ANOVA) techniques. Shapiro-Wilk´s Test on Normal Distribution was performed. The mean for each concentration was compared with solvent control means using an appropriate multiple comparison test method. Williams's t-test was used if variance-homogeneity requirements are fulfilled. The Multiple Sequentially rejective Median (2x2-table) test for non-homogeneous variances with Bonferroni-Holm-adjustment was performed. As a test for homogeneity of variances, Levene's test was done. All statistical analysis was performed with ToxRat Professional version 3.2.1 (02.11.2015).

## Results and discussion

Environmental parameters (pH and temperature) remained within acceptable limits throughout the study. The cultures showed a pH of 7.39-7.95 during the course of the study and were exposed at 22.6 – 23.2°C under continuous illumination (111 µE *m-2*s-1).

The measured concentrations of zoxamide remained within a range of 81 – 117% of nominal in the freshly prepared test solutions at test start and at each renewal in the freshly prepared test solutions. The zoxamide concentrations in the spent test solutions were determined at 72 – 131% of nominal at each renewal and at the end of the study (day 7). The calculated study endpoints were based on nominal and geometric mean measured test item concentrations.

**Table A 38: Test item concentrations**

| **Nominal test item concentration (mg/L)** | | **Concentration** µg/L test item | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **Control** | **Solvent control** | **0.33** | **1.00** | **3.01** | **9.01** | **27.0** | **81.1** | **243.0** |
| day 0 (fresh) | measured | <LOQ | <LOQ | 0.270 | 1.015 | 3.177 | 8.925 | 26.85 | 74.24 | 191.7 |
| | **% of nominal a.i.** | **-** | **-** | **83** | **104** | **108** | **101** | **102** | **94** | **81** |
| day 3 (spent) | Measured | <LOQ | <LOQ | 0.297 | 0.893 | 2.940 | 8.195 | 25.84 | 73.65 | 187.8 |
| | **% of nominal a.i.** | **-** | **-** | **91** | **91** | **100** | **93** | **98** | **93** | **79** |
| day 3 (fresh) | Measured | <LOQ | <LOQ | 0.312 | 1.017 | 3.423 | 9.348 | 27.79 | 87.09 | 205.9 |
| | **% of nominal a.i.** | **-** | **-** | **95** | **104** | **116** | **106** | **105** | **110** | **87** |
| day 5 (spent) | Measured | <LOQ | <LOQ | 0.429 | 1.050 | 3.243 | 10.57 | 25.20 | 80.15 | 170.6 |
| | **% of nominal a.i.** | **-** | **-** | **131** | **107** | **110** | **120** | **95** | **101** | **72** |
| day 5 (fresh) | Measured | <LOQ | <LOQ | 0.296 | 1.071 | 3.182 | 8.934 | 28.2 | 82.11 | 266.6 |
| | **% of nominal a.i.** | **-** | **-** | **91** | **109** | **108** | **102** | **107** | **104** | **112** |
| day 7 (spent) | Measured | <LOQ | <LOQ | 0.253 | 0.896 | 2.485 | 8.358 | 24.07 | 72.42 | 224.9 |
| | **% of nominal a.i.** | **-** | **-** | **77** | **92** | **85** | **95** | **91** | **92** | **95** |
| geometric mean measured a.i. concentration over 7 days | | - | - | 0.30 | 0.98 | 3.06 | 8.95 | 26.29 | 77.49 | 203.37 |
| **% of nominal a.i.** | | **-** | **-** | **90** | **98** | **102** | **99** | **97** | **96** | **84** |

<LOQ: not detected or detected at concentration below LOQ = Limit of quantification (0.055 µg/L zoxamide)

The two untreated controls (with and without solvent (vehicle)) were compared by Student's t-test. As a result, there were no statistically significant differences between control and solvent control. Therefore, the solvent control was used for comparison with the treated samples.

**Table A 39: Effects of Zoxamide technical on growth rate and yield for *Lemna gibba***

| **Treatment group** µg/L test item nominal | **Final frond number** replicate mean day 7 | **Biomass (dry weight)** replicate mean day 7 (mg) | **% Inhibition** | | | |
|---|---|---|---|---|---|---|
| | | | **Average specific growth rate (% $I_r$)** | | **yield (% $I_y$)** | |
| | | | frond number | biomass | frond number | biomass |
| Control | 70.3 | 11.3 | - | - | - | - |
| Solvent control | 72.0 | 11.2 | - | - | - | - |
| 0.33 | 74.3 | 12.2 | -1.5* | -3.1* | -3.7* | -9.0* |
| 1.00 | 73.3 | 11.1 | -0.9* | 0.3 | -2.1* | 1.0 |
| 3.01 | 71.7 | 10.4 | 0.3 | 3.3 | 0.5 | 8.4 |
| 9.01 | 58.7 | 7.6 | 9.9 $^{+}$ | 15.7 $^{+}$ | 21.2 $^{+}$ | 34.7 $^{+}$ |
| 27.03 | 23.7 | 5.4 | 53.6 $^{+}$ | 28.7 $^{+}$ | 76.7 $^{+}$ | 55.9 $^{+}$ |
| 81.05 | 19.7 | 2.6 | 62.4 $^{+}$ | 57.7 $^{+}$ | 83.1 $^{+}$ | 83.3 $^{+}$ |
| 243.02 | 19.0 | 2.5 | 64.2 $^{+}$ | 58.9 $^{+}$ | 84.1 $^{+}$ | 83.9 $^{+}$ |

* negative values show an increase compared to the solvent control
$^{+}$ significantly different to the solvent control (Williams t-test; $p \leq 0.05$, one-sided)

No statistically significant effect on the average specific growth rate and yield of *Lemna* based on frond number and biomass was observed at the nominal concentrations ≤ 3.01µg/L test item, whereas statistically significant effects ($p \leq 0.05$) were calculated for nominal concentrations ≥ 9.01 µg/L test item. As a result, the NOEC for average specific growth rate and yield for frond number and biomass was determined to be 3.01 µg/L test item (equivalent to 3.06 µg/L test item/L, mean measured) and the LOEC was determined to be 9.01 µg/L test item (equivalent to 8.95 µg/L test item/L, mean measured), based on nominal concentrations.

The frond number EC-values (0-7 d) were 7.59, 9.70 and 15.5 µg/L test item for growth rate ($E_rC_{10}$, $E_rC_{20}$ and $E_rC_{50}$) and 4.52, 7.01 and 16.2 µg/L test item for yield ($E_yC_{10}$, $E_yC_{20}$ and $E_yC_{50}$), based on nominal concentrations, respectively.

The frond number EC-values (0-7 d) were 7.57, 9.62 and 15.2 µg/L test item for growth rate ($E_rC_{10}$, $E_rC_{20}$ and $E_rC_{50}$) and 4.40, 6.86 and 16.0 µg/L test item for yield ($E_yC_{10}$, $E_yC_{20}$ and $E_yC_{50}$), based on mean measured a.i. concentrations, respectively.

The biomass EC-values (0-7 d) were 7.09, 10.7 and 23.7 µg/L test item for growth rate ($E_rC_{10}$, $E_rC_{20}$ and $E_rC_{50}$) and 2.83, 4.67 and 12.2 µg/L test item for yield ($E_yC_{10}$, $E_yC_{20}$ and $E_yC_{50}$), based on nominal concentrations, respectively.

The biomass EC-values (0-7 d) were 6.93, 10.5 and 23.1 µg/L test item for growth rate ($E_rC_{10}$, $E_rC_{20}$ and $E_rC_{50}$) and 2.79, 4.60 and 12.0 µg/L test item for yield ($E_yC_{10}$, $E_yC_{20}$ and $E_yC_{50}$), based on mean measured a.i. concentrations, respectively.

**Table A 40: 7-days LOEC, NOEC and effect concentrations $EC_x$ of zoxamide technical for growth rate and yield based on frond number and biomass for *Lemna gibba***

| Effect concentration | Zoxamide, µg/L | | | |
|---|---|---|---|---|
| | average specific growth rate inhibition | | yield inhibition | |
| | Frond number | Biomass | Frond number | Biomass |
| **NOEC** | | | | |
| test item, nominal | **3.01** | **3.01** | **3.01** | **3.01** |
| Zoxamide, mean measured | 3.06 | 3.06 | 3.06 | 3.06 |
| **LOEC** | | | | |
| test item, nominal | **9.01** | **9.01** | **9.01** | **9.01** |
| Zoxamide, mean measured | 8.95 | 8.95 | 8.95 | 8.95 |
| **$EC_{10}$** | **$E_rC_{10}$** | **$E_rC_{10}$** | **$E_yC_{10}$** | **$E_yC_{10}$** |
| (95th confidence interval) | | | | |
| test item, nominal | **7.59** | **7.09** | **4.52** | **2.83** |
| | (6.33 – 9.11) | (4.13 – 12.2) | (2.64 – 7.75) | (2.31 – 3.48) |
| Zoxamide, mean measured | 7.57 | 6.93 | 4.40 | 2.79 |
| | (6.33 – 9.05) | (4.01 – 12.0) | (2.57 – 7.52) | (2.59 – 3.00) |
| **$EC_{20}$** | **$E_rC_{20}$** | **$E_rC_{20}$** | **$E_yC_{20}$** | **$E_yC_{20}$** |
| (95th confidence interval) | | | | |
| test item, nominal | **9.70** | **10.7** | **7.01** | **4.67** |
| | (8.19 – 11.5) | (6.42 – 18.0) | (4.19 – 11.7) | (3.87 – 5.67) |
| Zoxamide, mean measured | 9.62 | 10.5 | 6.86 | 4.60 |
| | (8.15 – 11.4) | (6.22 – 17.7) | (4.11 – 11.4) | (4.30 – 4.93) |
| **$EC_{50}$** | **$E_rC_{50}$** | **$E_rC_{50}$** | **$E_yC_{50}$** | **$E_yC_{50}$** |
| (95th confidence interval) | | | | |
| test item, nominal | **15.5** | **23.7** | **16.3** | **12.2** |
| | (12.7 – 18.9) | (12.5 – 44.1) | (8.66 – 30.2) | (9.80 – 15.3) |
| Zoxamide, mean measured | 15.2 | 23.1 | 16.0 | 12.0 |
| | (12.6 – 18.6) | (12.0 – 43.6) | (8.58 – 29.7) | (11.0 – 13.0) |

No chlorotic effects were observed in the control and solvent group during the test. Some chlorotic effects

of zoxamide technical were observed at nominal test item concentrations ≥ 27.03 µg/L on day 5 and 7.

According to the guideline, the doubling time of the frond number in the control must be less than 2.5 days (60 h), corresponding to approximately a 7-fold increase in biomass in 7 days and an average specific growth rate of 0.275 $d^{-1}$. The measured doubling time of the frond numbers in the control was on average 2.36 days (1.9 days for dry weight), corresponding to a 7.8-fold increase in frond number over the 7-day study period (mean of 9 to 70.3 fronds in the control vessels) and a 13.0-fold increase in dry weight (0.867 mg to 11.3 mg dry weight). The average specific growth rate in the control was 0.294 $d^{-1}$ for frond number and 0.367 $d^{-1}$ for dry weight. Therefore, all validity criteria were met in this study.

The $E_rC_{50}$ (growth rate based on frond number) value for the reference item (toxic standard) 3,5 - dichlorophenol was 3.04 mg/L. This value is included in the range 2.2 - 3.8 mg/L 3,5 - dichlorophenol as stated in Guideline ISO 20079, demonstrating that the test system was sensitive.

## Conclusion

Three replicate cultures of *Lemna gibba* L. were exposed under semi-static conditions to zoxamide technical at nominal concentrations of 0.33, 1.00, 3.01, 9.01, 27.03, 81.05, 243.02 µg test item/L over a period of 7 days. Dimethylformamide saved as solvent for the active substance.

Since the 0-7 days measured test item concentrations amounted to 84-102 % of nominal and thus were within the required 80-120% range, the study results were based on nominal test item concentrations.

The 7-days $E_rC_{50}$ and $E_yC_{50}$ values based on nominal test item concentrations were 23.7 and 16.3 µg/L.
The 7-days $E_rC_{10}$ and $E_yC_{10}$ values based on nominal test item concentrations were 7.09 and 4.52 µg/L test item. The corresponding 7-days NOEC values were 3.01 µg/L test item for both yield and growth rate.

All validity criteria were met, the study is valid.

(Juckeland D. 2020)

## A 2.2.1.14 Study 14 – CYMOXANIL 33% + ZOXAMIDE 33% WG: Fish acute toxicity

| | |
|---|---|
| Comments of zRMS: | Study evaluated and accepted by zRMS UK. Please refer to product fRR dated January 2012. |

| | |
|---|---|
| **Reference:** | **KCP 10.2.1/14** |
| Report | xxx., 2007: Acute toxicity of Cymoxanil 33% + Zoxamide 33% WG to rainbow trout (*Oncorhynchus mykiss*), determined under flow-through conditions.<br>Oxon Italia S.p.A., Italy<br>xxx, Report No. CH-E–023/2006, GLP, Not published |
| Guideline(s): | OECD 203 (1992)<br>EU Commission Directive 92/69/EEC, C1 (1992)<br>OPPTS 850.1075 (1996) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

Duplication (if vertebrate study) No

**Executive Summary**

The acute toxicity of 'Cymoxanil 33% + Zoxamide 33% WG' to rainbow trout (*Oncorhynchus mykiss*) was tested by a 96-hour flow-through test on juvenile fishes. They were exposed to five nominal concentrations of test item (0.10 0.32, 1.00, 3.20, 10.00 mg/L) under defined conditions. The test organisms were checked for mortality after 2, 24, 48, 72 and 96 hours from test beginning.
96 h $LC_{50}$ was estimated to be 0.83 mg nominal concentration/L, NOEC was 0.24 mg/L and LOEC 0.93 mg/L.

## I. MATERIALS AND METHODS

### A. MATERIALS

| | |
|---|---|
| **1.Test Material:** | |
| **Description:** | Granules |
| **Lot/Batch #:** | Batch code: BPL 212 |
| **Purity:** | Nominal: Cymoxanil 33 %, Zoxamide 33 % WG<br>Declared according to CoA: Cymoxanil 32.29%, Zoxamide 33.18% |
| **Retest date** | June 2008 |
| **2. Vehicle and/or positive control** | Not relevant |
| **3. Test animals** | Fish |
| **Species:** | *Oncorhynchus mykiss* |
| **Strain** | Not relevant |
| **Age:** | n.a |
| **Weight at dosing:** | 2 g approximately |
| **Source:** | Commercial fish supplier |
| **Acclimation period** | 20 days |
| **Diet:** | Commercial fish food until 48 hours before test initiation |
| **Water:** | Not relevant |
| **Housing:** | 60 L capacity glass aquaria, 24 h renewal of test medium. |
| **4. Environmental conditions** | |
| **Test medium** | Reconstituted test water, pH 7.07-7.32 |
| **Temperature:** | 16.3 – 17.9°C |
| **Humidity:** | Not relevant |
| **Air changes:** | Not relevant |
| **Photoperiod:** | 16 h light |

### B. STUDY DESIGN AND METHODS

| | |
|---|---|
| **1. In life dates** | 17 July – 21 July 2006 |

| | |
|---|---|
| **2. Animal assignment and treatment** | The test concentrations were chosen on the basis of a preliminary test; the final study was performed at 0.10, 0.32, 1.00, 3.20 and 10 mg test item/L. 7 fish were placed into 60 L glass aquaria containing either prepared test or control medium water. The flow of reconstructed water was of 15 L/h, the one of test item concentrated solution of 4 ml/min. Fishes were exposed for 96 h and during the whole period they were not fed.<br>Mortality and any sub-lethal effects were observed at 2, 24, 48, 72 and 96 hours after test start.<br>The concentrations of test item were measured as Zoxamide active ingredient content by GC/MS. Samples of the test water and control were taken at time 0, 48, 96 h, samples from refurnishing solution at the beginning and at the end of test period. |
| **3. Statistics** | $LC_{50}$ 96 h was calculated by Trimmed Spearman-Karber analysis |

## II. RESULTS AND DISCUSSION

### A. MORTALITY

Mortality was noted starting from 1.0 mg/L of nominal concentration after 96 h of treatment; 100% of mortality was observed at 3.20 mg/L of nominal test and above.

**Table IIIA 10.2.2.1-1: % of mortality of fishes in the main test**

| | | | n. dead fishes | | | | |
|---|---|---|---|---|---|---|---|
| **Nominal test concentration (mg/L)** | **Calculated test concentration (mg/L)** | **No. exposed fish** | **2 h** | **24 h** | **48 h** | **72 h** | **96 h** |
| 0 (control) | < LOD | 7 | 0 | 0 | 0 | 0 | 0 |
| 0.10 | 0.07 | 7 | 0 | 0 | 0 | 0 | 0 |
| 0.32 | 0.24 | 7 | 0 | 0 | 0 | 0** | 0** |
| 1.00 | 0.93 | 7 | 0 | 0 | 0 | 0** | 4* |
| 3.20 | 3.32 | 7 | 0 | 0 | 3 | 6* | 7 |
| 10.00 | 7.96 | 7 | 0 | 0* | 5 | 7 | 7 |

* all the survivors showed signs of distress such as apathy and immobility
** two of the survivors showed signs of distress such as apathy and immobility

### B. OBSERVATIONS

The test medium was normal during all testing period for lowest concentration; for the others it showed an increasing turbidity with the raising of concentrations, with filamentous formations on the bottom of the aquaria.

### C. TEST CONCENTRATIONS

The mean concentration of the test item in the refurnishing solutions was determined to be 84% of nominal concentration, calculate from the average concentrations of Zoxamide content.

## III. CONCLUSIONS

The 96h $LC_{50}$ was estimated to be 0.83 mg/L nominal, NOEC was observed at 0.24 mg/L and LOEC at 0.93 mg/L.

## A 2.2.1.15 Study 15 – CYMOXANIL 33% + ZOXAMIDE 33% WG: Effects on *Daphnia magna*

| | |
|---|---|
| Comments of zRMS: | Study evaluated and accepted by zRMS UK. Please refer to product fRR dated January 2012. |

| | |
|---|---|
| **Reference:** | **KCP 10.2.1/15** |
| Report | xxx, 2007: Acute toxicity of Cymoxanil 33% + Zoxamide 33 % WG to Daphnia magna in a 48-hour immobilization test under semi-static exposure-limit test. Oxon Italia S.p.A., Italy<br>xxx, Report No. CH-001/2007, GLP, Not published |
| Guideline(s): | OECD 202 (adopted April 13 2004)<br>OECD 202 (1984)<br>92/69/EEC, C.2 (1992) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | No |

**Executive Summary**

The acute toxicity of 'Cymoxanil 33% + Zoxamide 33% WG' to *Daphnia magna* was tested by exposure of juvenile daphnids in a 48 h semi-static test, with a loading rate of aqueous medium of nominal 100 mg testing item/L. The immobilization of the organisms was checked after 24 and 48 h after the beginning of exposure.
No mortality was observed after 24 and 48 h, therefore the NOEC of 'Cymoxanil 33% + Zoxamide 33% WG' to *Daphnia magna* was considered to be 100 mg/L nominal (equivalent to 44.64 mg/L) or higher; also LOEC and $EC_{50}$ at 48 h were assessed to be greater than 44.64 mg/L of the product.

### I. MATERIALS AND METHODS

**A. MATERIALS**

**1.Test Material:**

| | |
|---|---|
| **Description:** | Granules |
| **Lot/Batch #:** | Batch code: BPL 212 |
| **Purity:** | Nominal: Cymoxanil 33 %, Zoxamide 33 % WG<br>Declared according to CoA: Cymoxanil 32.29% , Zoxamide 33.18% |
| **Retest date** | June 2008 |
| **2. Vehicle and/or positive control** | Not relevant |
| **3. Test animals** | |
| **Species:** | *Daphnia magna* STRAUS |

| | |
|---|---|
| **Strain** | Not relevant |
| **Age:** | 6-24-hour old |
| **Weight at dosing:** | Not relevant |
| **Source:** | Italian Health Department |
| **Acclimation period** | Not relevant |
| **Diet:** | Green algae *Pseudokirchneriella subcapitata* and suspension of the yeast *Saccaromices cerevisiae.* |
| **Water:** | Not relevant |
| **Housing:** | 50 ml beakers filled with 40 ml of test medium, covered with glass plates. |

**4. Environmental conditions**

| | |
|---|---|
| **Test medium** | Reconstituted test water. pH 7.21 – 7.48 |
| **Temperature:** | 21.8- 25.1 °C |
| **Humidity:** | Not relevant |
| **Air changes:** | Not relevant |
| **Photoperiod:** | 16h photoperiod daily |

**B. STUDY DESIGN AND METHODS**

| | |
|---|---|
| **1. In life dates** | March 2007 |
| **2. Animal assignment and treatment** | The study was performed at nominal concentration of 100 mg/L. Four replicates of five individuals were put in the vessels with the test item either the control and tested. The dapnhids were exposed to 40 ml of test medium for 48 h, with daily renewal of test media<br>Immobility of organisms was observed after 24 and 48 h of exposure.<br>Test item concentrations were measured by determining Zoxamide concentration by GC/MS at time 0 (fresh media), 24, (fresh and aged media) and 48 h (aged media). |
| **3. Statistics** | None required |

## II. RESULTS AND DISCUSSION

**A. MORTALITY**

After 48 h no immobilization was observed, therefore the NOEC was assessed at 44.64 mg formulation/L, and LOEC and $EC_{50}$ were determined to be higher than that value.

**Table 10.2.2.2-1: Immobilization of Daphnia in the main test**

| | | no. of immobile daphnids | |
|---|---|---|---|
| **Actual concentration (mg/L)** | **No. exposed daphnids** | **24 h** | **48 h** |
| 0 (control) | 20 | 0 | 0 |
| 44.64 | 20 | 0 | 0 |

**B. OBSERVATIONS**

Test medium was a clear solution during the whole test.

**C. TEST CONCENTRATIONS**

The biological results were referred to the geometric mean concentration of 'Cymoxanil 33% + Zoxamide 33% WG' test solution, that was 44.64 mg/L, calculated on the basis of Zoxamide content.

**III. CONCLUSIONS**

The test item 'Cymoxanil 33% + Zoxamide 33% WG' didn't cause immobilization to *Daphnia magna*, so that the NOEC value can be considered 44.64 mg/L or higher; LOEC and $EC_{50}$ values at 48 h were assessed to be greater than 44.64 mg/L of the product, corresponding to 100 mg/L as nominal concentration.

(xxx. 2007)

## A 2.2.1.16 Study 16 - CYMOXANIL 33% + ZOXAMIDE 33% WG: Effects on alga

| | |
|---|---|
| Comments of zRMS: | Study evaluated and accepted by zRMS UK. Please refer to product fRR dated January 2012. |

| | |
|---|---|
| **Reference:** | **KCP 10.2.1/16** |
| Report | xxx, 2007: Toxicity of 'Cymoxanil 33% + Zoxamide 33% WG' to green algae *Pseudokirchneriella subcapitata* determined in a growth inhibition study. Oxon Italia S.p.A., Italy<br>xxx, Report No. CH-002/2007, GLP, Not published |
| Guideline(s): | OECD 201 (adopted 23 March 2006) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | No |

**Executive Summary**

The effects of 'Cymoxanil 33% + Zoxamide 33% WG' on the growth of green algae *Pseudokirchneriella subcapitata* was assessed through a 72 hours test, under defined conditions. Aqueous medium contained test item at five nominal concentrations: 0.03, 0.07, 0.17, 0.41 and 1.00 mg/L.
Algal cell density was measured every 24 hours in order to evaluate the percentage of inhibition.
$EC_{50}$ for algal yield after 72 h was 0.055 mg/L, $EC_{50}$ for the growth rate was 0.151 mg/L (nominal concentrations).

**I. MATERIALS AND METHODS**

**A. MATERIALS**

| | |
|---|---|
| **1.Test Material:** | |
| **Description:** | Granules |
| **Lot/Batch #:** | Batch code BPL 212 |
| **Purity:** | Nominal: Cymoxanil 33 %, Zoxamide 33 % WG<br>Declared according to CoA: Cymoxanil 32.29%, Zoxamide 33.18% |
| **Retest date** | June 2008 |
| **2. Vehicle and/or positive control** | Not relevant |
| **3. Test animals** | Algae |

| | |
|---|---|
| **Species:** | *Pseudokirchneriella subcapitata* |
| **Strain** | Not relevant |
| **Age:** | Not relevant |
| **Weight at dosing:** | Not relevant |
| **Source:** | IRSA – CNR (Water Research Insitute- -Italian Research Council) |
| **Acclimation period** | Not relevant |
| **Diet:** | Not relevant |
| **Water:** | Not relevant |
| **Housing:** | The algae were cultured under continuous illumination. The stock cultures were weekly transferred to fresh medium and continuously shacked. |

**4. Environmental conditions**

| | |
|---|---|
| **Test medium** | Sterile algal nutrient medium |
| **Temperature:** | 24 ± 2°C |
| **Humidity:** | Not relevant |
| **Air changes:** | Not relevant |
| **Photoperiod:** | Continuous illumination |

**B. STUDY DESIGN AND METHODS**

| | |
|---|---|
| **1. In life dates** | March 2007 |
| **2. Animal assignment and treatment** | The study was performed at five nominal concentrations of test item: 0.03, 0.07, 0.17, 0.41, and 1.00 mg/L. Flasks with three replicates for each concentration and six controls were filled with 50 ml of solution. Algae were inoculated in these flasks in order to obtain a starting cell density of around $10^4$ cells/ml and one replica for each concentration was not inoculated. The test was carried out under continuous illumination for 72 hours.<br>Cell density was measured every 24 hours with a spectrofluorophotometer for the evaluation of the percentage of inhibition of cell growth as biomass and as growth rate.<br>Test water concentrations were measured by GC/MS at time 0 and after 72 hours, both from inoculated and negative replicates. |
| **3. Statistics** | End-points were calculated by the mean of the CETIS elaboration software v1.026D, using linear interpolation. |

**II. RESULTS AND DISCUSSION**

**A. ALGAL GROWTH**

In the test solutions the algal growth rate after 72 hours was inhibited from a minimum value of 5% to a maximum of 62% in comparison with the control. The initial algal biomass had a decreasing from 27 % (corresponding to the lowest concentration) to 98 % (corresponding to highest concentration) up to the end of the test.

**Table IIIA 10.2.2.3-1: Algal yield and growth inhibition - 72 hours of exposure**

| Nominal test item concentration (mg/L) | Mean biomass at 72h (cell/ml) | Mean growth inhibition % |
|---|---|---|
| 0 (control) | 3973677 | - |
| 0.03 | 2915627 | 27 |
| 0.07 | 1420127 | 64 |
| 0.17 | 129908 | 97 |
| 0.41 | 111533 | 97 |
| 1.00 | 99440 | 98 |

**B. OBSERVATIONS**

No microscopic abnormalities of cells were detected

**C. TEST CONCENTRATIONS**

The nominal concentrations were used for the assessment of the end-points, since the measured concentrations were expected to be not reliable due to the high variable ratio nominal/measured concentrations. This was probably due to the non–homogeneous distribution of the test item in the flasks, which did not allow to collect representative samples for the analysis, and to the low concentrations of product tested.

**III. CONCLUSIONS**

72 h $EC_{50}$ was estimated to be 0.055 mg/L and 0.151 mg/L (nominal concentrations) for biomass growth ($E_bC_{50}$) and growth rate ($E_rC_{50}$) respectively.

(xxx. 2007)

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

### A 2.2.2.1 Study 1 – Zoxamide tech.: Fish ELS study on sheepshead minnow

An additional fish ELS study with sheepshead minnow (xxx, 1998) is available from the authorisation of zoxamide and its products in the US. The study has been evaluated by US EPA, but not yet by European authorities. It is therefore provided with this submission to complete the picture on chronic toxicity of zoxamide to fish and to be used in the aquatic risk assessment (i.e. for a species sensitivity distribution).

The study has been performed at Wildlife International in the US. To support the report of xxx (1998), the legal successor of Wildlife International in the US has been asked to re-evaluate the findings with regard to current guidelines based on the report data and the raw data still available in the laboratory. Please refer to the "Final Report Addendum for RH-117,281 Technical: An Early Life-stage Toxicity Test with The Sheepshead Minnow (*Cyprinodon variegatus*)" (Milligan et al., 2020), which is presented in the following together with the summary of the original report findings.

The following study has been provided to Latvia as RMS for zoxamide and cMSs for interzonal evaluation in July 2020.

| | |
|---|---|
| Comments of zRMS: | The study was not crucial for finalization of the risk assessment, thus was not evaluated by zRMS. |

| | |
|---|---|
| **Reference:** | **KCP 10.2.2/01** |
| Report | xxx, 2020: Final report addendum for RH-117,281 technical: An early life-stage toxicity test with the sheepshead minnow (*Cyprinodon variegatus*)<br>Gowan Crop Protection Ltd., UK<br>xxx, USA, Report No. 129A-143A, GLP, Not published |
| Guideline(s): | OCSPP 850.1400 (2016)<br>SANCO 3029/99 rev. 4 (2000) |
| Deviations: | No |
| Acceptability: | Yes |

**and**

**Reference:** **KCP 10.2.2/02**

Report: xxx., 1998: RH-117,281 technical: An early life-stage toxicity test with the sheepshead minnow (*Cyprinodon variegatus*)
xxx, Report No. 97RC-0078
Gowan Crop Protection Ltd., UK
xxx, Report No. 129A-143A, GLP, Not published

Guideline(s): 850.1500 (1996)

Deviations: Two sets of Day 0 analytical samples were collected. The first set of samples contained an interference in the chromatography. An additional set of samples was collected and analysed using a direct injection method which removed the interference.
The protocol states that method validation which bracelets the concentrations of the study will be conducted and approved by Dr. Sandra Ferris prior to initiation of the definitive. The method was actually validated after test initiation since the first set of samples contained an interference in the chromatography. A new analytical method was developed concurrently with the Day 0 samples. However, formal validation of the method occurred after test initiation.
The deviations did not adversely affect the results and the integrity of the study.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): No

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | RH-1l7,281 Technical (Lot No. DSR-9510; TD No.95-161) |
| **Active substance content or purity** | 92.3 % (w/w) |
| **Test organism** | sheepshead minnow *(Cyprinodon variegatus)* |
| Age: | eggs < 24 hours old |
| Number of test organisms: | 80 embryos |
| Weight: | Biomass loading (the total wet weight of the fish in the negative control replicate A test chamber per liter of test water) at the end of the test was calculated to be 0.034 g of fish per liter of test water that passed through the test chamber during a 24-hour period. Instantaneous loading was 0.22 g of fish per liter of test water in the test chamber at any given time. |
| Source: | Wildlife International Ltd. cultures<br>Easton, Maryland 21601 |
| Acclimation period: | not relevant |
| Diet: | Live brine shrimp nauplii *(Artemia sp.)* |
| Feeding: | Newly-hatched larvae were fed live brine shrimp nauplii *(Artemia* |

| | |
|---|---|
| | *sp.)* 3 times per day during the first 7 days post-hatch. On Days 8 through 26 post-hatch, all fish were fed live brine shrimp nauplii 3 times daily on weekdays and at least 2 times daily on weekends. On Day 27 and 28 post-hatch, fish were not fed to allow for clearance of the digestive tracts before weight measurements were made. |
| Housing: | 9-L glass aquaria filled with approximately 7 L of test solution |
| **Environmental conditions** | |
| Temperature: | 25 ± 2 °C |
| Photoperiod: | 16 hours light (640 lux), 8 hours dark, with 30 min transition period |
| Test medium: | filtered salt water (sand filtered 25 µm), particle filter (0.2 µm), UV sterilised |
| pH: | 8.0 – 8.3 |
| Dissolved oxygen: | ≥ 64 % of saturation (4.7 mg/L) |
| Salinity: | 20 % |
| **Application rate(s)** | 0.019, 0.040, 0.078, 0.15 and 0.25 mg a.i./L (flow-through conditions) |
| Negative control: | filtered salt water<br>solvent control (acetone; 0.10 mL/L) |
| **Post exposure observation period** | 34 days |
| **Remarks** | None |

The objective of this study was to determine the effects of a test substance, RH-117,281 (zoxamide) technical (92.3 % w/w pure) on the sheepshead minnow, *Cyprinodon variegatus*, during early life-stage development. Groups of 80 sheepshead minnow embryos were exposed to a geometric series of five test concentrations, a solvent (acetone) control and a negative (filtered saltwater) control for 34 days.

A primary stock solution of RH-117,281 was prepared by dissolving the test substance in acetone at a concentration of 3.00 mg a.i./mL. The primary stock was sonicated and inverted to aid in solubilisation of the test substance. Aliquots were proportionally diluted with acetone to prepare four additional stocks at concentrations of 1.50,0.750,0.375 and 0.188 mg a.i./mL. The five working stocks were injected into the diluter mixing chambers (at a rate of 0.0125 mL/minute) where they were mixed with dilution water (at a rate of 125 mL minute) to achieve the desired test concentrations. Acetone was injected into the mixing chamber for the solvent control. Nominal test concentrations used in the study were 0.019, 0.038, 0.075, 0.15 and 0.30 mg a.i./L. The concentration of acetone in the solvent control and all treatment groups was 0.10 mL/L in order to maximise the solubility of RH-117,281 technical in saltwater. All test solutions appeared clear and colorless.

A continuous-flow diluter was used to deliver each concentration of the test substance, a solvent control and a negative (dilution water) control. Syringe pumps (Harvard Apparatus) were used to deliver the five test substance stock solutions and the solvent for the solvent control into mixing chambers assigned to each treatment and the solvent control. The stock solutions were diluted with filtered saltwater in the mixing chambers in order to obtain the desired test concentrations. The flow of dilution water to the mixing chambers was controlled by rotameters. Rotameters were calibrated prior to test initiation and at approximately weekly intervals thereafter. The flow of test water from each mixing chamber was split and allowed to flow into four replicate test chambers. The proportion of the test water that was split into each

replicate was checked prior to the test, and at weekly intervals thereafter to ensure that flow rates varied by no more than ±10% of the mean for the four replicates.

The diluter was adjusted so that each test chamber received approximately 6.4 volume additions of test water every 24 hours. The test substance delivery pumps were calibrated before the test. The general operation of the diluter was checked visually at least two times per day during the test and once at the end of the test. The delivery of test substance to test chambers began approximately 53 hours prior to test initiation in order to establish equilibrium of the concentrations of the test substance.

The test chambers were 9-L glass aquaria filled with approximately 7 L of test solution. The depth of the test water in a representative test chamber was approximately 16 cm, they were impartially positioned in a temperature-controlled environmental chamber.

The embryo incubation cups were suspended in the water column of each test chamber and attached to a rocker arm. The reciprocating motion of the rocker arm (approximately 2 rpm) facilitated circulation of test water around the embryos during incubation. The incubation cups were constructed from glass cylinders approximately 50 mm in diameter with 425 J.im Nytex screen mesh attached to the bottom with silicone sealant.

The water used for testing was natural seawater collected at Indian River Inlet, Delaware, and diluted to a salinity of approximately 20% with Wildlife International Ltd. well water. The freshly-collected seawater was passed through a sand filter to remove particles greater than approximately 25 µm, and pumped into a 37,800-L storage tank and aerated with spray nozzles. The water again was filtered (0.2 µm) to remove microorganisms and particles. Prior to use, a UV sterilizer was provided as an additional method of water treatment.

Lighting used to illuminate test chambers during culturing and testing was provided by fluorescent tubes that emitted wavelengths similar to natural sunlight (Colortone® 50). A photoperiod of 16 hours of light and 8 hours of dark was controlled with an automatic timer. A 30-minute transition period of low light intensity was provided when lights went on and off to avoid sudden changes in lighting. Light intensity at test initiation was approximately 640 lux over a negative control replicate at the surface of the water. Light intensity was measured using a SPER Scientific Ltd. light meter. Temperature was measured in each test chamber at the beginning and end of the test and at weekly intervals during the test using a liquid-in-glass thermometer. Temperature also was measured continuously in one negative control replicate using a Fulscope ERIC recorder. The target test temperature during the study was 25±2°C. The pH of the water was measured in alternate replicates at the beginning and end of the test and at weekly intervals during the test. Dissolved oxygen content was measured daily in alternate replicates of each treatment and control group during the first 7 days of the test, at weekly intervals during the test, and at test termination. Salinity was measured in alternating replicates of the negative control at the beginning of the test, once a week during the test, and at test termination. Measurements of pH were made using a Fisher Accumet Model 915 pH meter and dissolved oxygen was measured using a Yellow Springs Instrument Model 51B dissolved oxygen meter. Salinity was measured using an Aquafauna Bio-Marine, Inc. refractometer.

Sheepshead minnow embryos used in this test were less than 24 hours old and were obtained from a culture maintained by Wildlife International Ltd., Easton, Maryland. Newly-hatched larvae were fed live brine shrimp nauplii (*Artemia sp.*) 3 times per day during the first 7 days post-hatch. On days 8 through 26 post-hatch, all fish were fed live brine shrimp nauplii 3 times daily on weekdays and at least 2 times daily on weekends. On day 27 and 28 post-hatch, fish were not fed to allow for clearance of the digestive tracts before weight measurements were made. Brine shrimp nauplii were obtained by hatching cysts purchased from Bonneville Artemia International Inc., Salt Lake City, Utah. Rations were adjusted each week to account for losses due to mortality and growth of the fish.

Biomass loading (the total wet weight of the fish in the negative control replicate test chamber per liter of test water) at the end of the test was calculated to be 0.034 g fish per liter of test water that passed through

the test chamber during a 24-hour period. Instantaneous loading was 0.22 g fish per liter of test water in the test chamber at any given time.

Concentrations of RH-117,281 technical in the test solutions were measured at pretest, days 0, 7, 14,21, 28 and at the end of the test by HPLC-UV with a method validated by the laboratory. Water samples were collected during the test and analyzed for RH-117,281 by HPLC. Water samples were collected from one replicate test chamber of each treatment and control group prior to the test (pre-test) to evaluate diluter performance. Water samples were also collected from one alternating replicate test chamber of each treatment and control group on Days 0, 7, 14, 21, 28 and at test termination. An additional set of samples was collected on Day 0 due to an analytical interference. All water samples were collected from mid-depth of each test chamber. The analytical method and the results of the measurements were verified in an addendum to the report (Milligan et al., 2020).

Observations of mortality were made twice during the first 24 hours of the embryo exposure period and daily until hatch. After hatching, larvae were observed daily to evaluate the number of mortalities. The number of individuals exhibiting clinical signs of toxicity or abnormal behavior during the 28-day post-hatch exposure period were also evaluated. From these observations, hatching success, time to hatch, and post-hatch growth and survival were evaluated. Hatching success was calculated as the percentage of viable embryos that hatched successfully. Post-hatch survival was calculated as the number of larvae surviving to test termination divided by the number of embryos which hatched successfully. Post-hatch growth of the sheepshead minnows was evaluated at the conclusion of the 28-day post-hatch exposure period. Total lengths for each surviving fish were determined with the SIGMA SCANTM scientific measurement system and wet and dry weights were measured using an analytical balance.

In the original study, hatching success, post-hatch survival, wet weight, dry weight, and total length of the juvenile fish were test endpoints that were analyzed statistically. Negative and solvent control groups were compared using either Student's t-test or 2X2 contingency tables. When no differences were detected between the two control groups ($p \leq 0.05$), those data were pooled and used to assess treatment-level effects. Survival data were analysed using 2X2 contingency tables and the chi-square test to identify treatment groups that showed a statistically significant difference ($p \leq 0.05$) from the pooled control group. Growth data were evaluated for normality using the Shapiro-Wilks' test and for homogeneity of variance using Bartlett's test. For data with unequal replicate sample sizes which passed both homogeneity of variance and normality tests, the Bonferroni t-test was used to evaluate differences between treatment and control means. The results of the statistical analyses were used to aid in the determination of the NOEC and the LOEC. All statistical tests were performed on a personal computer using SPSSIPC Version 2.0 (5) or TOXSTAT® 3.4 (6) statistical software.

In the addendum to the report (Milligan et al, 2020), the test endpoints were analysed again statistically. The statistical analyses used to estimate the $LC/EC_x$ values were based on the procedures provided in current study guidelines and the OECD (2006). It was performed in the light of approaches in the statistical analysis of ecotoxicity data guidance. It was checked if LC/ECx values could be estimated (e.g., $LC_{10}$, $LC_{20}$ and $LC_{50}$ for survival (hatching success and post-hatch larval survival); $EC_{10}$, $EC_{20}$ and $EC_{50}$ for growth). These values were determined from a regression model, and calculations were based on the following conditions: the test concentrations must bracket the LC/ECx so that the LC/ECx comes from interpolation rather than extrapolation; the LC/ECx was estimated so that (i) the 95% confidence interval reported for LC/ECx does not contain zero and is not overly wide, (ii) the 95% confidence interval for the predicted mean at LC/ECx does not contain the control mean, and (iii) there is no significant lack-of-fit of regression model to the data. The LCx values for hatching success were estimated with the Log-Gompertz model, while the LCx values for post-hatch survival were estimated with the linear interpolation method, using CETIS software (version 1.9.3.0) if possible. The ECx values for the growth (total length, wet weight and dry weight) data were estimated with the Bruce-Versteeg method in CETIS software, when possible. When reliable LCx or ECx values together with their 95% confidence intervals could not be

determined, an explanation was provided – in line with data requirements of Commission Regulation (EU) No. 283/2013 and EFSA Technical Report (2019) on general recurring issues in ecotoxicology.

## Results and discussion

All water quality measurements remained within acceptable limits throughout the test. Measurements of salinity in the negative control test chambers were consistently 20 parts per thousand. Measurements of pH ranged from 8.0 to 8.3 and showed no obvious difference between control and treatment groups. Dissolved oxygen concentrations remained ≥60 percent of saturation and all temperature measurements were within the desired range of 25 ± 2°C.

Nominal concentrations selected for use in this study were 0.019,0.038,0.075, 0.15 and 0.30 mg a.i./L. Mean measured concentrations were 0.019, 0.040, 0.078, 0.15 and 0.25 mg a.i./L. These mean values represent 100, 105, 104, 100 and 83%, respectively, of the nominal test concentrations. No precipitates were observed in the test solutions.

**Table A 41: Mesured test item concentrations**

| Nominal test concentration mg a.i./L) | Measured concentration (mg a.i./L) | | | | | | Mean | % of nominal |
|---|---|---|---|---|---|---|---|---|
| | Day 0 | Day 7 | Day 14 | Day 21 | Day 28 | Day 34 | | |
| Negative control | < LOQ[1] | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | -- | -- |
| Solvent control | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | -- | -- |
| 0.019 | 0.0183 | 0.0218 | 0.0175 | 0.0200 | 0.0193 | 0.0200 | 0.019 | 100 |
| 0.038 | 0.0403 | 0.0405 | 0.0371 | 0.0434 | 0.0390 | 0.0417 | 0.040 | 105 |
| 0.075 | 0.0744 | 0.0727 | 0.0791 | 0.0783 | 0.0799 | 0.0816 | 0.078 | 104 |
| 0.15 | 0.147 | 0.126 | 0.141 | 0.155 | 0.153 | 0.155 | 0.15 | 100 |
| 0.30 | 0.242 | 0.210 | 0.216 | 0.209 | 0.297 | 0.302 | 0.25 | 83 |

[1] limit of quantificatio (LOQ) = 0.010 mg a.i./L

Hatching success and time to hatch

Sheepshead minnow embryos either hatched on day 5 or day 6. Hatching success in the negative and solvent control groups averaged 88 and 89%, respectively. There were no statistically significant ($p>0.05$) differences in hatching success between the negative and solvent control groups and the controls were pooled for comparisons among the treatment groups. No statistically significant ($p>0.05$) differences in hatching success existed between the pooled control group and any RH-117,281 treatment group. Daily observations of embryos and newly hatched larvae indicated that there were no apparent differences in time to hatch between the control groups and any of the treatments tested.

**Table A 42: Hatching success of sheepshead minnow embryos exposed to the test substance**

| Mean measured test concentration (mg a.i./L) | Replicate | No. of eggs exposed | Total number of hatched embryos | | | | | | | No. hatched | Replicate % hatching success[1] | Treatment % hatching success |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | | | |
| Negative Control | A | 20 | 0 | 0 | 0 | 0 | 0 | 10 | 18 | 18 | 90 | 88 |
| | B | 20 | 0 | 0 | 0 | 0 | 0 | 10 | 19 | 19 | 95 | |
| | C | 20 | 0 | 0 | 0 | 0 | 0 | 6 | 15 | 15 | 75 | |
| | D | 20 | 0 | 0 | 0 | 0 | 0 | 7 | 18 | 18 | 90 | |
| Solvent Control | A | 20 | 0 | 0 | 0 | 0 | 0 | 5 | 19 | 19 | 95 | 89 |
| | B | 20 | 0 | 0 | 0 | 0 | 0 | 6 | 18 | 18 | 90 | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | 20 | 0 | 0 | 0 | 0 | 0 | 6 | 18 | 18 | 90 | |
| | D | 20 | 0 | 0 | 0 | 0 | 0 | 5 | 16 | 16 | 80 | |
| 0.019 | A | 20 | 0 | 0 | 0 | 0 | 0 | 5 | 16 | 16 | 80 | 83 |
| | B | 20 | 0 | 0 | 0 | 0 | 0 | 5 | 17 | 17 | 85 | |
| | C | 20 | 0 | 0 | 0 | 0 | 0 | 5 | 18 | 18 | 90 | |
| | D | 20 | 0 | 0 | 0 | 0 | 0 | 6 | 15 | 15 | 75 | |
| 0.040 | A | 20 | 0 | 0 | 0 | 0 | 0 | 6 | 15 | 15 | 75 | 81 |
| | B | 20 | 0 | 0 | 0 | 0 | 0 | 8 | 16 | 16 | 80 | |
| | C | 20 | 0 | 0 | 0 | 0 | 0 | 7 | 16 | 16 | 80 | |
| | D | 20 | 0 | 0 | 0 | 0 | 0 | 6 | 18 | 18 | 90 | |
| 0.078 | A | 20 | 0 | 0 | 0 | 0 | 0 | 6 | 15 | 15 | 75 | 86 |
| | B | 20 | 0 | 0 | 0 | 0 | 0 | 6 | 16 | 16 | 80 | |
| | C | 20 | 0 | 0 | 0 | 0 | 0 | 5 | 18 | 18 | 90 | |
| | D | 20 | 0 | 0 | 0 | 0 | 0 | 10 | 20 | 20 | 100 | |
| 0.15 | A | 20 | 0 | 0 | 0 | 0 | 0 | 10 | 17 | 17 | 85 | 84 |
| | B | 20 | 0 | 0 | 0 | 0 | 0 | 8 | 16 | 16 | 80 | |
| | C | 20 | 0 | 0 | 0 | 0 | 0 | 10 | 18 | 18 | 90 | |
| | D | 20 | 0 | 0 | 0 | 0 | 0 | 12 | 16 | 16 | 80 | |
| 0.25 | A | 20 | 0 | 0 | 0 | 0 | 0 | 6 | 14 | 14 | 70 | 80 |
| | B | 20 | 0 | 0 | 0 | 0 | 0 | 5 | 14 | 14 | 70 | |
| | C | 20 | 0 | 0 | 0 | 0 | 0 | 4 | 17 | 17 | 85 | |
| | D | 20 | 0 | 0 | 0 | 0 | 0 | 4 | 19 | 19 | 95 | |

[1] Percent Hatching Success = Number Hatched / Number Exposed

Clinical observations and post-hatch survival

There were no effects on hatching success or post-hatch survival in any RH-117,281 treatment group and all surviving organisms appeared normal throughout the test.

All fish were observed to determine the number of mortalities. The numbers of individuals exhibiting clinical signs of toxicity or abnormal behavior also were evaluated. Surviving fish in the negative and solvent control groups appeared normal throughout the test. The survival percentages in the negative and solvent control groups during the post-hatch period were 97 and 96%, respectively. There were no statistically significant ($p > 0.05$) differences in post-hatch survival between the negative and solvent control groups and the controls were pooled to evaluate treatment-related effects. All surviving fish in the RH-117,281 treatments appeared normal throughout the test and no statistically significant ($p > 0.05$), differences in post-hatch survival existed when compared to the pooled control group. During the test, 4 fish were unaccounted for and were assumed dead.

**Table A 43: Survival of larvae exposed to the test substance for 28 days post-hatch**

| Mean measured test concentration (mg a.i/L) | Replicate | Initial number of larvae | Approximate live counts[1] (days post-hatch) | | | | | Replicate percent survival[2] | Treatment percent survival |
|---|---|---|---|---|---|---|---|---|---|
| | | | 0 | 7 | 14 | 21 | 28 | | |
| **Negative Control** | A | 18 | 18 | 16 | 16 | 16 | 16 | 89 | 97 |
| | B | 19 | 19 | 19 | 19 | 19 | 19 | 100 | |
| | C | 15 | 15 | 15 | 15 | 15 | 15 | 100 | |
| | D | 18 | 18 | 18 | 18 | 18 | 18 | 100 | |
| **Solvent Control** | A | 19 | 19 | 19 | 19 | 19 | 19 | 100 | 96 |
| | B | 18 | 18 | 17 | 17 | 17 | 17 | 94 | |
| | C | 18 | 18 | 17 | 17 | 17 | 16 | 89 | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | D | 16 | 16 | 16 | 16 | 16 | 16 | 100 | |
| **Pooled Controls** | - | 141 | 141 | 137 | 137 | 137 | 136 | - | 96 |
| **0.019** | A | 16 | 16 | 15 | 15 | 15 | 15 | 94 | 97 |
| | B | 17 | 17 | 16 | 16 | 16 | 16 | 94 | |
| | C | 18 | 18 | 18 | 18 | 18 | 18 | 100 | |
| | D | 15 | 15 | 15 | 15 | 15 | 15 | 100 | |
| **0.040** | A | 15 | 15 | 15 | 15 | 15 | 15 | 100 | 98 |
| | B | 16 | 16 | 16 | 16 | 16 | 16 | 100 | |
| | C | 16 | 16 | 16 | 16 | 16 | 16 | 100 | |
| | D | 18 | 18 | 18 | 18 | 18 | 17 | 94 | |
| **0.078** | A | 15 | 15 | 15 | 15 | 15 | 14 | 93 | 96 |
| | B | 16 | 16 | 16 | 16 | 16 | 15 | 94 | |
| | C | 18 | 18 | 17 | 17 | 17 | 17 | 94 | |
| | D | 20 | 20 | 20 | 20 | 20 | 20 | 100 | |
| **0.15** | A | 17 | 17 | 17 | 17 | 17 | 17 | 100 | 99 |
| | B | 16 | 16 | 16 | 16 | 16 | 15 | 94 | |
| | C | 18 | 18 | 18 | 18 | 18 | 18 | 100 | |
| | D | 16 | 16 | 16 | 16 | 16 | 16 | 100 | |
| **0.25** | A | 14 | 14 | 14 | 14 | 14 | 14 | 100 | 94 |
| | B | 14 | 14 | 12 | 12 | 12 | 12 | 86 | |
| | C | 17 | 17 | 16 | 16 | 16 | 16 | 94 | |
| | D | 19 | 19 | 18 | 18 | 18 | 18 | 95 | |

[1] Live counts made on Days 0 through 21 post-hatch were variable due to the difficulty in counting large numbers of living fish. The counts made on Day 28 were exact counts of all fish surviving at the end of the test (i.e., the number of fish weighed and measured).

[2] Percent Survival = $\frac{\text{Live Counts on Day 28 Post-Hatch}}{\text{Initial Number of Larvae}} \times 100$

Growth

Growth was evaluated at test termination by measuring the total length, wet weight and dry weight of each surviving fish. Total length, wet weight and dry weight measurements of the negative and solvent control fish were compared using Student's t-test. No statistically significant differences between the two control groups were found ($p>0.05$) for total length, wet weight and dry weight measurements. Therefore, those data were pooled for evaluation of treatment-related growth effects.

There were no apparent differences in fish growth between the pooled control group and the 0.019 and 0.040 mg a.i./L treatment groups. Any differences in mean total lengths, mean wet weights or mean dry weights were slight and not statistically different ($p > 0.05$) from the pooled control group. However, wet weight was reduced in a dose-dependent, statistically significant ($p > 0.05$), manner in the 0.078, 0.15 and 0.25 mg a.i./L treatment groups when compared to the pooled control group. Length and dry weight of sheepshead minnows in the 0.25 mg a.i./L treatment group were also reduced in comparison to the pooled controls ($p > 0.05$). Therefore, the LOEC for growth was 0.078 mg a.i.1L and the NOEC was 0.040 mg a.i./L.

**Table A 44: Total length, wet weight and dry weight of sheepshead minnow larvae at the end of the 28-day post-hatch observation period**

| Mean measured test concentration (mg a.i./L) | Total length (mm) Mean (± SD) | Wet weight (mg) Mean (± SD) | Dry weight (mg) Mean (± SD) |
|---|---|---|---|
| Negative control | 18.3 ± 1.94 | 93.8 ± 27.4 | 20.8 ± 6.1 |

| | | | |
|---|---|---|---|
| Solvent control | 18.6 ± 1.79 | 91.9 ± 26.6 | 20.2 ± 6.0 |
| Pooled controls | 18.5 ± 0.38 | 93.0 ± 3.62 | 20.6 ± 1.4 |
| 0.019 | 18.4 ± 0.40 | 88.5 ± 5.76 | 20.5 ± 1.19 |
| 0.040 | 18.5 ± 0.38 | 88.8 ± 3.16 | 19.7 ± 0.97 |
| 0.078 | 17.9 ± 0.53 | 84.6 ± 8.11* | 19.1 ± 1.94 |
| 0.15 | 18.0 ± 0.39 | 80.3 ± 5.85* | 18.6 ± 1.21 |
| 0.25 | 17.5 ± 0.43* | 76.5 ± 6.44* | 17.3 ± 1.87* |

* Indicates a significant difference from the pooled controls using the Bonferroni t-test ($p \leq 0.05$).

Growth was the most sensitive biological factor. The wet weight of sheepshead minnows in the 0.078, 0.15 and 0.25 mg a.i./L treatment groups were statistically reduced ($p \leq 0.05$) when compared to the pooled control group. Length and dry weight of sheepshead minnows in the 0.25 mg a.i./L treatment group were also statistically reduced *(*$p \leq 0.05$) when compared to the pooled control group.

For the study, the no-observed-effect-concentration (NOEC) was 0.040 mg a.i./L, while the lowest-observed-effect-concentration (LOEC) was 0.078 mg a.i./L. The maximum acceptable toxicant concentration (MATC) was 0.056 mg a.i./L (the geometric mean of the NOEC and LOEC). The results of the study were based on mean measured test concentrations.

**Table A 45: Study results with regard to total length, wet weight and dry weight of sheepshead minnow larvae at the end of the 28-day post-hatch observation period**

| | |
|---|---|
| NOEC | 0.040 mg a.i./L |
| LOEC | 0.078 mg a.i./L |
| Maximum acceptable toxicant concentration (MATC) | 0.056 mg a.i./L |

In the addendum to the report (Milligan et at., 2020), the $EC_{10}$ value for fish wet weight was estimated to be 0.093 mg a.i./L, with a 95 % confidence interval of 0.054 to 0.14 mg a.i./L. The $EC_{20}$ and $EC_{50}$ values for wet weight were not reportable, since the ECx values were extrapolated above the highest mean measured test concentration and the 95 % confidence intervals were overly wide. The decreases in wet weight in the treatment groups were ≤17 % compared to the pooled control group. The lack of a strong concentration dependent response supports the inability to reliably estimate $EC_{20}$ and $EC_{50}$ values for fish wet weight.

## Conclusion

The effects of RH-117,281 technical (zoxamide technical) on the early life stage development of sheepshead minnows *(Cyprinodon variegatus)* were assessed during a 34-day toxicity test. Up to a nominal concentration of 0.30 mg a.i./L (mean measured concentration of 0.25 mg a.i./L, or 83% of the nominal test concentration) were tested. Acetone (100 µL acetone/L) served as a solvent for zoxamide. As a result of the study, no effects from the untreated control and the solvent control were found. As treatment-related effects of zoxamide, statistically significant decreases were evident in total length and fish dry weight at a mean measured concentration of 0.25 mg a.i./L, and a statistically significant decrease in wet weight at mean measured concentrations of 0.078, 0.15 and 0.25 mg a.i./L ($p \leq 0.05$). The effect on wet weight was slight (<10%) in the 0.078 mg a.i./L treatment group, while the effects on all growth endpoints in the 0.25 mg a.i./L treatment group were more pronounced. Based on effects on wet weight, the overall LOEC for the study was identified as 0.078 mg a.i./L (or 78 µg a.i./L).

The NOEC for this study was 0.040 mg a.i./L. The LOEC, based on wet weight, was 0.078 mg a.i/L. The MATC was calculated to be 0.056 mg a.i./L.

The $EC_{10}$ value for fish wet weight was estimated to be 0.093 mg a.i./L, with a 95 % confidence interval of 0.054 to 0.14 mg a.i./L. The $EC_{20}$ and $EC_{50}$ values for wet weight were not reportable, since the ECx

values were extrapolated above the highest mean measured test concentration and the 95 % confidence intervals were overly wide.

The study is regarded valid.

(xxx. 1998)

## A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

## A 2.3 KCP 10.3 Effects on arthropods

## A 2.3.1 KCP 10.3.1 Effects on bees

## A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

### A 2.3.1.1.1 Study 1 - Cymoxanil 33% + Zoxamide 33% WG: Acute toxicity to honey bees

| | |
|---|---|
| Comments of zRMS: | This study was previously evaluated and considered to be acceptable. Please refer to product fRR dated January 2012. |

| | |
|---|---|
| **Reference:** | **KCP 10.3.1.1/01** |
| Report | Colli, M., 2006: Effects, acute oral and acute contact toxicity of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' on the honeybee, Apis mellifera, L., in laboratory (Limit test).<br>Oxon Italia S.p.A., Italy<br>Biotecnologie BT S.r.l., Italy, Report No. BT026/06, GLP, Not published |
| Guideline(s): | EPPO 170 (2001)<br>OECD 213 (1998)<br>OECD 214 (1998) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication<br>(if vertebrate study) | No |

**Executive Summary**

Oral acute toxicity of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' was tested on young adult of honeybee *Apis mellifera.* Five replicates of 10 bees were treated with the product at the dose of 100 µg/bee, the control group received water only.
Dimethoate was used as reference test item.
Mortality and behavioural abnormalities were assessed at 3, 24, 48 and 72 h after the application.
No mortality neither abnormalities occurred after oral exposure, so oral $LD_{50}$ of the product is > 100 µg formulation/bee.

## I. MATERIALS AND METHODS

### A. MATERIALS

| | |
|---|---|
| **1.Test Material:** | |
| **Description:** | Granules |
| **Lot/Batch #:** | Batch code BPL 212 |
| **Purity:** | Nominal: Cymoxanil 330 g/kg, Zoxamide 330 g/kg<br>Measured: Cymoxanil 322.9 g/kg, Zoxamide 333.2 g/kg |
| **Stability of test compound** | June 2008 (expiry date) |
| **2. Vehicle and/or positive control** | Solutions prepared in sucrose solution, dimethoate (Perfekthion) used as positive control |
| **3. Test animals** | Honeybees |
| **Species:** | *Apis mellifera* |
| **Strain** | Not relevant |
| **Age:** | Young adult |
| **Weight at dosing:** | Not relevant |
| **Source:** | BIOTECNOLOGIE BT - Todi (PG) – Italy |
| **Acclimation period** | 2 hours |
| **Diet:** | Aqueous sucrose solution |
| **Water:** | Not relevant |
| **Housing:** | Plastic cages (5.5 x 11 x 10.5 cm) with a piece of wax held in suspension |
| **4. Environmental conditions** | |
| **Temperature:** | 25-25.33°C |
| **Humidity:** | 52.5 – 59.5% |
| **Air changes:** | Ventilation |
| **Photoperiod:** | Constant darkness |

### B. STUDY DESIGN AND METHODS

| | |
|---|---|
| **1. In life dates** | 4 – 7 July 2006 |
| **2. Experimental treatment** | Five replicates of 10 bees were treated with the product at the dose of 100 µg/bee, the control group received water only. 200 µl of 50 % sucrose – water solution containing different doses of test item solution were given to each cage for 3 hours.<br>Simultaneously a test with reference item dimethoate (Perfekthion) at dose of 0.005 µl formulation/bee was performed. |
| **3. Observations** | During the exposure bees were observed for mortality and abnormal behaviour at 3, 24, 48 and 72 h. |
| **4. Statistics** | Anova – Manova and Student's T – Test |

## II. RESULTS AND DISCUSSION

### A. FINDINGS

No mortality or behavioural abnormalities occurred after 72 h of exposure; no repellent effect of the test item on bees was also noticed.

## III. CONCLUSIONS

Oral 72 h $LD_{50}$ of 'Cymoxanil 33% + Zoxamide 33% WG' is > 100 µg formulation/bee.

(Colli M. 2006)

**A 2.3.1.1.2 Study 2 - CYMOXANIL 33% + ZOXAMIDE 33% WG: Acute effects on**

**bumblebees**

| | |
|---|---|
| Comments of zRMS: | The study was conducted to guideline and according to the principles of GLP. The study is considered to be valid as mortality in the control was 0% and in reference item was 100%. |

**Reference:** **KCP 10.3.1.1/02**

Report: Amsel, K., 2017: Acute toxicity of Cymoxanil 33% + Zoxamide 33% to the bumblebee *Bombus terrestris* L. under laboratory conditions
Gowan Crop Protection Ltd., UK, Oxon Italia S.p.A, Italy
BioChem Agrar, Gerichshain, Germany, Report No. 17 48 BBA 0003, GLP, Not published

Guideline(s): Proposal for a New OECD Guideline for the Testing of Chemicals: Bumblebee, acute contact and oral toxicity test (Draft, August 2016)

Deviations: No

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): No

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | Zoxamide 33% + Cymoxanil 33% (Reboot / GWN 9823) (30AL5020) |
| **Active substance content (analysed)** | 33.0% w/w zoxamide<br>32.8% w/w cymoxanil |
| **Species:** | *Bumblebee – Bombus terrestris* L. (Hymenoptera, Apidae) |
| Age: | Adult bumblebee workers |
| Number of animals: | 60 replicates with 1 bumblebee each (= 60 bumblebees) for control and treatment<br>30 bumblebees for reference item |
| Weight: | about 150-300 mg |
| Source: | Biobest Belgium N.V., Ilse Velden 18, 2260 Westerlo, Belgium; Delivered from Katz Biotech AG |
| Acclimation period: | 17 hours (starving period of 4 hours) |
| Feeding: | 50% (w/v) sucrose solution |
| Housing: | Nicot cages (part of the Nicot queen bee rearing system) with a length of 7 cm and a diameter of 2 cm. |
| **Environmental conditions** | |
| Temperature: | 23.4 – 24.5°C |
| Photoperiod: | Constant darkness (diffuse artificial light of about 100 lux only during handling and assessments) |

| | |
|---|---|
| Relative humidity: | 50 - 78% |
| **Application rate(s)** | Oral test:<br>Test item: 547.2 µg product/bumblebee in sucrose solution<br>Control: Sucrose solution<br>Contact test:<br>Test item: 608 µg product/bumblebee<br>Control: deionised water<br>Vehicle control: 0.5% v/v TritonX |
| **Positive control** | Dimethoate EC 400 (405.2 g/L dimethoate; analysed content) |
| **Post exposure observation period** | 48 hours |
| **Remarks** | None |

The acute toxicity of Cymoxanil 33% + Zoxamide 33% WG (WG formulation containing 33% w/w of each cymoxanil and zoxamide) to the bumblebee *Bombus terrestris* L. in a laboratory test after oral and contact exposure for 48 hours.

Based on the results of range-finders, the final tests were done as limit tests with a topical application rate of 608.0 µg Cymoxanil 33% + Zoxamide 33% WG/bumblebee (equivalent to 200.6 µg cymoxanil and 199.4 µg zoxamide) and a nominal oral application rate of 608.0 µg Cymoxanil 33% + Zoxamide 33% WG/bumblebee (equivalent to 200.6 µg cymoxanil and 199.4 µg zoxamide) with a mean consumed oral application rate of 547.2 µg Cymoxanil 33% + Zoxamide 33% WG/bumblebee (equivalent to 180.6 µg cymoxanil and 179.5 µg zoxamide, considering analysed active substance concentrations in the product).

In the contact test, deionised water as well as deionised water with a wetting agent (0.5% (v/v) Triton X) - used as vehicle for the test and reference item - served as controls. In the oral test, the control group was treated with 50% (w/v) sucrose solution. In both tests, Dimethoate EC 400 (containing 405.2 g/L dimethoate analysed content) served as reference item.

In both studies, assessments of mortality and behavioural effects were done after 4, 24 and 48 hours. The statistical calculations were performed with the computer program ToxRat Professional 3.2.1 (2015).

## Results and discussion

Environmental parameters (temperature, light intensity and relative humidity) remained within acceptable limits throughout the study. The cultures were exposed at 23.4 – 24.5°C under continuous illumination (100 lux) with relative humidity 50 - 78%.

**Table A 46: Applied and consumed dosages in the oral toxicity test**

| **Treatment group** | **Test solution ID** | **Item applied** | **Applied dosages** | | **Actual intake of the applied test item** | | **Applied volume (µL/bumblebee)** |
|---|---|---|---|---|---|---|---|
| Control | AC | Sucrose solution | - | | | | 40 |
| | | | **[µg product/ bumblebee]** | **[µg a.s./ bumblebee]** | **[µg product/ bumblebee]** | **[µg a.s./ bumblebee]** | |
| Test item | AT | Cymoxanil 33% + Zoxamide 33% WG* | 608.0 | 400.1 | 547.2 | 360.1 | 40 |
| Reference | AR | Dimethoate | 4.00 | 1.51 | 3.82 | 1.44 | 40 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| item | | EC 400* | | | | | |

* based on analysed content of total a.s., Calculations are performed with non-rounded values.

The test and reference item were solved in 50% (w/v) sucrose solution.

In the oral toxicity test, no mortality occurred in the control group fed with 50% (w/v) sucrose solution. In the test item treatment, no mortality occurred after mean oral consumption of 547.2 µg Cymoxanil 33% + Zoxamide 33% WG within 48 hours. No behavioural effects of bumblebees were noted at the tested dose rate in the oral toxicity test. The resulting $LD_{50}$ after 48 hours was estimated to be > 547.2 µg consumed Cymoxanil 33% + Zoxamide 33% WG/bumblebee.

**Table A 47: Mortality and behaviour of bumblebees in the oral toxicity test**

| Treatment group (dosage unit) | Dosage consumed | After 4 hours | | | | After 24 hours | | | | After 48 hours | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mortality mean % | BA sum | | | Mortality mean % | BA sum | | | Mortality mean % | BA sum | | |
| | | total | ∑ | A | M | total | ∑ | A | M | total | ∑ | A | M |
| Control | Sucrose | 0.0 | 0 | 0 | 0 | 0.0 | 0 | 0 | 0 | 0.0 | 0 | 0 | 0 |
| **Cymoxanil 33% + Zoxamide 33% WG** [µg product/ bumblebee] | 547.2 | 0.0 | 0 | 0 | 0 | 0.0 | 0 | 0 | 0 | 0.0 | 0 | 0 | 0 |
| **Reference item** [µg a.s./ bumblebee] | 1.44 | 0.0 | 0 | 0 | 0 | 90.0 | 0 | 0 | 02 | 100.0 | - | - | - |

Mortality results are averages based on 60/30 replicates consisting of 1 bumblebee each
BA: Behavioural abnormalities
A: affected
M: moribund
Calculations are performed with non-rounded values

In the contact toxicity test, no mortality occurred in the control groups treated either with deionised water or with Triton X solution. In the test item treatment, mortality was 3.3% after 48 hours after thoracic application of 608.0 µg Cymoxanil 33% + Zoxamide 33% WG/bumblebee. No behavioural effects of bumblebees occurred in the contact toxicity test. Also, the range finder test showed no mortality and behaviour effects up to the highest tested dose rate of 608.0 µg Cymoxanil 33% + Zoxamide 33% WG/bumblebee within 48 hours. The resulting $LD_{50}$ after 48 hours was estimated to be > 608.0 µg Cymoxanil 33% + Zoxamide 33% WG/ bumblebee.

**Table A 48: Mortality and behaviour of bumblebees in the contact toxicity test**

| Treatment group (dosage unit) | Dosage applied | After 4 hours | | | | After 24 hours | | | | After 48 hours | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mortality mean % | BA sum | | | Mortality mean % | BA sum | | | Mortality mean % | BA sum | | |
| | | total | ∑ | A | M | total | ∑ | A | M | total | ∑ | A | M |
| Control | Water | 0.0 | 0 | 0 | 0 | 0.0 | 0 | 0 | 0 | 0.0 | 0 | 0 | 0 |
| | 0.5% Triton X | 0.0 | 0 | 0 | 0 | 0.0 | 0 | 0 | 0 | 0.0 | 0 | 0 | 0 |
| **Cymoxanil 33% + Zoxamide** | 608.0 | 0.0 | 0 | 0 | 0 | 0.0 | 0 | 0 | 0 | 0.0 | 0 | 0 | 0 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **33% WG** [µg product/ bumblebee] | | | | | | | | | | | | | |
| **Reference item** [µg a.s./ bumble-bee] | 10.0 | 0.0 | 0 | 0 | 0 | 100.0 | - | - | - | 100.0 | - | - | - |

Mortality results are averages based on 60/30 replicates consisting of 1 bumblebee each
BA: Behavioural abnormalities
A: affected
M: moribund
Calculations are performed with non-rounded values

**Table A 49: $LD_{50}$-values of the contact and oral toxicity test**

| **$LD_{50}$** | **Contact test** | | **Oral test[1]** | |
|---|---|---|---|---|
| | **24 h** | **48 h** | **24 h** | **48 h** |
| **$LD_{50}$ (µg product/bumblebee)** | > 608.0 | > 608.0 | > 547.2 | > 547.2 |
| **$LD_{50}$ (µg total a.s./bumblebee)** | > 400.1 | > 400.1 | > 360.1 | > 360.1 |

[1] Doses of the oral toxicity test are referring to mean consumed dose

Mortality in the reference item treatment in the contact and oral test was 100.0% after 48 hours. Mortality in all control groups was below 10%. Thus, all validity criteria were met.

## Conclusion

The acute toxicity of Cymoxanil 33% + Zoxamide 33% WG was determined after contact and oral exposure to bumblebees. Based on the results of range-finder studies, the main tests were performed as limit tests with the following results:

The $LD_{50}$ (48 h) after thoracic application was found at > 608.2 µg Cymoxanil 33% + Zoxamide 33% WG/bumblebee (equivalent to 200.6 µg cymoxanil and 199.4 µg zoxamide) and the $LD_{50}$ (48 h) after oral consumption was > 547.2 µg Cymoxanil 33% + Zoxamide 33% WG/bumblebee (equivalent to 180.6 µg cymoxanil and 179.5 µg zoxamide, considering analysed active substance concentrations in the product).

(Amsel K. 2017)

## A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

### A 2.3.1.2.1 Study 1 - CYMOXANIL 33% + ZOXAMIDE 33% WG: Chronic toxicity to adult honey bees

| | |
|---|---|
| Comments of zRMS: | The study was conducted to guideline and according to the principles of GLP. The study is considered to be valid as mortality after 10 days in the control was 3.3% in group AC and 6.7% in group BC. The mean mortality after 10 days in reference item was 100%. |

**Reference:** **KCP 10.3.1.2/01**

Report Ruhland, S., 2018: Chronic toxicity of Cymoxanil 33% + Zoxamide 33% WG to the honey bee *Apis mellifera* L. under laboratory conditions
Gowan Crop Protection Ltd., UK, Oxon Italia S.p.A, Italy
BioChem agrar, Germany, Report No. 17 48 BAC 0005, GLP, Not published

| | |
|---|---|
| Guideline(s): | Revised proposal for a new OECD Guideline for the testing of chemicals: Honey bee (*Apis mellifera* L.), chronic oral toxicity test (10 day feeding test in the laboratory) (October 2016)<br>OECD 213 (1998)<br>SANCO/3029/99 rev. 4 (2000) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | No |

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | Reboot (GWN-9823) (30AL5020) |
| **Active substance content (nominal)** | 33.00% (w/w) cymoxanil (analysed)<br>32.8% (w/w) zoxamide (analysed) |
| **Species:** | Honey bee - *Apis mellifera* L. subspecies Buckfast |
| Age: | 2 days old |
| Number of animals: | 3 replicates of 10 bees each for control<br>3 replicates of 10 bees each oer test item dose<br>3 replicates of 10 bees each per reference item dose |
| Weight: | not relevant |
| Source: | BioChem agrar, Germany |
| Acclimation period: | 2 – 3 days under test conditions<br>photoperiod: darkness<br>temperature: 33 ± 2 °C |
| Food: | 50% (w/v) aqueous sucrose solution<br>The bees were fed with 50% (w/v) aqueous sucrose solution including the test item or the reference item. The control treatments were fed with 50% (w/v) aqueous sucrose solution. The treated/untreated food was provided *ad libitum* in a plastic syringe, which had been weighed before application. |
| Housing: | Aluminium cages with the dimensions: 95 mm x 60 mm x 70 mm; with holes in the lateral walls for ventilation and two glass plates (one in front and one in the back) for observation of the bees. |
| **Environmental conditions** | |
| Temperature: | 31.2-33.5 °C |
| Photoperiod: | Darkness (except during assessments) |
| Relative humidity | 58.0-66.5% |
| **Application rate(s)** | Control AC: untreated diet (50% (w/v) aqueous sucrose solution)<br>Control BC: untreated diet (50% (w/v) aqueous sucrose solution + |

| | |
|---|---|
| | 0.1% (w/v) xanthan)<br>Test item (T) = 150, 75.2, 37.6, 18.8 and 9.41 µg product/bee/day<br>3 additional test units without bees but with filled syringes for evaluation of evaporation of each control solution |
| **Post exposure observation period** | 10 days |
| **Remarks** | None |

Based on the results of a range finder, the chronic oral toxicity of Cymoxanil 33% + Zoxamide 33% WG (containing nominally 33% of each cymoxanil and zoxamide) to adult worker bees of *Apis mellifera* L. has been tested under laboratory conditions in a 10-day chronic toxicity feeding test. For this study, 2 days old worker honey bees (*Apis mellifera* L. subspecies Buckfast) were exposed to a daily application of Cymoxanil 33% + Zoxamide 33% WG diluted in the bee food (50% (w/v) aqueous sucrose solution + 0.1% (w/v) xanthan).

Young adults of of *Apis mellifera* L. were daily exposed to 5 doses of Cymoxanil 33% + Zoxamide 33% WG in treated food (50% (w/v) aqueous sucrose solution + 0.1% (w/v) xanthan). The following treatment groups were set up: 5 doses of the test item, 1 untreated control group (50% (w/v) aqueous sucrose solution (=AC)), 1 untreated viscosifier control group (50% (w/v) aqueous sucrose solution (=BC)) + 0.1% (w/v) xanthan and 1 dose of the toxic standard (Dimethoate EC 400 at a nominal dose of 27.3 ng a.i./bee/day) with 3 replicates per dose and 10 bees per replicate. The chronic toxicity of the test item was determined at nominal doses of 150, 75.2, 37.6, 18.8 and 9.41 µg product/bee/day, corresponding to concentrations of 3.832, 1.916, 0.958, 0.479 and 0.239 g product/kg food. 3 additional test units without bees but with filled syringes were set up for evaluation of evaporation of each control solution.

Bee mortality and behavioural effects were daily assessed. Environmental conditions were observed. The concentrations of the active ingredients in the highest and lowest test item feeding concentration of the first day of application (D0) were determined with a method validated according to SANCO/3029/99 rev. 4 (2000). The active ingredients in sucrose solution were analysed by high pressure liquid chromatography (HPLC) with UV-detection (UV) under analytical phase report no. 17 48 BAC 0005. The limit of quantification (LOQ) was defined in the context of this study as the lowest successfully validated fortification level, i.e. 46.40 mg/L of cymoxanil and 46.69 mg/L of zoxamide.

Statistical evaluation was performed by descriptive statistics (Step-down Cochran-Armitage Test) for analysis of the mortality data (one-sided greater, $\alpha = 0.05$) and NOEDD/NOEC. Logit analysis using linear max. likelihood regression was applied for the determination of $LDD_{50/20/10}$ and $LC_{50/20/10}$ (lethal dietary doses/concentration). The statistical program ToxRat Professional 3.2.1 (2015) was used.

## Results and Discussion

Environmental parameters (temperature and relative humidity) remained within acceptable limits throughout the study. The cultures were exposed at 31.2 – 33.5°C with relative humidity 58 – 66.5%. The honeybees were kept under constant darkness except during the assessments.

The application doses were analytically confirmed. The recoveries of cymoxanil in the specimens were 103% and 104%, the recoveries of zoxamide were 86%. No active ingredient was detected in the control specimens.

After 10 days, mortalities of 3.3% were observed in control group AC and 6.7% in control group BC. Taking into account the actual food uptake and the evaporated amount of feeding solution, the bees effectively consumed doses of 72.9, 43.1, 26.6, 18.0 and 10.5 µg product/bee/day - which caused mortalities of 96.7, 80.0, 30.0, 3.3 and 6.7%, respectively after 10 days. Mortalities in the treatment groups with bees

consuming doses of 72.9, 43.1 and 26.6 µg product/bee/day were statistically significantly increased compared to the control group BC.

In the final assessment no treatment related behavioural abnormalities were observed in the test item treatments.

The effective reference dosage in the study was 12.9 ng a.i./bee/day, which caused a mean mortality of 100%.

**Table A 50: Mean mortality and behaviour of bees in the chronic toxicity feeding test after 10 days**

| Treatment group | Treatment group ID | Daily dose | | Concentration [g product/ kg food] | After 10 days | | |
|---|---|---|---|---|---|---|---|
| | | nominal | consumed° | | Mean mortality | | Number of bees with behavioural abnormalities** |
| | | [µg product/bee/day] | | | Absolute [%] | Corrected [%] | |
| Control | AC | - | - | - | 3.3 | - | 0 out of 29 |
| | BC | - | - | - | 6.7 | - | 0 out of 28 |
| Test item | AT | 150 | 72.9 | 3.832 | 96.7* | 96.4 | 0 out of 1 |
| | BT | 75.2 | 43.1 | 1.916 | 80.0* | 78.6 | 0 out of 6 |
| | CT | 37.6 | 26.6 | 0.958 | 30.0* | 25.0 | 0 out of 21 |
| | DT | 18.8 | 18.0 | 0.479 | 3.3 | 0.0 | 0 out of 29 |
| | ET | 9.41 | 10.5 | 0.239 | 6.7 | 0.0 | 0 out of 28 |
| | | [ng a.i./bee/day] | | [mg a.i./ kg food] | | | |
| Reference item | AR | 27.3 | 12.9 | 0.696 | 100 | 100 | - |

Results are averages based on 3 replicates, containing 10 bees each; Calculations are performed with non-rounded values and corrected for evaporation
corrected: corrected mortality (according to SCHNEIDER-ORELLI 1947) for mortality of untreated control group BC, negative values are treated as "0"
° Corrected by subtracting the mean evaporation figure of each day of application
* Statistically significant difference in pairwise comparison between treatment and untreated solvent control (Step-down Cochran-Armitage Test Procedure, one-sided greater, $\alpha = 0.05$)
** Number of bees with behavioural abnormalities referring to number of remaining bees

**Table A 51: Toxicity of Cymoxanil 33% + Zoxamide 33% WG in a chronic toxicity feeding test**

| | Endpoints | 10 d |
|---|---|---|
| Test item doses | $LDD_{50}$ [µg consumed product/bee/day][1] | 34.2 (30.6 – 38.4) |
| | $LDD_{20}$ [µg consumed product/bee/day][1] | 26.4 (22.3 – 29.5) |
| | $LDD_{10}$ [µg consumed product/bee/day][1] | 22.6 (18.2 – 25.9) |
| | NOEDD [µg consumed product/bee/day][2] | 18.0 |
| | $LDD_{50}$ [µg consumed total a.i./bee/day][1] | 22.5 (20.1 – 25.3) |
| | $LDD_{20}$ [µg consumed total a.i./bee/day][1] | 17.4 (14.7 – 19.5) |
| | $LDD_{10}$ [µg consumed total a.i./bee/day][1] | 14.9 (12.0 – 17.1) |
| | NOEDD [µg consumed total a.i./bee/day][2] | 11.9 |
| Test item concentrations | $LC_{50}$ [g product/kg food][1] | 1.356 (1.151 – 1.602) |
| | $LC_{20}$ [g product/kg food][1] | 0.931 (0.722 – 1.103) |

| | | |
|---|---|---|
| | $LC_{10}$ [g product/kg food][1] | 0.747 (0.534 – 0.913) |
| | NOEC [g product/kg food][2] | 0.479 |
| | $LC_{50}$ [g total a.i./kg food][1] | 0.892 (0.757 – 1.054) |
| | $LC_{20}$ [g total a.i./kg food][1] | 0.613 (0.475 – 0.726) |
| | $LC_{10}$ [g total a.i./kg food][1] | 0.492 (0.351 – 0.601) |
| | NOEC [g total a.i./kg food][2] | 0.315 |

[1] Lethal dietary doses/concentrations (95%-cl lower/upper) were calculated using logit analysis (linear max. likelihood regression)

[2] No observed effect dietary dose/concentration was calculated using Step-down Cochran-Armitage Test Procedure; α = 0.05; one sided greater

All validity criteria were met: Control mortality was < 15% and mortality in the reference item group was > 50% after 10 days of exposure. Thus, the study is valid.

## Conclusion

Based on the results of a range finder, the chronic oral toxicity of Cymoxanil 33% + Zoxamide 33% WG (containing nominally 33% of each cymoxanil and zoxamide) to adult worker bees of *Apis mellifera* L. has been tested under laboratory conditions in a 10-day chronic toxicity feeding test.

The chronic oral toxicity of Cymoxanil 33% + Zoxamide 33% WG on young adult honey bees (*Apis mellifera* L.) was investigated in a 10-day chronic, dose-response feeding study under laboratory conditions.

The $LDD_{50}$ was determined to be 34.2 µg consumed product/bee/day (equivalent to 22.5 µg consumed total a.i./bee/day) and the $LC_{50}$ to be 1.356 g product/kg food (equivalent to 0.892 g total a.i./kg food), respectively.

The $LDD_{20}$ was determined to be 26.4 µg consumed product/bee/day (equivalent to 17.4 µg consumed total a.i./bee/day) and the $LC_{20}$ to be 0.931 g product/kg food (equivalent to 0.613 g total a.i./kg food), respectively.

The $LDD_{10}$ was determined to be 22.6 µg consumed product/bee/day (equivalent to 14.9 µg consumed total a.i./bee/day) and the $LC_{10}$ to be 0.747 g product/kg food (equivalent to 0.492 g total a.i./kg food), respectively.

The NOEDD was determined to be 18.0 µg consumed product/bee/day (equivalent to 11.9 µg consumed total a.i./bee/day) and the NOEC to be 0.479 g product/kg food (equivalent to 0.315 g total a.i./kg food), respectively.

(Ruhland S. 2018)

## A 2.3.1.3 KCP 10.3.1.3 Effects on honey bee development and other honey bee life stages

### A 2.3.1.3.1 Study 1 – Zoxamide: Chronic toxicity to honey bee larvae

EFSA (2017) requested *"Further information to address the risk to bee larvae (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5)."* A study to address this data requirement is presented in the following.

The following study has been provided to Latvia as RMS for zoxamide and cMSs for interzonal evaluation in July 2020.

| | |
|---|---|
| Comments of zRMS: | The study was not crucial for finalization of the risk assessment, thus was not evaluated by zRMS. |

**Reference:** **KCP 10.3.1.3/01**

Report xxx., 2018: Zoxamide: Honey bee (*Apis mellifera* L.) larval toxicity, repeated exposure
xxx, A Gowan Group Company, USA
xxx, Report No. 12791.6307, GLP, Not published

Guideline(s): OECD 239 (2016)

Deviations: The protocol states that the cell culture plates will be located within a larval box inside the incubator and that the larval box assists in maintaining a relative humidity ≥90%. On test day 7 (16 July 2018), the humidity in the larval plates was recorded at 84% during a 15-minute interval. The decrease in humidity coincided with documented routine maintenance of the surrogate larval box where the temperature was monitored. During this maintenance it was necessary to disconnect the HOBO data logger probe, which likely resulted in the low humidity reading. The test itself was conducted in an identical larval box that had not been disturbed and it can be surmised that the relative humidity was maintained ≥90% for the duration of the larval stage. In addition, the control groups met the referenced acceptability criteria, the decrease in humidity was short in duration and well within the tolerance limits for the test organism. Therefore, this deviation is considered to not impact the integrity of the study and its results.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) No

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | Zoxamide tech. (2015081302) |
| **Active substance content or purity** | 99.1 % (w/w) |
| **Species** | *Apis mellifera* |
| Age: | ≤ 24 hours old larvae |
| Number of larvae: | 36 larvae (3 replicates of 12 larvae each) |
| Source: | Wood´s Beekeeping Supply, Lincoln, Rhode Island |
| Acclimation: | 2 days |
| Diet: | The deionised water used in the diet preparation was boiled and then cooled to room temperature before use. Three untreated diets were prepared prior to test initiation and were stored frozen until use. The diets were prepared as follows: |

| Component (g) | Diet A[a] | Diet B[b] | Diet C[c] |
|---|---|---|---|
| Deionised water | 800 | 800 | 720 |
| D-glucose[d] | 95 | 120 | 220 |
| D-fructose[d] | 95 | 120 | 220 |

| | |
|---|---|
| | <table><tr><td>Yeast extract[de]</td><td>16</td><td>24</td><td>48</td></tr><tr><td>Royal jelly[f]</td><td>800</td><td>800</td><td>1200</td></tr></table><br>[a] Diet fed on day 1<br>[b] Used for treated diet fed on exposure day 3<br>[c] Used for treated diet fed on exposure days 4, 5 and 6<br>[d] Supplier: Sigma Aldrich, Saint Louis, Missouri<br>e Yeast extract is made from *Saccharomyces cerevisiae* species of yeast<br>[f] Supplier: Stakich, Inc., Troy, Michigan |
| **Test system** | larval test vessels: sterile, 48-well cell culture plates (1.6 mL/well; Corning) containing a plastic queen cup grafting cell (Mann Lake) in 32 wells during acclimation and in 18 wells during exposure; the perimeter wells within each plate not containing larvae partially filled with deionized water to assist in maintaining the relative humidity at >90%<br>pupation plates: sterile, 24-well cell culture plates (3.4 mL/well; Corning), each containing two layers of sterilized dust-free Kim-wipes |
| **Environmental conditions** | |
| Temperature: | Larval phase (days 1-8): 31° - 34°C<br>Pupal phase (days 9-22): 32° - 34°C |
| Photoperiod: | near darkness, laboratory lighting only for approximately 30 minutes each day during observations and renewal of the diet |
| Relative humidity: | Larval phase (days 1-8): 95% - 99% (once 84%, see study plan deviation)<br>Pupal phase (days 9-22): 68% - 78% |
| **Application rate(s)** | 38, 78, 150, 300 and 610 µg a.i./g (nominal diet concentration)<br>36, 67, 150, 300 and 640 µg a.i./g (mean measured diet concentration)<br>5.9, 11, 24, 49, and 110 µg a.i./larva (calculated dose) |
| Negative control: | deionised water<br>solvent control (acetone) |
| Positive control: | Dimethoate |
| **Test duration** | 22 days |
| **Remarks** | None |

The objective of this study was to evaluate the effect of zoxamide technical to honey bee larvae and successive pupae survival, adult emergence, and adult weight in an artificial *in vitro* testing design. The 22-day exposure of larvae to zoxamide treated diet was initiated on day 3 and continued through day 6, resulting in both dermal and oral exposure until pupation (typically day 7 to 8).

First instar larvae were transferred to 48-well plates for a 2-day acclimation phase and then exposed to zoxamide during four days of the larval treatment phase (days 3, 4, 5, and 6) as part of the 22-day period including larval, pupal, and adult stages. The test was conducted in near total darkness. Organisms were Only exposed to laboratory lighting for approximately 30 minutes each day during observations and diet renewal. Larvae were kept in an incubator at 33 ± 2 °C with a relative humidity of ≥90%.

Representative samples of the royal jelly diet, comprised of water and royal jelly and additional ingredients, were analysed periodically to ensure that they are free of toxic concentrations of PCBs, toxic metals, common pesticides, and antibiotics.

Based on the results of a range-finder, nominal cumulative test item concentrations of 38, 78, 150, 300, and 610 µg a.i./g diet, equivalent to doses of 6.3, 13, 25, 50, and 100 µg a.i./larva, a negative control, and a solvent (acetone) control were selected for the definitive exposure.

For test item treatment, a 150 mg a.i./mL primary stock solution was prepared by dissolving 15.0985 g of zoxamide (14.9626 g pure active ingredient) in 100 mL of acetone as solvent. This stock solution was used to prepare the treated royal jelly diets. The solvent control diet contained an equivalent amount of untreated acetone (0.48%) as each treated diet. Untreated diet was used for the negative control. An 8-day reference item test was included in the test and was conducted concurrently with the definitive exposure using larvae obtained from the same hives as those used to initiate the definitive exposure. Dimethoate served as reference item since it is known to be toxic to the honey bee larvae.

On day 3, individual larvae in all plates were fed by adding 20 µL of the appropriate treated diet B to each cell; on days 4, 5, and 6, respectively, larvae in all plates were fed by adding 30, 40, and 50 µL of the appropriate treated diet C to each cell. Larvae that were observed to completely consume their diet on day 7 or 8 were transferred to the appropriate labelled pupation plates; larvae that did not consume the entire diet were considered dead and not transferred to pupation plates. The number of replicates with uneaten diet was recorded. The health of the larvae was observed and recorded daily. Mortality of a larva was defined by lack of movement.

Upon transfer to the pupal plates, the plates were maintained within an incubator at the same temperature with as before, but with a lower relative humidity of 50 to 85%.

After pupation (non-feeding, developmental stage), test organisms were allowed to complete development to adulthood.

Survival of pupae was initially checked on day 15 to avoid disturbing the fragile pre-pupal stage earlier. Larvae that failed to develop into pupae by day 15 were classified as dead. Starting on day 15, the number of emerged adults was recorded. At the time of emergence, each adult bee was removed from the well plate, frozen, and individually weighed. The test was terminated on day 22. At test termination, after health observations and remaining individual bee weights were recorded. Pupae that had not emerged by day 22 were considered dead.

The 8-day (larval) and 22-day (pupal, adult emergence, and adult weight) No-Observed-Effect Dose (NOED), the dose which demonstrated no statistically adverse effect on survival, emergence, or body weight when compared to the control and the Lowest-Observed-Effect Dose (LOED), which is the lowest dose that demonstrated a statistically significant effect on survival, emergence, or adult weight compared to the control, were determined. The $ED_{10}$, $ED_{20}$, and $ED_{50}$ (for sublethal endpoints) and the $LD_{10}$, $LD_{20}$, and $LD_{50}$ (for survival endpoints) were determined. These statistical endpoints were also determined based on measured diet concentrations (i.e., NOEC, LOEC, $LC_X$, and $EC_X$). Calculations were performed using CETIS Version 1.8, applying applicable statistical tests. The results of the negative control and solvent control data for each endpoint were compared using Fisher's Exact Test for percent survival/emergence and Equal Variance two sample t-Test for adult weight data. Since no significant difference was observed between the controls, the negative control was used to evaluate treatment performance, per current U.S. EPA guidance.

The concentration of test substance was measured in the stock solutions on day 3 and in the treated diets on days 3 through 6 using liquid chromatography with tandem mass spectrometry detection (LC-MS/MS). The method was fully validated according to SANCO/3029/99 rev. 4 at a LOQ of 0.500 µg/g.

The dose rates within this study were based on the cumulative dose over four days. The resulting mean measured diet concentrations were used to adjust the nominal cumulative dosages. The results of this study were based on mean measured diet concentrations (µg a.i./g diet) and calculated dose (µg a.i./larva) that reflect the mean measured diet concentrations.

## Results and discussion

Environmental parameters (relative humidity and temperature) remained within acceptable limits throughout the study. The humidity was in the range for the larval phase (days 1-8): 84% - 99% and for pupal phase (days 9-22): 68% - 78%values, temperature ranged from 31° - 34°C (larval phase (days 1-8)) and 32° - 34°C (pupal phase (days 9-22)). Based on historical data, these parameters were considered acceptable for the survival and growth of the test organisms.

Prior to conducting the definitive exposure, the solubility, homogeneity, and stability of zoxamide in royal jelly diet has been confirmed in a non-GLP diet trial. Based on the results of this trial, zoxamide was applied at nominal concentrations of 610 µg a.i./g (equivalent to a dose of 100 µg a.i./larva) in royal jelly diet. The storage stability of zoxamide in refrigerated royal jelly diet was confirmed for at least four days.

Analysis of the stock solutions resulted in measured concentrations ranging from 86 to 95% of nominal concentrations. These results confirmed the appropriate amount of zoxamide in the stock solution. Mean measured diet concentrations of zoxamide in royal jelly diets on day 3, 4, 5, and 6 for the nominal concentrations of 38, 78, 150, 300, and 610 µg a.i./g were 36, 67, 150, 300, and 640 µg a.i./g, respectively, and ranged from 86 to 110% of nominal concentrations. These mean measured diet concentrations (µg a.i./g diet) were used to adjust the nominal cumulative dose (µg a.i./larva) for each treatment and were expressed as calculated doses of 5.9, 11, 24, 49, and 110 µg a.i./larva.

The mean larval survival (days 3 to 8) was 97, 97, 97, 92, 97, 97, and 94% in the negative control, solvent control, 5.9, 11, 24, 49, and 110 µg a.i./larva treatments, respectively.

Fisher's Exact Test indicated that the days 3 to 8 larval survival data for the negative control and solvent control data were not significantly different; therefore, treatment data were compared to the negative control data to define treatment effects.

Fisher's Exact Test with Bonferroni-Holm's Adjustment determined no significant reduction in larval survival among honey bees exposed to any of the treatments compared to the negative control. Therefore, the 8-day larval survival NOED and LOED values for zoxamide to honey bees were determined to be 110 and >110 µg a.i./larva, respectively. Since no concentration resulted in ≥10, 20, or 50% mortality, the 8-day $LD_X$ values of zoxamide to honey bee larvae were all empirically estimated to be >110 µg a.i./larva, the highest calculated dose tested.

The mean pupal survival (days 8 to 22) was 91, 97, 89, 91, 97, 94, and 94% in the negative control, solvent control, 5.9, 11, 24, 49, and 110 µg a.i./larva treatments, respectively. Fisher's Exact Test indicated that the pupal survival data for the negative control and solvent control data were not significantly different; therefore, treatment data were compared to the negative control data to define treatment effects.

Fisher's Exact Test with Bonferroni-Holm's Adjustment determined no significant reduction in pupal survival among honey bees exposed to any of the treatments compared to the negative control. Therefore, the 22-day pupal survival NOED and LOED values for zoxamide to honey bees were determined to be 110 and >110 µg a.i./larva, respectively. Since no concentration tested resulted in ≥10, 20, or 50% mortality, the 22-day $LD_X$ values of zoxamide to honey bee pupae were empirically estimated to be >110 µg a.i./larva, the highest calculated dose tested.

**Table A 52: Larval survival and mortality**

| Mean measured diet concentration (µg a.i./g diet) | Calculated dose (µg a.i./ larval) | % survival (number of surviving larvae) | | | | | Day 8 | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Day 3 | Day 4 | Day 5 | Day 6 | Day7 | % survival (no. of surviving larvae) | % mortality (no. of dead larvae) | Corrected % mortality[ab] |
| Negative Control | Negative Control | 100 (36) | 100 (36) | 100 (36) | 100 (36) | 100 (36) | 97 (35) | 3 (1) | NA[c] |
| Solvent Control | Solvent Control | 100 (36) | 100 (36) | 100 (36) | 100 (36) | 100 (36) | 97 (35) | 3 (1) | 0 |
| 36 | 5.9 | 100 (36) | 100 (36) | 100 (36) | 100 (36) | 100 (36) | 97 (35) | 3 (1) | 0 |
| 67 | 11 | 100 (36) | 100 (36) | 100 (36) | 100 (36) | 100 (36) | 92 (33) | 8 (3) | 6 |
| 150 | 24 | 100 (36) | 100 (36) | 100 (36) | 100 (36) | 100 (36) | 97 (35) | 3 (1) | 0 |
| 300 | 49 | 100 (36) | 100 (36) | 100 (36) | 100 (36) | 100 (36) | 97 (35) | 3 (1) | 0 |
| 640 | 110 | 100 (36) | 100 (36) | 100 (36) | 100 (36) | 100 (36) | 94 (34) | 6 (2) | 3 |

a per the study guideline, mortality values were also corrected and reported using Abbott´s formula (Abbott, 1925)
b Relative to the negative control.
c NA = not applicable

At test termination, the mean adult percent emergence (days 3 to 22) in the negative control, solvent control, 5.9, 11, 24, 49, and 110 µg a.i./larva treatments was 89, 94, 86, 83, 94, 92, and 89%, respectively. Fisher's Exact Test indicated that the day 22 adult percent emergence data for the negative control and solvent control data were not significantly different; therefore, treatment data were compared to the negative control data to define treatment effects. Fisher's Exact Test with Bonferroni-Holm's Adjustment determined no significant reduction in adult percent emergence for honey bees exposed to any of the levels tested compared to the negative control. Therefore, the 22-day adult emergence NOED and LOED values for zoxamide to honey bees were determined to be 110 and >110 µg a.i./larva, respectively. Since no concentration tested resulted in ≥10, 20, or 50% reduction in emergence, the 22-day $ED_X$ values of zoxamide to honey bee larvae through adulthood were empirically estimated to be >110 µg a.i./larva, the highest calculated dose tested.

**Table A 53: Pupal survival and adult percent emergence**

| Mean measured diet concentration (µg a.i./g diet) | Calculated dose (µg a.i./larva) | No.[a] | Day 8-22 | | | Day 3-22 | | |
|---|---|---|---|---|---|---|---|---|
| | | | Cumulative % survival (no. of surviving pupae) | Cumulative % mortality (no. of dead pupae) | Abbott's corrected % mortality[bc] | Cumulative % emergence (no. of emerged adults) | Cumulative % mortality[d] (no. of dead organisms) | Abbott's corrected percent mortality[bc] |
| Negative control | Negative control | 35 | 91 (32) | 9 (3) | NA[e] | 89 (32) | 11 (4) | NA |
| Solvent control | Solvent Control | 35 | 97 (34) | 3 (1) | -6 | 94 (34) | 6 (2) | -6 |
| 36 | 5.9 | 35 | 89 (31) | 11 (4) | 3 | 86 (31) | 14 (5) | 3 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 67 | 11 | 33 | 91 (30) | 9 (3) | 1 | 83 (30) | 17 (6) | 6 |
| 150 | 24 | 35 | 97 (34) | 3 (1) | -6 | 94 (34) | 6 (2) | -6 |
| 300 | 49 | 35 | 94 (33) | 6 (2) | -3 | 92 (33) | 8 (3) | -3 |
| 640 | 110 | 34 | 94 (32) | 6 (2) | -3 | 89 (32) | 11 (4) | 0 |

a number of larvae transferred
b Per the study guideline, mortality values were also corrected and reported using Abbott's formula (Abbott, 1925).
c Relative to the negative control
d Based on 36 larvae at initiation
e NA = not applicable

The mean adult weight at emergence in the negative control, solvent control, 5.9, 11, 24, 49, and 110 µg a.i./larva treatments was 0.1138, 0.1100, 0.1098, 0.1082, 0.0998, 0.1104, and 0.1023 g, respectively. Statistical analysis (Equal Variance Two-Sample t-Test) determined no significant difference in weight between the negative control and solvent control. Therefore, the negative control data was used to determine the treatment weight data effects.

Dunnett's Multiple Comparison Test determined a significant reduction in weight for adults at emergence among honey bees exposed to the 24 and 110 µg a.i./larva treatments, compared to the negative control. Due to the lack of a clear dose response as indicated by no significant reduction in weight at the 49 µg a.i./larva dose rate, the effect at the 24 µg a.i./larva dose rate was not considered toxicant related and was likely a function of biological variability. Therefore, the NOED and LOED values were determined to be 49 and 110 µg a.i./g larva, respectively. Since no concentration tested resulted in ≥10, 20, or 50% reduction, the $ED_X$ values were empirically estimated to be >110 µg a.i./larva, the highest calculated dose tested.

**Table A 54: Adult weight at emergence**

| Mean measured diet concentration (µg a.i./g diet) | Calculated dose (µg a.i./larva) | No. [a] | Mean adult weight at emergence[b] (g) |
|---|---|---|---|
| Negative control | Negative control | 32 | 0.1138 (0.0118) |
| Solvent control | Solvent control | 34 | 0.1100 (0.0110) |
| 36 | 5.9 | 31 | 0.1098 (0.0110) |
| 67 | 11 | 30 | 0.1082 (0.0115) |
| 150 | 24 | 34 | 0.0998[c] (0.0148) |
| 300 | 49 | 33 | 0.1104 (0.0089) |
| 640 | 110 | 32 | 0.1023[c] (0.0088) |

[a] number of adults weighed
[b] Standard deviations are presented in parentheses.
[c] Significantly reduced, compared to the negative control, based on Dunnett's Multiple Comparison Test. Due to the lack of a clear dose response as indicated by no significant reduction in weight at the 49 µg a.i./larva dose rate, the effect at the 24 µg a.i./larva dose rate was not considered toxicant related and was likely a function of biological variability.

The nominal cumulative dose rate of dimethoate maintained during the reference test was 7.9 µg a.i./larva, equivalent to 48 µg a.i./g diet. Mortality during the larval stage (days 3 to 8) was 69% for honey bee larvae exposed to 7.9 µg a.i./larva nominal cumulative dose. These results confirm the sensitivity of the test system.

All validity criteria were met:

- Larval mortality from days 3 to 8 in the negative control, and solvent control, if present, should be ≤15% prior to pupation (larval mortality was 3 and 3%, respectively).
- Percent emergence in the negative control, and solvent control, if present, should be ≥70% at termination (emergence in the negative control and solvent control was 89 and 94%, respectively).
- Larval mortality in the reference toxicant treatment level (7.9 µg a.i. dimethoate/larva) should be ≥50% on day 8 (larval mortality in the 7.9 µg a.i. dimethoate/larva treatment was 69%.).

**Table A 55: Summary of study endpoints**

| Endpoint | NOEC / NOED | LOEC / LOED | $LC_{10}/EC_{10}$ / $LD_{10}/ED_{10}$ (95% CI[a]) | $LC_{20}/EC_{20}$ / $LD_{20}/ED_{20}$ (95% CI) | $LC_{50}/EC_{50}$ / $LD_{50}/ED_{50}$ (95% CI) |
|---|---|---|---|---|---|
| **based on mean measured diet concentrations (µg a.i./g diet)** | | | | | |
| 3-8-day larval survival | 640 | > 640 | > 640 (NA[d]) | > 640 (NA) | > 640 (NA) |
| 8-22-day pupal survival | 640 | > 640 | > 640 (NA) | > 640 (NA) | > 640 (NA) |
| 3-22-day adult emergence | 640 | > 640 | > 640 (NA) | > 640 (NA) | > 640 (NA) |
| Adult weight at emergence | 300 | 640 | > 640 (NA) | > 640 (NA) | > 640 (NA) |
| **based on calculated dose (µg a.i./larvae)** | | | | | |
| 3-8-day larval survival | 110 | > 110 | > 110 (NA[d]) | > 110 (NA) | > 110 (NA) |
| 8-22-day pupal survival | 110 | > 110 | > 110 (NA) | > 110 (NA) | > 110 (NA) |
| 3-22-day adult emergence | 110 | > 110 | > 110 (NA) | > 110 (NA) | > 110 (NA) |
| Adult weight at emergence | 49 | 110 | > 110 (NA) | > 110 (NA) | > 110 (NA) |

[a] CI = Confidence interval
[d] NA = not applicable. $LC_x/EC_x$ value was empirically estimated; therefore, corresponding 95% confidence intervals could not be calculated

## Conclusion

The objective of this study was to evaluate the effect of zoxamide to honey bee larvae and successive pupae survival, adult emergence, and adult weight in an artificial in vitro testing design. The 22-day exposure of larvae to zoxamide treated diet was initiated on day 3 and continued through day 6, resulting in both dermal and oral exposure until pupation.

The results from the stock solution and royal jelly diet analyses indicate appropriate exposure concentrations.

The 8-day NOED and LOED values for zoxamide to honey bees were determined to be 110 and >110 µg a.i./larva, respectively. The 22-day NOED and LOED values for zoxamide to honey bee pupal percent survival were 110 and >110 µg a.i./larva, respectively. The 22-day percent emergence NOED and LOED values for zoxamide to honey bees were determined to be 110 and >110 µg a.i./larva, respectively. The weight for adults at emergence NOED and LOED values for zoxamide values were 49 and >110 µg a.i./larva, respectively.

The 22-d $LD_{50}$ was > 110 µg a.s/larvae, the 22-d $LC_{50}$ > 640 µg a.s./g diet with regard to larval and pupal survival, adult emergence and adult weight at emergence.

The study is valid.

(xxx R. 2018)

### A 2.3.1.3.2 Study 2 - CYMOXANIL 33% + ZOXAMIDE 33% WG: Chronic toxicity to honey bee larvae

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD 239 guideline and according to the principles of GLP. The study is considered to be valid. |

| | |
|---|---|
| **Reference:** | **KCP 10.3.1.3/02** |
| Report | Scheller, K., 2020: Cymoxanil 33% + Zoxamide 33% WG - Repeated exposure of honey bee (*Apis mellifera* L.) larvae under laboratory conditions (*in vitro*) Gowan Crop Protection Ltd., UK, SIPCAM OXON S.p.A, Italy BioChem agrar, Germany, Report No. 17 48 BLC 0005, GLP, Not published |
| Guideline(s): | OECD 239 (2016)<br>SCHMEHL et *al.* (2016) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | No |

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | Cymoxanil 33% + Zoxamide 33% WG / Reboot (30AL5020) |
| **Active substance content (measured)** | Zoxamide: 32.8 % (w/w) (analyzed)<br>Cymoxanil: 33.0 % (w/w) (analyzed) |
| **Species:** | Honey bee *Apis mellifera L., ssp. Buckfast (*Hymenoptera, Apoidea*)* |
| Age: | First instar larvae (L1) |
| Number of larvae: | 12 larvae/replicate; 3 replicates (placed on one culture plate) |
| Source: | BioChem agrar, Germany |
| Acclimation: | |
| Diet: | 50% aqueous sugar solution and 50% royal jelly.<br>The aqueous sugar solutions as one component of the artificial diets were prepared prior to the test and stored in a fridge until use. The sugar solutions were mixed with royal jelly every day before each feeding occasion. Each larva was fed separately using a sterile pi-pette. The food drop was placed next to the larvae to avoid drowning. Before feeding, the final diets were warmed up to 34.5°C. During the process, the culture plate in operation was placed on a warming plate set to 34.5 °C. |
| **Test system** | 36 crystal polystyrene grafting cells (e.g. CNE Nicotplast, internal diameter 9 mm) were placed in three groups on each 48-well plate, |

| | |
|---|---|
| | which has been labelled with at least study number and treatment group replicate number. The plates were placed on an adjustable heating plate (e.g. stretching table), which was set to 34.5 °C. Artificial diet A was pipetted into the grafting cells, followed by placing one freshly grafted larva per cell. |
| **Environmental conditions** | |
| Temperature: | 34.1° - 34.7°C |
| Photoperiod: | Constant darkness throughout the test (diffuse artificial light only during handling and assessments) |
| Relative humidity: | D1-D8: 99-100%<br>D8-D15: 80-88%<br>D15-D22: 55-59% |
| **Application rate(s)** | Control:<br>AC: Untreated diet B/C (50 % aqueous sugar solution with 50 % royal jelly)<br>BC: Untreated diet B/C containing 1% v/v acetone<br>Test item:<br>AT: Treated diet B/C at a concentration of 365 mg product/kg food<br>BT: Treated diet B/C at a concentration of 183 mg product/kg food<br>CT: Treated diet B/C at a concentration of 91 mg product/kg food<br>DT: Treated diet B/C at a concentration of 46 mg product/kg food<br>ET: Treated diet B/C at a concentration of 23 mg product/kg food<br>Reference item:<br>AR: Treated diet B/C at a concentration of 47 mg a.i./kg food |
| Negative control: | deionised water<br>solvent control (acetone) |
| Positive control: | Dimethoate |
| **Test duration** | 22 days |
| **Remarks** | None |

In a chronic toxicity test, honeybee (Apis mellifera L) larvae were repeatedly exposed over a period of 4 days to Cymoxanil 33% + Zoxamide 33% WG.

Based on the results of a range-finding test, the toxicity of the test item was determined at total doses of nominally 57.8, 28.9, 14.4, 7.2 and 3.6 µg product/larva. The test item concentrations in the diet were 365, 183, 91, 46 and 23 mg product/kg food. Acetone served as solvent for Cymoxanil 33% + Zoxamide 33% WG. Additionally, honey bee larvae were treated with Dimethoate tech. as reference item at a total dose of 7.4 µg Dimethoate/larva (concentration: 47 mg a.i./kg) or with an untreated diet as control.

One-day-old honeybee larvae (D1) of Apis mellifera L. were transferred from brood combs to polystyrene grafting cells in 48-well cell culture plates 3 days before the start of the treatment. On four successive days (D3 to D6), the larvae were chronically exposed to Reboot diluted in the larval food (aqueous sugar solution mixed with royal jelly). After the applications, no additional feedings of the larvae took place.

In total, three treatment groups with 3 replicates per dose and 12 larvae per replicate were set up: one group with 5 doses of the test item, one untreated control group (control and solvent control) and one dose of the reference item.

Assessments of larval/pupal mortality were done on D4, D5, D6, D7, D8, D15 and D22. Additionally, other observations such as smaller body size or remaining food after 96 and 120 hours (on D7 and D8) were noted.

In an analytical phase of the study, the concentrations of the active ingredients zoxamide and cymoxanil in the test item stock solutions A (highest concentration) and E (lowest concentration) and in the control2 (C) were analytically verified with a fully validated method according to SANCO/3029/99 rev. 4 using high performance liquid chromatography (HPLC) with diode-array detector (DAD).

## Results and discussion

Environmental parameters (temperature and relative humidity) remained within acceptable limits throughout the study. The cultures were exposed at 34.1 – 34.7°C with relative humidity D1-D8: 99-100%, D8-D15: 80-88% and D15-D22: 55-59%. The honeybee larvae were kept under constant darkness except during the assessments.

The concentrations of zoxamide and cymoxanil in the test item stock solutions A (highest rate) and E (lowest rate) resulted in recovery rates of 83-91% and 100-104%, respectively. No active ingredient was detected in the solvent control samples. Thus, the nominal zoxamide and cymoxanil test concentrations were analytically verified.

**Table A 56: Test item concentrations**

| Treatment | StA | StE | Control |
|---|---|---|---|
| Cymoxanil | | | |
| Nominal conc. (mg/L) | 275.5 | 17.22 | 0.000 |
| Dilution factor | 50 | 3.125 | 3.125 |
| **Analysed concentration (mg/L)** **Recovery** | | | |
| Day 3 | 277.2 | 17.63 | < LOQ |
| | 101% | 102% | |
| Day 4 | 288.0 | 17.28 | < LOQ |
| | 105% | 100% | |
| Day 5 | 278.5 | 17.47 | < LOQ |
| | 101% | 101% | |
| Day 6 | 283.3 | 17.68 | < LOQ |
| | 103% | 103% | |
| Zoxamide | | | |
| Nominal conc. (mg/L) | 277.2 | 17.33 | 0.000 |
| Dilution factor | 50 | 3.125 | 3.125 |
| **Analysed concentration (mg/L)** **Recovery** | | | |
| Day 3 | 242.3 | 14.35 | < LOQ |
| | 87% | 83% | |
| Day 4 | 251.8 | 15.28 | < LOQ |
| | 91% | 88% | |
| Day 5 | 239.7 | 15.00 | < LOQ |

| | | | |
|---|---|---|---|
| | 86% | 87% | |
| Day 6 | 251.5 | 14.99 | < LOQ |
| | 91% | 87% | |

Conc. = concentration
<LOQ: not detected or detected concentration below LOQ (8.594 mg/L) for cymoxanil and LOQ (8.646 mg/L) for zoxamide

On D8 of the test (120 hours after first exposure), a mortality of 8.3% was observed un the untreated control AC, and no mortality was observed in the solvent control BC.

In the test item group, cumulative mortalities ranged between 8.3 and 91.7%. The cumulative mortality in the reference item group was 91.7%. In the test item treatment groups, where larvae were exposed to 57.8, 28.9 and 7.2 µg product/larva, 100.0%, 11.1% and 4.2% of the remaining larvae did not complete-ly ingest the whole amount of the provided food.

After 22 days, the adult emergence rate in the untreated control AC as well as in the solvent control BC was 91.7% (mortality 8.3% each). In the test item treatment groups, adult emergence rate was 0.0%, 11.1%, 52.8%, 72.2% and 77.8% (from the highest to the lowest dose/concentration). The respective cumulative mortality was 100.0%, 88.9%, 47.2%, 27.8%, and 22.2% (corrected for solvent control: 100.0%, 87.9%, 42.4%, 21.2% and 15.2%).

Statistically significant differences in adult emergence/cumulative mortality on D22 compared to the control occurred in the test item doses where each larva was treated with 57.8 µg product/larva (100.0% mortality; corrected for solvent control: 100.0%), with 28.9 µg product/larva (88.9% mortality; corrected for solvent control: 87.9%), with 14.4 µg product/lava (47.2% mortality; corrected for solvent control: 42.4%), and with 7.2 µg product/larva (27.8% mortality; corrected for solvent control: 21.2%).

Mortality in the reference (AR) group was above 50 % across all replicates on D8, being 91.7% on average (corrected for control: 90.9%).

**Table A2.3.1-1: Sublethal effects of Reboot to Apis mellifera L. after repeated exposure**

| Treatment group | Test solution ID | Dose | Concentration | On D8 | | | On D22 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Mean mortality of larvaeD3 to D8[%] | | Mean OO | Mean mortality of pupae[3]D8-D22[%] | | Mean mortality of larvae & pupae D3-D22[%] | | Adult emergence rate [%] |
| | | [µg product/ larva] | [mg product/kg food] | abs. | corr. | | abs. | corr. | abs. | corr. | abs. |
| Control | AC | - | - | 8.3 | - | 0.0 | 0.0 | - | 8.3 | - | 91.7 |
| | BC | - | - | 0.0 | - | 0.0 | 8.3 | - | 8.3 | - | 91.7 |
| Test item | AT | 57.8 | 365 | 91.7 | 91.7 | 100.0 | 100.0 | 100.0 | 100.0* | 100.0 | 0.0 |
| | BT | 28.9 | 183 | 63.9 | 63.9 | 11.1 | 69.2 | 66.4 | 88.9* | 87.9 | 11.1 |
| | CT | 14.4 | 91 | 38.9 | 38.9 | 0.0 | 13.6 | 5.8 | 47.2* | 42.4 | 52.8 |
| | DT | 7.2 | 46 | 19.4 | 19.4 | 4.2 | 10.3 | 2.2 | 27.8* | 21.2 | 72.2 |
| | ET | 3.6 | 23 | 8.3 | 8.3 | 0.0 | 15.2 | 7.4 | 22.2 | 15.2 | 77.8 |
| Reference item | AR | [µg a.i./larva] | [mg a.i./kg food] | | | | | | | | |
| | | 7.4 | 47 | 91.7 | 90.9 | 0.0 | 100.0 | 100.0 | 100.0 | 100.0 | 0.0 |

Results are averages based on 3 replicates, containing 12 larvae each; see appendix 4 for details
corr.: corrected mortality (according to Schneider-Orelli 1947): test item corrected by solvent control BC and reference item corrected for control AC
abs.: absolute mortality as counted from the results; negative values are set to "0"
Calculations are performed with non-rounded values.
OO: Other observations (remaining food, small body size)
* Statistically significant compared to the untreated control (Step-down Cochran-Armitage Test)
CL: 95%-Confidence limits

**Table A 57: Summary of study endpoints**

| Treatment | Endpoints | On D22[3] (19 days after first application) | |
|---|---|---|---|
| | | **Based on product** | **Based on total a.i.[4]** |
| **Test item doses** | $ED_{50}$ [µg product/larva][2] (lower/upper CL) | 14.1 (11.7 – 17.1) | 9.3 (7.7 – 11.3) |
| | $ED_{20}$ [µg product/larva][2] (lower/upper CL) | 6.4 (4.7 – 8.9) | 4.2 (3.1 – 5.9) |
| | $ED_{10}$ [µg product/larva][2] (lower/upper CL) | 3.8 (2.5 – 5.9) | 2.5 (1.6 – 3.9) |
| | NOED [µg product/larva][1] | 3.6 | 2.4 |
| **Test item concentrations** | $EC_{50}$ [mg product/kg food][2] (lower/upper CL) | 90 (74 – 108) | 59 (49 – 71) |
| | $EC_{20}$ [mg product/kg food][2] (lower/upper CL) | 41 (30 – 56) | 27 (20 – 37) |
| | $EC_{10}$ [mg product/kg food][2] (lower/upper CL) | 24 (16 – 38) | 16 (11 – 25) |
| | NOEC [mg product/kg food][1] | 23 | 15 |

[1] Step-down Cochran-Armitage Test (α=0.05)
[2] Determined following the Weibull analysis using linear maximum likelihood regression (α=0.05)
[3] Endpoints are based on the sum of analysed content of the a.i. in the formulated product.
[4] Endpoints expressed in a.i. were calculated by multiplying the endpoint expressed in product with the analysed content of a.i., divided by the test item's density. Analysed content: 658 g/kg (32.8% w/w for zoxamide, 33.0% w/w for cymoxanil); Density: set to 1 as test item is solid

All validity criteria of the study were met:

- control mortality on D8 was ≤ 15 % across all control replicates (control AC: 8.3%, solvent control BC: 0.0%))
- mortality in the reference item on D8 was ≥ 50 % for larvae exposed to a total dose of 7.4 µg a.i./larva across all reference replicates (91.7%)
- adult emergence rate on D22 was ≥ 70% across all control replicates (91.7%)

## Conclusion

In a chronic toxicity study with repeated exposure of honeybee larvae to Cymoxanil 33% + Zoxamide 33% WG, the $ED_{50}$ after 22 days (D22) was determined at 14.1 µg product/larva, the $ED_{20}$ at 6.4 µg product/larva, and the $ED_{10}$ at 3.8 µg product/larva. The NOED (D22) was 3.6 µg product/larva.

These endpoints amount to $ED_{50}$ values after 22 days (D22) of 9.3 µg total a.i./larva, $ED_{20}$ values of 4.2 µg total a.i./larva, and $ED_{10}$ values of 2.5 µg total a.i./larva for both zoxamide and cymoxanil based on their analysed contents in the formulated product. The NOED (D22) was 2.4 µg total a.i./larva.

The $EC_{50}$ after 22 days (D22) was determined at 90 mg product/kg food, the $EC_{20}$ at 41 mg product/kg food, and the $EC_{10}$ at 24 mg product/kg food. The NOEC (D22) was 23 mg product/kg food.

These values amount to $EC_{50}$ values after 22 days (D22) of 59 mg total a.i./kg food, $ED_{20}$ values of 27 mg total a.i./kg food, and $ED_{10}$ values of 16 mg total a.i./kg food for both zoxamide and cymoxanil based on the analysed content of the active substances in the formulated product. The NOEC (D22) was 15 mg total a.i./kg food.

(Scheller K. 2020)

## A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

## A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

## A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

### A 2.3.1.6.1 Study 1 - Cymoxanil 33% + Zoxamide 33% WG: Honey bee field study 2018 in CEU

| | |
|---|---|
| Comments of zRMS: | Under field conditions, the Cymoxanil 33% + Zoxamide 33% WG was applied twice at a rate of each 1.35 kg product/ha with interval of 7 days on full-flowering *Phacelia*, during active foraging of honeybees. The start of the exposure was on the day of the second application (including pollen composition analyses). The residue analyses confirmed the exposure of honeybee colonies to the product in flowers, pollen, nectar and bees. The residues declined rapidly within 7 days after the last test item treatment (on DAT 0) to amounts ≤ 0.5% of initial in pollen (trap). Furthermore, cymoxanil and zoxamide were not detectable any more (i.e. below the LOD of 0.001 mg/kg) within 2 days after the last test item application in nectar (forager bees and in-hive).<br><br>In the conclusion, the 3-fold max. single application rate of 0.45 kg product/ha, caused no adverse effects on the behaviour, the mortality, the foraging activity, the weight development, the condition of the colonies and the bee brood development over an observation period of 42 days after the second application.<br><br>The study is considered to be reliable and suitable for the risk assessment. |

**Reference:** **KCP 10.3.1.6/01**

Report: Schnurr, A., 2020: Effects of Cymoxanil 33% + Zoxamide 33% on the honeybee *Apis mellifera* L. under field conditions with additional assessments on colony and brood development
Gowan Crop Protection Ltd., UK, SIPCAM OXON S.p.A, Italy
BioChem agrar, Germany, Report No. 18 48 BFB 0001, GLP, Not published

Guideline(s): OECD 75 (2007)
EPPO Standard PP 1/170(4) (2010)
Recommendations of the AG Bienenschutz (2011)
Recommendations of the EFSA Guidance Document on the risk assessment of plant protection products on bees (2013)

Deviations: Additional specimen numbers were generated for nectar storage stability testing (1848BFB0001-126, 1848BFB0001-127, 1848BFB0001-128) due to missing specimen numbers for nectar storage stability in the study plan.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) No

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | Cymoxanil 33% + Zoxamide 33% (GSOL7019) |
| **Active substance content (measured)** | Cymoxanil: 33.0% w/w (nominal), 32.75 ± 0.15 % (analysed)<br>Zoxamide: 33.0% w/w (nominal), 32.91 ± 0.17% (analysed)<br>Zoxamide isomers: 50.65 ± 0.10 % R-isomer, 49.35 ± 0.10 % S-isomer |
| **Species:** | *Apis mellifera* L. Buckfast (Hymenoptera, Apoidea), healthy bee colonies |
| Age: | Adult and juvenile bees of all brood stages |
| Number of animals: | 12713-16200 bees per hive on DAT 2 |
| Number of bee colonies (replicates)/treatment group: | 7 (+ 1 colony for residue analysis only)/2 |
| Weight: | 24.9 and 24.8 kg/colony |
| Source: | BioChem agrar, Germany |
| Acclimation: | 3 days pre-exposure phase |
| Field size: | Control: 2.01 ha<br>Test item: 2.0 ha |
| Feeding: | On the treated field.<br>The food status of all colonies was acceptable throughout the trial, no artificial food was offered and all assessments of colony represented the natural state |
| **Environmental conditions** | |
| Temperature: | 22.4 - 24.1°C (first application)<br>19.9 – 23.9°C (second application) |
| Relative humidity: | 56.6 – 58.7% (first application)<br>62.2 – 73.9 % (first application) |
| **Application rates** | Control: left untreated<br>Test item: 2 x 1.35 kg product/ha (the 3-fold max. single application rate of 0.45 kg product/ha) at a minimum interval of 7±1 days (equivalent to 2 x 891 g total a.i./ha) |
| **Test duration** | 3 days pre-exposure<br>8 days exposure<br>42 days post-exposure |
| **Remarks** | None |

The purpose of this study was to determine potential effects of Cymoxanil 33 % + Zoxamide 33 % WG (containing nominally 33 % of each active ingredient cymoxanil and zoxamide) on the honeybee (*Apis*

*mellifera*) after two consecutive foliar applications of each 1.35 kg product/ha (the 3-fold max. single application rate of 0.45 kg product/ha) at an interval of 7 days during flowering of *Phacelia (Phacelia tanacetifolia*) and during bee flight under field conditions. The second application was conducted at the beginning of full-flowering of *Phacelia (Phacelia tanacetifolia*) (BBCH 65) and during bee flight. The control field was left untreated. Main endpoints were the mortality, foraging activity, bee behaviour, colony and brood development. Special attention was paid on detailed brood development during two brood cycles via photo documentation of initially labelled eggs. Additionally, residues of cymoxanil and zoxamide were determined in spray solution, flowers, pollen (pollen from pollen traps and in-hive pollen), nectar (nectar from forager bees and in-hive nectar), dead bees and alive bees with an analytical method fully validated according to SANCO/3029/99 rev. 4 and SANCO/825/00 (2010).

7 replicates (bee colonies) honeybees of the species *Apis mellifera* L. were exposed to untreated or test item treated and highly attractive crop *Phacelia tanacetifolia* under field conditions. The size of the untreated control and test item treated field was 2.00 and 2.01 ha, respectively. Two consecutive foliar applications of each 1.35 kg product/ha (the 3-fold max. single application rate of 0.45 kg product/ha) at an interval of 7 days were carried out shortly before flowering (DAT -7) and during full flowering (DAT 0) of Phacelia (*Phacelia tanacetifolia*), respectively. During the entire test period, the climatic data (temperature, relative humidity, rainfall and cloud cover (at time of evaluation) were recorded continuously. The growth stage of *Phacelia tanacetifolia* was recorded from the first application to the end of exposure phase on each assessment day.

Assessments of mortality and bee behavior were carried out at 3 days prior to the second application (at monitoring site), on the day of the second application and on the following 42 days after application.

Foraging activity assessments were carried out on the day of the second application and on the following 7 days after the second application during exposure.

Behavior: daily recording during entire study period

Colony development (colony strength and brood development) during two consecutive brood cycles: DAT -2, DAT 2, DAT 9; DAT 14, DAT 20, DAT 26, DAT 30, DAT 35 and DAT 42 which is equal to BFDB 0, BFD 4, BFD 11, BFD 16, BFD 22, BFD 28, BFD 32, BFD 37 and BFD 44

Detailed brood assessments: BFD 0, BFD 4, BFD 11, BFD 16, BFD 22 (BFD 0C), BFD 28 (BFD 6C), BFD 32 (BFD 10C), BFD 37 (BFD 15C) and BFD 44 (BFD 22C) for initially labelled eggs.

Colony weight: DAT -2, DAT 2, DAT 9; DAT 14, DAT 20, DAT 26, DAT 30, DAT 35 and DAT 42Colony strength, colony weight and general brood assessments according to IMDORF et al. (1987) and IMDORF & GERIG (1999) were carried out on DAT -2, DAT 2, DAT 9, DAT 14, DAT 20, DAT 26, DAT 30, DAT 35 and DAT 42 (assessments of the pollen and nectar storage area, area containing eggs, larvae and capped cells as well number of bees/colony) covering two consecutive brood cycles.

Next to the general brood evaluation, detailed brood assessments were carried out by photo documentation. Assessments were performed during the two brood cycles of the general brood assessments; here, 300 eggs on one or more brood frames were marked before application (DAT -2/BFD 0). The development of these eggs was checked within the first brood cycle until expected hatching of worker bees (DAT 20/BFD 22). The detailed brood assessment of another 300 eggs was carried out in a second brood cycle, starting with the brood fixing on (DAT 20/BFD 22) (= second BFD 0) and lasting until (DAT 42/BFD 44).

Additionally, residues of cymoxanil and zoxamide were determined in spray solution, flowers, pollen (pollen from pollen traps and in-hive pollen), nectar (nectar from forager bees and in-hive nectar), dead bees and alive bees with an analytical method fully validated according to SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 8.1 (2010). The analysis was carried out following the QuEChERS extraction method inclusive clean-up as recommended by Anastassiades et al. (2003) using high performance liquid

chromatography (HPLC) with mass-spectrometric (MS-MS) for the detection of both cymoxanil and zoxamide in one run. The LOQ was 0.005 mg a.i./L for spray solution and nectar and 0.005 mg/kg for bees, pollen and flowers.

## Results and discussion

Environmental parameters (temperature relative humidity) remained within acceptable limits throughout the study.

The test item concentrations in the spray solutions were analytically confirmed. All control samples or samples before test item application showed no residues above the limit of quantification (i.e. no residues above the LOQ of 0.005 mg a.i./kg). However, residues of zoxamide and cymoxanil on/in flowers, bees, pollen and nectar confirmed a proper and (unrealistic) worst-case test item loading of bees and their feed items in the treated field part.

**Table A 58: Residue analysis of cymoxanil and zoxamide in spray solution**

| Matrix | Treatment group | Sampling day | Recovery of cymoxanil (%) | Recovery of zoxamide (%) |
|---|---|---|---|---|
| Spay solution | Test item | DT I | 97 | 97 |
| | Test item | DAT 0 | 94 | 100 |

DT I: Day of Treatment; DAT: Days after second treatment

**Table A 59: Residue analysis of cymoxanil and zoxamide in flowers**

| Matrix | Treatment group | Sampling day | Concentration of cymoxanil mg/kg) * | Concentration of zoxamide (mg/kg) * |
|---|---|---|---|---|
| Flowers | Control | DT I ba | n.d. | n.d. |
| | | DT I | n.d. | n.d. |
| | | DAT 0 | n.d. | n.d. |
| | | DAT 2 | n.d. | n.d. |
| | | DAT 5 | n.d. | n.d. |
| | | DAT 7 | n.d. | n.d. |
| | Test item | DT I ba | n.d. | n.d. |
| | | DT I | 28.27 | 39.42 |
| | | DAT 0 | 34.51 | 52.21 |
| | | DAT 2 | 6.220 | 30.85 |
| | | DAT 5 | 0.465 | 7.469 |
| | | DAT 7 | 0.155 | 4.570 |

* moist weight (as received)
n.d. = not determinable or ≤ LOD (LOD = 0.001 mg/kg of cymoxanil and zoxamide)
ba: before application
DT I: Day of Treatment
DAT: Days after second treatment

**Table A 60: Residue analysis of cymoxanil and zoxamide in pollen**

| Matrix | Treatment group | Sampling day | Concentration of cymoxanil mg/kg) * | Concentration of zoxamide (mg/kg) * |
|---|---|---|---|---|
| Pollen (trap) | Control | DAT 0 | n.d. | n.d. |
| | | DAT 2 | n.d. | n.d. |

| | | | | |
|---|---|---|---|---|
| | | DAT 5 | n.d. | n.d. |
| | | DAT 7 | n.d. | n.d. |
| Pollen (in-hive) | | DAT 2 | n.d. | n.d. |
| | | DAT 7 | n.d. | n.d. |
| Pollen (trap) | Test item | DAT 0 | 16.77 | 15.56 |
| | | DAT 2 | 3.215 | 5.389 |
| | | DAT 5 | 0.165 | 0.399 |
| | | DAT 7 | 0.029 | 0.071 |
| Pollen (in-hive) | | DAT 2 | 1.519 | 3.526 |
| | | DAT 7 | 0.108 | 0.665 |

* moist weight (as received)
n.d. = not determinable or ≤ LOD (LOD = 0.001 mg/kg of cymoxanil and zoxamide)
DAT: Days after second treatment

**Table A 61: Residue analysis of cymoxanil and zoxamide in nectar**

| Matrix | Treatment group | Sampling day | Concentration of cymoxanil mg/kg) * | Concentration of zoxamide (mg/kg) * |
|---|---|---|---|---|
| Nectar (forager bees) | Control | DAT 0 | n.d. | n.d. |
| | | DAT 2 | n.d. | n.d. |
| | | DAT 5 | n.d. | n.d. |
| | | DAT 7 | n.d. | n.d. |
| Nectar (in-hive) | | DAT 2 | n.d. | n.d. |
| | | DAT 7 | n.d. | n.d. |
| Nectar (forager bees) | Test item | DAT 0 | 0.292 | 0.511 |
| | | DAT 2 | 0.006 | n.d. |
| | | DAT 5 | n.d. | n.d. |
| | | DAT 7 | n.d. | n.d. |
| Nectar (in-hive) | | DAT 2 | n.d. | n.d. |
| | | DAT 7 | n.d. | n.d. |

* moist weight (as received)
n.d. = not determinable or ≤ LOD (LOD = 0.001 mg/kg of cymoxanil and zoxamide)
DAT: Days after second treatment

**Mortality**: The mortality of honeybees at the monitoring site before the second application was on a low and similar level in the control and the test item treatment, indicating comparable colonies. No statistically significant differences were observed between the two treatment groups, neither based on daily comparisons nor on overall comparison before application (Student test, two-sided, $p>0.05$). Also, during the exposure phase (beginning on the day of the second test item application) and during post-exposure phase no differences in mean mortality were observed between both treatment groups. Mortality was on a low and comparable level throughout the study, without any statistically significant differences (with the exceptions for DAT 20 and DAT 31).

No mortality of honeybee pupae was observed in both treatment groups during the entire test.

**Foraging activity:** Shortly before the second application, the foraging activity was on an adequate and similar level in all test groups, indicating that bees had well adapted to the new environmental conditions and that they were well exposed during the test item applications. After the second application and during

the following days of the exposure phase the mean foraging activity was on a comparable level in the control and the test item treatment group.

**Bee behavior:** No behavioral changes of the bees after test item treatment and compared to the control colonies were observed during the assessments between DAT 0 until DAT 42. Honeybees showed no different or abnormal behaviors.

**Colony weight:** At the colony assessment conducted two days before the second application (DAT -2), the average weight per colony was similar, amounting to 24.9 and 24.8 kg/colony in the control and the test item treatment group, respectively. This difference was not statistically significant (Student-t test, two-sided, $p<0.05$). The development of the colony weight in the control and the test item treatment group during the post-application period, which covered two consecutive brood cycles, was very similar. At the last assessment on BFD 44 the mean colony weight was 27.4 kg (± 2.6 kg; relative increase of +9.8 % compared to the first assessment on BFD 0) and 29.5 kg/colony (± 2.1 kg; relative increase of +18.9 % compared to the first assessment on BFD 0) in the control and the test item treatment, respectively. Resulting in even a higher increase in the test item group compared to the control.

Consequently, the statistical analysis revealed no significant differences between control and test item treatment group during the entire post-application period (Student-t test, one-sided smaller, $p>0.05$).

**General brood assessments**

Colony strength: Two days before the second application (DAT -2/BFD 0), the average colony strength was 14014 and 14641 bees/colony in the control and the test item treatment group, respectively, and was thus similar and without any statistically significant difference (Student-t test, two-sided, $p>0.05$). In the course of the study, which covered two consecutive brood cycles, the colony strength in the control and test item treatment group developed in a comparable way with a relative increase of 22% and 20 % at the last assessment if compared to the pre-exposure assessment in the control and test item treatment group, respectively. No statistically significant differences were observed between control and test item treatment group throughout both investigated brood cycles (Student-t test, one-sided smaller, $p>0.05$).

Brood area: At the first assessment (BFD 0) at the monitoring site, the mean brood areas of the single stages, i.e. eggs, larvae and capped cells (= pupae) as well as that of the total brood (total area occupied by eggs, larvae and capped cells) was similar in both treatment groups without any statistically significant differences (Student-t test, two sided, $p>0.05$). During the course of the study, which covered two consecutive brood cycles, the mean areas of the single stages as well as the mean total brood nest area of the control and the test item treatment group displayed a similar brood development within the range of natural variability. At the end of the first brood cycle/beginning of the second brood cycle on BFD 22, the total mean brood nest size (eggs, larvae, capped cells) in the control and test item treatment showed a relative increase of 17 % and 23 %, respectively. Due to natural reduction of brood activity after the bee season and shift to winter bee production, the brood nest started to decrease slightly during the second investigated brood cycle by -28 % and by -23 % in the control and the test item treatment group, respectively. However, this decrease was comparable in both treatment groups. Furthermore, the relative decrease of the test item colonies in the test item group was even lower than in the control. The assessment of the areas covered with pollen and nectar/honey during pre-exposure (BFD 0) revealed similar levels and a sufficient supply of all colonies with food. Further assessments displayed a good food supply with no limitation during the course of the study. Statistical analysis revealed no significant differences between control and test item treatment group in total brood (eggs+larvae+pupae)/total food (nectar+pollen) throughout both investigated brood cycles (Student-t test, one-sided smaller, $p>0.05$).

**Brood development of individually labelled brood cells**

Brood termination rate [BTR]: The mean BTR of initially labelled eggs amounted to 6.6 and 2.9 % for the control and the test item groups, respectively, at evaluation on BFD 22, the last assessment of the first

brood cycle. Therefore, the termination of labelled eggs was on a similar level without any statistical significance. Due to natural reduction of brood activity after the bee season and to a shift to winter bee production, the brood nests started to decrease slightly during the second investigated brood cycle. On BFD 44 the mean BTR of initially labelled eggs amounted to 14.1 % and 12.1 % for the control and the test item treatment group, respectively. Also, in this brood cycle, no statistically significant differences of the BTRs in the control and the test item treatment occurred.

Brood index [BI]: The BI displays a negative correlation with the BTR: the higher the BTR the lower the BI and vice versa. Therefore, the BI of initially labelled eggs was slight lower in the test item treatment and amounted to 4.6 and 4.9 at BFD 22. However, no statistically significant differences were detected. Generally, the brood-index (BI), which is an indicator of the bee brood development and facilitates a comparison between different treatments, display a negative correlation with the BTR: the higher the BTR the lower the BI and vice versa. At the last assessment on BFD 44 the mean BIs amounted to 4.3 and 4.4 in the control and test item treatment, respectively, without any statistically significant differences (STUDENT t-test, one-sided smaller, $p>0.05$).

Brood compensation index [BCI]: The brood compensation index (BCI) is an indicator for brood development including recovery after termination of brood in the marked cells. Generally, the BCIs in all treatment groups were slightly higher than the corresponding BIs on most assessment days, indicating that at least some cells with terminated brood were refilled with new eggs and subsequently developed. The mean BCI of initially labelled eggs at BFD 22 was 4.7 and 4.9 in the control and test item treatment, respectively, without any statistically significant differences. Also, during the second brood cycle, mean BCIs were almost the same in both treatment groups, without any statistically significant differences. At the last assessment on BFD 44 the mean BCI was 4.5 in both treatment groups.

**Table A 62: Summary of study endpoints**

| **Evaluation / Assessment** | | **Treatment group** | | | |
|---|---|---|---|---|---|
| | | **Control** | | **Test item Cymoxanil 33% + Zoxamide 33% WG** | |
| | | **mean[1)]** | **SD** | **mean[1)]** | **SD** |
| **Adult mortality** (bees/colony/day) | Pre-exposure phase [3)] (DAT -3 to DAT -1) | **7.3** | 3.7 | **8.6** | 3.0 |
| | Exposure phase [2)] after first application (DAT 0ba to DAT -1) | **5.2** | 1.8 | **8.7** | 0.4 |
| | Exposure phase2) after second application (DAT 0aa to DAT -1) | **4.5** | 1.7 | **8.7** | 0.4 |
| | Post-exposure phase [3)] (DAT 8 to DAT 42) | **2.2** | 0.7 | **1.8** | 0.5 |
| | Overall after first application (DAT 0ba to DAT 42) | **2.8** | 0.7 | **2.3** | 0.5 |
| | Overall after second application (DAT 0aa to DAT 42) | **2.8** | 0.7 | **2.2** | 0.5 |
| **Pupal mortality** (bees/colony/day) | Pre-exposure phase [3)] (DAT -3 to DAT -1) | **0.0** | 0.0 | **0.0** | 0.0 |
| | Exposure phase [2)] after first application (DAT 0ba to DAT -1) | **0.0** | 0.0 | **0.0** | 0.0 |
| | Exposure phase [2)] after second application (DAT 0aa to DAT -1) | **0.0** | 0.0 | **0.0** | 0.0 |
| | Post-exposure phase [3)] (DAT 8 to DAT 42) | **0.0** | 0.0 | **0.0** | 0.0 |
| | Overall after first application (DAT 0ba to DAT 42) | **0.0** | 0.0 | **0.0** | 0.0 |

| | | | | | |
|---|---|---|---|---|---|
| | Overall after second application (DAT 0aa to DAT 42) | **0.0** | 0.0 | **0.0** | 0.0 |
| **Foraging activity** [bees/m²/ colony/day] | Exposure phase after first application | **9.2** | 0.9 | **8.7** | 0.4 |
| | Exposure phase after second application | **9.1** | 0.8 | **8.7** | 0.4 |
| **Colony weight** [kg/colony] | Before application (BFD 0) | **24.9** | 1.9 | **24.8** | 2.6 |
| | Overall post-application phase (BFD 44) | **27.4** | 2.6 | **29.5** | 2.1 |
| **Brood termination rate** [%] | Eggs (BFD 22) [4] | **6.6** | 4.8 | **2.9** | 1.3 |
| **Brood termination rate** [%] | Eggs (BFD 44) [4] | **14.1** | 10.8 | **12.1** | 10.9 |
| **Brood index** [n] | Eggs (BFD 22) [4] | **4.6** | 0.3 | **4.9** | 0.1 |
| **Brood index** [n] | Eggs (BFD 44) [4] | **4.3** | 0.5 | **4.4** | 0.5 |
| **Brood compensation index** [n] | Eggs (BFD 22) [4] | **4.7** | 0.2 | **4.9** | 0.1 |
| **Brood compensation index** [n] | Eggs (BFD 44) [4] | **4.5** | 0.4 | **4.5** | 0.4 |

[1] mean of seven replicates
[2] sum of dead honeybees found in dead bee trap and on gauze sheets in front of each hive
[3] dead honeybees found in dead bee trap only
[4] at the last relevant assessment when development is expected to be completed, i.e. BFD 22/44 for marked eggs
ba: before second application; aa: after second application
* = statically significant different when comparing treatment against control via Student or Welch t-test (α=0.05); at pre-application period: two-sided; post application: one-sided greater (mortality; BTR); one-sided smaller (colony strength; total brood; total food; colony weight; brood index; brood compensation index)

The mean BI of the initially labelled eggs at BFD 22 amounted to 4.6 and 4.9 in the control and test item treatment, respectively. No statistically significant differences were detected on BFD 4, 11, 16 and 22 (STUDENT or WELCH t-test, one-sided smaller, p > 0.05).
Also for the second investigated brood cycle no statistically significant differences were detected on BFD 28, 32, 37 and 44 (STUDENT t-test, one-sided smaller, p > 0.05). At the last assessment on BFD 44 the mean Bis amounted to 4.3 and 4.4 in the control and test item treatment, respectively.

Table 17: Summary of honeybee brood index in the course of the study

| Assessment Day | | Mean brood index [n] | | | |
|---|---|---|---|---|---|
| | | Control | | Test item | |
| | | Mean[1] | ± SD | Mean[1] | ± SD |
| **BFD 4** | 1st brood cycle | 2.2 | 0.4 | 2.6 | 0.3 |
| **BFD 11** | | 3.7 | 0.2 | 3.9 | 0.1 |
| **BFD 16** | | 3.7 | 0.2 | 3.9 | 0.1 |
| **BFD 22** | | 4.6 | 0.3 | 4.9 | 0.1 |
| **BFD 28** | 2nd brood cycle | 2.5 | 0.4 | 2.6 | 0.2 |
| **BFD 32** | | 3.5 | 0.4 | 3.5 | 0.4 |
| **BFD 37** | | 3.4 | 0.4 | 3.5 | 0.4 |
| **BFD 44** | | 4.3 | 0.5 | 4.4 | 0.5 |

BFD: Brood area fixing day; [1] mean of seven replicates

## Conclusion

The test item Cymoxanil 33% + Zoxamide 33% WG was applied twice at a rate of each 1.35 kg product/ha (the 3-fold max. single application rate of 0.45 kg product/ha; corresponding to 2x 445.5 mg cymoxanil/ha and 445.5 mg zoxamide/kg; based on nominal content of a.i.) at an interval of 7 days to full-flowering *Phacelia tanacetifolia* during active foraging of honeybees in the field. The start of the exposure was on the day of the second application. Pollen composition analyses of pollen baskets after the second application confirmed the use of the test item treated flowering *Phacelia* fields by honeybees as their main food source, and the use of contaminated feed items (like *Phacelia* pollen). Furthermore, residue analyses confirmed the worst-case exposure of honeybee colonies to the test item in flowers, pollen, nectar and bees.

Overall, two applications of Cymoxanil 33% + Zoxamide 33% WG caused no adverse effects on the behavior, the mortality, the foraging activity, the weight development, the condition of the colonies and the bee brood development over an observation period of 42 days after the second application.

(Schnurr A. 2020)

### A 2.3.1.6.2 Study 2 - Cymoxanil 33% + Zoxamide 33%: Honey bee field study 2019 in SEU

| | |
|---|---|
| Comments of zRMS: | The study was evaluated to comprehensively assess the effect of the Cymoxanil 33% + Zoxamide 33% WG on the development of bee colonies.<br><br>Under field conditions, the Cymoxanil 33% + Zoxamide 33% WG was applied twice at a rate of each 1.35 kg product/ha with interval of 7 days on full-flowering *Phacelia*, during active foraging of honeybees. The start of the exposure was on the day of the second application (including pollen composition analyses). The residue analyses confirmed the exposure of honeybee colonies to the product in flowers, pollen, nectar and bees.<br><br>In the conclusion, the 3-fold max. single application rate of 0.45 kg product/ha, caused no adverse effects on the behaviour, the mortality, the foraging activity, the weight development, the condition of the colonies and the bee brood development over an observation period of 42 days after the second application.<br><br>The study is considered to be reliable and suitable for the risk assessment. |

| | |
|---|---|
| **Reference:** | **KCP 10.3.1.6/02** |
| Report | Schnurr, A., 2020: Effects of Cymoxanil 33% + Zoxamide 33% on the honeybee *Apis mellifera* L. under field conditions in Spain (Southern Europe) with additional assessments on colony and brood development<br>Gowan Crop Protection Ltd., UK, SIPCAM OXON S.p.A, Italy<br>BioChem agrar, Germany, Report No. 19 48 BFB 0001, GLP, Not published |
| Guideline(s): | OECD 75 (2007)<br>EPPO Standard PP 1/170(4) (2010)<br>Recommendations of the AG Bienenschutz (2011)<br>Recommendations of the EFSA Guidance Document on the risk assessment of plant protection products on bees (2013) |
| Deviations: | No sampling of in-hive pollen on DAT 2. Retain samples of Control and Test item could not be generated: |

1948BFB0001-46
1948BFB0001-47
1948BFB0001-64
1948BFB0001-65, there were not enough in-hive pollen available.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): No

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | Cymoxanil 33% + Zoxamide 33% / Reboot (GSOL7019) |
| **Active substance content (measured)** | Cymoxanil: 33.0% w/w (nominal), 32.75 ± 0.15 % (analysed)<br>Zoxamide: 33.0% w/w (nominal), 32.91 ± 0.17% (analysed)<br>Zoxamide isomers: 50.65 ± 0.10 % R-isomer, 49.35 ± 0.10 % S-isomer |
| **Species:** | *Apis mellifera* L. *iberiensis Engel* (Hymenoptera, Apoidea), |
| Age: | Adult and juvenile bees of all brood stages |
| Number of animals: | 6325-10725 bees per hive on BFD 0 |
| Number of bee colonies (replicates)/treatment group: | 7 (+ 1 colony for residue analysis only)/2 |
| Weight: | 34.91 and 33.75 kg/colony |
| Source: | Beekeeper Joaquin Cordero, Paseo del Moro No. 19, 41370 Cazalla (Seville), Spain |
| Acclimation: | 3 days pre-exposure phase |
| Field size: | Control: 2.01 ha<br>Test item: 2.0 ha |
| Feeding: | On the treated field.<br>The food status of all colonies was acceptable throughout the trial, no artificial food was offered and all assessments of colony represented the natural state |
| **Environmental conditions** | |
| Temperature: | 22.8 - 23.1°C (first application)<br>15.3 – 15.5°C (second application) |
| Relative humidity: | 48 – 52% (first application)<br>73 – 75% (second application) |
| **Application rates** | Control: left untreated<br>Test item: 2 x 1.35 kg product/ha (the 3-fold max. single application rate of 0.45 kg product/ha) at a minimum interval of 7±1 days (equivalent to 2 x 891 g total a.i./ha) |
| **Test duration** | 3 days pre-exposure<br>8 days exposure phase |

| | |
|---|---|
| | 42 days post exposure phase |
| **Remarks** | None |

The purpose of this study was to determine potential effects of Cymoxanil 33 % + Zoxamide 33 % WG (containing nominally 33 % of each active ingredient cymoxanil and zoxamide) on the honeybee (A*pis mellifera*) after two consecutive foliar applications of each 1.35 kg product/ha (the 3-fold max. single application rate of 0.45 kg product/ha) at an interval of 7 days during flowering of Phacelia (*Phacelia tanacetifolia*) and during bee flight under field conditions. The first application was conducted at the beginning of flowering (BBCH 61) during daytime and the second application at full-flowering (BBCH 65) during daily bee flight. Main endpoints were the mortality, foraging activity, bee behaviour, colony and brood development. Special attention was paid on detailed brood development during two brood cycles via photo documentation of initially labelled eggs. Additionally, residues of cymoxanil and zoxamide were determined in spray solution, flowers, pollen (pollen from pollen traps and in-hive pollen), nectar (nectar from forager bees and in-hive nectar), dead bees and alive bees with an analytical method fully validated according to SANCO/3029/99 rev. 4 and SANCO/825/00 (2010).

7 replicates (bee colonies) honeybees of the species *Apis mellifera* L. were exposed to each untreated or test item treated and highly attractive crop *Phacelia tanacetifolia* under field conditions. The size of the untreated control and test item treated field was 2.01 and 2.00 ha, respectively. Two consecutive foliar applications of each 1.35 kg product/ha (the 3-fold max. single application rate of 0.45 kg product/ha) at an interval of 7 days were carried out shortly before flowering (DAT -7) and during full flowering (DAT 0) of Phacelia (*Phacelia tanacetifolia*), respectively. During the entire test period, the climatic data (temperature, relative humidity, rainfall and cloud cover (at time of evaluation) were recorded continuously. The growth stage of *Phacelia tanacetifolia* was recorded from the first application to the end of exposure phase on each assessment day.

Assessments of mortality and bee behavior were carried out 3 days prior to the second application (at monitoring site), on the day of the second application and on the following 42 days after application.

Foraging activity assessments were carried out on the day of the second application and on the following 7 days after the second application during exposure.

Behavior: daily recording during entire study period.

Colony development (colony strength and brood development) during two consecutive brood cycles: DAT -2, DAT 2, DAT 7; DAT 14, DAT 20, DAT 26, DAT 30, DAT 36 and DAT 42 which is equal to BFDB 0, BFD 4, BFD 9, BFD 16, BFD 22, BFD 28, BFD 32, BFD 38 and BFD 44. Additional overwintering assessments were carried out on BFD 185, BFD 214 and BFD 312

Detailed brood assessments on BFD 0, BFD 4, BFD 9, BFD 16, BFD 22 (BFD 0C), BFD 28 (BFD 6C), BFD 32 (BFD 10C), BFD 38 (BFD 16C) and BFD 44 (BFD 22C) for initially labelled eggs

Colony weight: DAT -2, DAT 2, DAT 7; DAT 14, DAT 20, DAT 26, DAT 30, DAT 36, DAT 42, DAT 183, DAT 212 and DAT 310

Additionally, residues of cymoxanil and zoxamide were determined in spray solution, flowers, pollen (pollen from pollen traps and in-hive pollen), nectar (nectar from forager bees and in-hive nectar), dead bees and alive bees with an analytical method fully validated according to SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 8.1 (2010) with a Limit of quantification (LOQ) of 0.005 mg/kg per analyte and a Limit of Detection (LOD) of 0.001 mg/kg per analyte. The analysis was following QuEChERS extraction method using high performance liquid chromatography (HPLC) with mass-spectrometric (MS-MS) detection of zoxamide and cymoxanil in one run.

## Results and discussion

Environmental parameters (temperature, relative humidity) remained within acceptable limits throughout the study.

The test item concentrations in the spray solutions were analytically confirmed. All control samples or samples before test item application showed no residues above the limit of quantification (i.e. no residues above the LOQ of 0.005 mg a.i./kg). However, residues of zoxamide and cymoxanil on/in flowers, bees, pollen and nectar confirmed a proper and (unrealistic) worst-case test item loading of bees and their feed items in the treated field part.

**Table A 63: Residue analysis of cymoxanil and zoxamide in spray solution**

| Matrix | Treatment group | Sampling day | Recovery of cymoxanil (%) | Recovery of zoxamide (%) |
|---|---|---|---|---|
| Spay solution | Test item | DT I | 93 | 90 |
| | Test item | DAT 0 | 86 | 80 |

DT I: Day of Treatment; DAT: Days after second treatment

**Table A 64: Residue analysis of cymoxanil and zoxamide in flowers**

| Matrix | Treatment group | Sampling day | Concentration of cymoxanil mg/kg) | Concentration of zoxamide (mg/kg) |
|---|---|---|---|---|
| Flowers | Control | DT I ba | < LOD | < LOD |
| | | DT I | < LOD | < LOD |
| | | DAT 0 | < LOD | < LOD |
| | | DAT 2 | < LOD | < LOD |
| | | DAT 5 | < LOD | < LOD |
| | | DAT 7 | < LOD | < LOD |
| | Test item | DT I ba | < LOD | < LOD |
| | | DT I | 40.71 | 48.49 |
| | | DAT 0 | 27.28 | 45.73 |
| | | DAT 2 | 13.31 | 33.23 |
| | | DAT 5 | 0.575 | 3.908 |
| | | DAT 7 | 0.248 | 3.059 |

LOD = 0.001 mg/kg
ba: before application
DT I: Day of Treatment
DAT: Days after second treatment

**Table A 65: Residue analysis of cymoxanil and zoxamide in pollen**

| Matrix | Treatment group | Sampling day | Concentration of cymoxanil mg/kg) | Concentration of zoxamide (mg/kg) |
|---|---|---|---|---|
| Pollen (trap) | Control | DAT 0 | < LOD | < LOD |
| | | DAT 2 | < LOD | < LOD |
| | | DAT 5 | < LOD | < LOD |
| | | DAT 7 | < LOD | < LOD |
| Pollen (in-hive) | | DAT 2 | < LOD | < LOD |
| | | DAT 7 | < LOD | < LOQ (0.0016) |
| Pollen (trap) | Test item | DAT 0 | 0.267 | 0.351 |
| | | DAT 2 | 0.381 | 1.544 |
| | | DAT 5 | 0.088 | 0.578 |
| | | DAT 7 | 0.196 | 0.583 |

| | | | | |
|---|---|---|---|---|
| Pollen (in-hive) | | DAT 2 | 0.114 | 0.779 |
| | | DAT 7 | 0.031 | 0.484 |

LOD = 0.001 mg/kg
LOQ = 0.005 mg/kg
DAT: Days after second treatment

**Table A 66: Residue analysis of cymoxanil and zoxamide in nectar**

| Matrix | Treatment group | Sampling day | Concentration of cymoxanil mg/kg) | Concentration of zoxamide (mg/kg) |
|---|---|---|---|---|
| Nectar (forager bees) | Control | DAT 0 | < LOD | < LOD |
| | | DAT 2 | < LOD | < LOD |
| | | DAT 5 | < LOD | < LOD |
| | | DAT 7 | < LOD | < LOD |
| Nectar (in-hive) | | DAT 2 | < LOD | < LOD |
| | | DAT 7 | < LOD | < LOQ |
| Nectar (forager bees) | Test item | DAT 0 | 0.126 | 0.043 |
| | | DAT 2 | 0.122 | 0.019 |
| | | DAT 5 | < LOD | < LOD |
| | | DAT 7 | < LOD | < LOD |
| Nectar (in-hive) | | DAT 2 | 0.033 | < LOD |
| | | DAT 7 | < LOD | < LOD |

LOD = 0.001 mg/kg
LOQ = 0.005 mg/kg
DAT: Days after second treatment

**Mortality**: The mortality of honeybees at the monitoring site before the second application was on a low and similar level in the control and the test item treatment indicating comparable colonies. No statistically significant differences were observed between the two treatment groups, neither based on daily comparisons nor on overall comparison before application (Student test, two-sided, $p>0.05$). Also, during the exposure phase (beginning on the day of the second test item application) and during post-exposure phase no differences in mean mortality were observed between both treatment groups (with the exception for DAT 5). Mortality was on a low and comparable level throughout the study, without any statistically significant differences (with the exceptions for DAT 12 and DAT 16).

No mortality of honeybee pupae was observed in both treatment groups during the entire test.

**Foraging activity:** Shortly before the second application, the foraging activity was on an adequate and similar level in all test groups, indicating that bees had well adapted to the new environmental conditions and that they were well exposed during the test item applications. After the second application and during the following days of the exposure phase the mean foraging activity was on a comparable level in the control and the test item treatment group

**Bee behavior:** No behavioral changes of the bees after test item treatment and compared to the control colonies were observed during the assessments between DAT -3 until DAT 42. Honeybees showed no different or abnormal behaviors.

**Colony weight:** At the colony assessment conducted two days before the second application (DAT -2), the average weight per colony was similar, amounting to 34.91 and 33.75 kg/colony in the control and the test item treatment group respectively. This difference was not statistically significant (Student-t test, two-sided, $p<0.05$). The development of the colony weight in the control and the test item treatment group during the post-application period, which covered two consecutive brood cycles was very similar. It resulted to an increase of +17.54 % and +12.63 % in the control and the test item treatment group at the last assessment if compared to the first assessment on BFD 0. Consequently, the statistical analysis revealed

no significant differences between control and test item treatment group during the entire post-application period (Student-t test, one-sided smaller, p>0.05). The additional overwintering assessment showed similar results. The colony weight in autumn (BFD 185) amounted to 23.18 and 21.07 kg/colony and increased to 23.10 and 24.94 kg/colony in spring (BFD 312) in the control and test item. The statistical analysis revealed a significant difference between control and test item treatment group at BFD 185 (Student-t test, one-sided smaller, p>0.05).

**General brood assessments**

Colony strength: Two days before the second application (DAT -2/BFD 0), the average colony strength was 8584 and 8977 bees/colony in the control and the test item treatment group, respectively and was thus very similar and without any statistically significant difference (Student-t test, two-sided, p>0.05). In the course of the study, which covered two consecutive brood cycles the colony strength in the control revealed an increased development in comparison to the test item treatment group with a relative increase of 79 % and 26 % at the last assessment (BFD 44), if compared to the pre-exposure assessment in the control and the test item treatment group, respectively. Statistically significant differences between control and test item treatment group were observed in the second investigated brood cycle on BFD 28, BFD 32 and BFD 38 (Student-t test, one-sided smaller, p>0.05). The additional overwintering assessment revealed a comparable development of the colony before and after winter in both treatment groups. During the first assessment in autumn (DAT 183/BFD 185), the average colony strength was 7739 and 7189 bees/colony in the control and the test item treatment group, respectively and therefore without any statistically significant difference (Student-t test, two-sided, p>0.05). Similar results were observed on the assessment in spring (DAT 310/BFD 312), the average colony strength amounted to 7621 and 7248 bees/colony in the control and the test item treatment group, respectively and this was without any statistically significant difference (Student-t test, two-sided, p>0.05).

Brood area: At the first assessment (BFD 0), the mean brood areas of the single stages, i.e. eggs, larvae and capped cells (= pupae) as well as that of the total brood (total area occupied by eggs, larvae and capped cells) was similar in both treatment groups without any statistically significant difference (Student-t test, two sided, p>0.05). During the course of the study, which covered two consecutive brood cycles the mean areas of the single stages as well as the mean total brood nest area of the control and the test item treatment displayed a comparable brood development within the range of natural variability. At the end of the first brood cycle/beginning of the second brood cycle on BFD 22, the total mean brood nest size (eggs, larvae, capped cells) in the control showed a relative increase of 20 % and test item treatment a slight decrease of -14 %, respectively. During the second investigated brood cycle brood area increased by 25 % and by 16 % in the control and the test item treatment, respectively. The assessment of the areas covered with pollen and nectar/honey during pre-exposure (BFD 0) revealed similar levels and a sufficient supply of all colonies with food. Further assessments displayed a good food supply with no limitation during the course of the study. Statistical analysis revealed no statistically significant differences between control and the test item treatment group in total food (nectar+pollen) throughout both investigated brood cycles (Student-t test, one-sided smaller, p>0.05). Nevertheless, statistical analysis revealed statistically significant differences between the control and the test item treatment group in total brood (eggs, larvae and capped cells) on BFD 22, BFD 28 and BFD 32 (Student-t test, one-sided smaller, p>0.05). At the first assessment in autumn (BFD 185), the mean brood areas of the single stages, i.e. eggs, larvae and capped cells (= pupae) as well as that of the total brood (total area occupied by eggs, larvae and capped cells) was similar in both treatment groups without any statistically significant difference (Student-t test, two sided, p>0.05). After winter during the assessment in spring (BFD 312), the colonies revealed an increased brood development in the total brood as well as the single brood stages, indicating a successful overwintering without statistically significant differences between control and test item (Student-t test, one-sided smaller, p>0.05).

**Brood development**

Brood termination rate [BTR]: The mean BTR of initially labelled eggs amounted to 20.0 and 22.3 % for the control and the test item group, respectively, at evaluation on BFD 22, the last assessment of the first brood cycle. Therefore, the termination of labelled eggs was on a similar level, without any statistical significance.

In the course of the study, the brood nest started to increase during the second investigated brood cycle and on BFD 44 the mean BTR of initially labelled eggs amounted to 9.3 % and 8.5 % for the control and the test item treatment group, respectively. Also, in this brood cycle, no statistically significant differences of the BTRs in the control and the test item treatment occurred.

Brood index [BI]: The BI displays a negative correlation with the BTR: the higher the BTR the lower the BI and vice versa. Therefore, the BI of initially labelled eggs in the control was equal to the test item treatment and amounted to 4.0 and 3.9 at BFD 22. However, no statistically significant differences were detected.

At the last assessment on BFD 44 the mean BIs amounted to 4.5 and 4.6 in the control and test item treatment, respectively. Also, for the second investigated brood cycle no statistically significant differences between control and test item treatment group were detected

Brood compensation index [BCI]: The brood compensation index (BCI) is an indicator for brood development including recovery after termination of brood in the marked cells. Generally, the BCIs in both treatment groups were comparable at all assessment days, indicating that at least some cells with terminated brood were refilled with new eggs and subsequently developed.

The mean BCI of initially labelled eggs at BFD 22 was 4.1 and 4.2 in the control and test item treatment, respectively, without any statistically significant differences

Also, during the second brood cycle, mean BCIs were almost the same in both treatment groups, without any statistically significant differences. At the last assessment on BFD 44 the mean BCI was 4.7 in both treatment groups.

**Table A 67: Summary of study endpoints**

| **Evaluation / Assessment** | | **Treatment group** | | | |
|---|---|---|---|---|---|
| | | **Control** | | **Test item Cymoxanil 33% + Zoxamide 33% WG** | |
| | | **mean[1)]** | **SD** | **mean[1)]** | **SD** |
| **Adult mortality** (bees/colony/day) | Pre-exposure phase [3)] (DAT -3 to DAT -1) | **12.2** | 3.9 | **19.5** | 14.0 |
| | Exposure phase [2)] after first application (DAT 0ba to DAT -1) | **12.1** | 5.0 | **13.9** | 8.8 |
| | Exposure phase2) after second application (DAT 0aa to DAT -1) | **11.4** | 4.8 | **13.1** | 8.8 |
| | Post-exposure phase [3)] (DAT 8 to DAT 42) | **9.9** | 4.4 | **13.2** | 11.1 |
| | Overall after first application (DAT 0ba to DAT 42) | **10.3** | 4.3 | **13.3** | 9.4 |
| | Overall after second application (DAT 0aa to DAT 42) | **10.1** | 4.3 | **13.2** | 9.4 |
| **Pupal mortality** (bees/colony/day) | Pre-exposure phase [3)] (DAT -3 to DAT -1) | **0.0** | 0.0 | **0.0** | 0.0 |
| | Exposure phase [2)] after first application (DAT 0ba to DAT -1) | **0.0** | 0.0 | **0.0** | 0.0 |
| | Exposure phase [2)] after second application (DAT | **0.0** | 0.0 | **0.0** | 0.0 |

| | | | | | |
|---|---|---|---|---|---|
| | 0aa to DAT -1) | | | | |
| | Post-exposure phase [3] (DAT 8 to DAT 42) | **0.0** | 0.0 | **0.0** | 0.0 |
| | Overall after first application (DAT 0ba to DAT 42) | **0.0** | 0.0 | **0.0** | 0.0 |
| | Overall after second application (DAT 0aa to DAT 42) | **0.0** | 0.0 | **0.0** | 0.0 |
| **Foraging activity** [bees/m²/ colony/day] | Exposure phase after first application | **10.7** | 0.6 | **10.7** | 0.6 |
| | Exposure phase after second application | **10.8** | 0.7 | **10.8** | 0.6 |
| **Colony weight** [kg/colony] | Before application (BFD 0) | **34.91** | 2.02 | **33.75** | 3.03 |
| | Overall post-application phase (BFD 44) | **41.03** | 4.82 | **38.01** | 5.55 |
| | Overwintering (Spring - BFD 312) | **23.10** | 2.56 | **24.94** | 3.93 |
| **Brood termination rate** [%] | Eggs (BFD 22) [4] | **20.0** | 27.8 | **22.3** | 16.7 |
| **Brood termination rate** [%] | Eggs (BFD 44) [4] | **9.3** | 6.6 | **8.5** | 5.8 |
| **Brood index** [n] | Eggs (BFD 22) [4] | **4.0** | 1.4 | **3.9** | 0.8 |
| **Brood index** [n] | Eggs (BFD 44) [4] | **4.5** | 0.3 | **4.6** | 0.3 |
| **Brood compensation index** [n] | Eggs (BFD 22) [4] | **4.1** | 1.4 | **4.2** | 0.7 |
| **Brood compensation index** [n] | Eggs (BFD 44) [4] | **4.7** | 0.2 | **4.7** | 0.2 |

[1] mean of seven replicates
[2] sum of dead honeybees found in dead bee trap and on gauze sheets in front of each hive
[3] dead honeybees found in dead bee trap only
[4] at the last relevant assessment when development is expected to be completed, i.e. BFD 22/44 for marked eggs
ba: before second application; aa: after second application

## Conclusion

The test item Cymoxanil 33% + Zoxamide 33% WG was applied twice at a rate of each 1.35 kg product/ha (the 3-fold max. single application rate of 0.45 kg product/ha; corresponding to 891 g/ha zoxamide and cymoxanil, respectively) at an interval of 7 days to full-flowering Phacelia tanacetifolia B. during active foraging of honeybees in the field. The start of the exposure was on the day of the second application. Pollen composition analyses of pollen baskets after the second application confirmed the use of the test item treated flowering Phacelia fields by honeybees as their main food source, and the use of contaminated feed items (like the Phacelia pollen) for foraging of the honeybee colonies. Furthermore, residue analyses confirmed the worst-case exposure of the bees as well as flowers, the pollen and the nectar to the test item.

Overall, two applications of Cymoxanil 33% + Zoxamide 33% WG caused no adverse effects on the behavior, the mortality, the foraging activity, the weight development, the condition of the colonies and the bee brood development over an observation period of 42 days and the additional overwintering phase in the field.

(Schnurr A. 2020)

## A 2.3.1.7 KCP 10.3.2 Effects on non-target arthropods other than bees

## A 2.3.1.8 KCP 10.3.2.1 Standard laboratory testing for non-target arthropods

## A 2.3.1.9 KCP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods

### A 2.3.1.9.1 Study 1 - CYMOXANIL 33% + ZOXAMIDE 33% WG: Effects on *Aphidius rhopalosiphi*

| | |
|---|---|
| Comments of zRMS: | Study evaluated and accepted by zRMS UK. Please refer to product fRR dated January 2012. |

| | |
|---|---|
| **Reference:** | **KCP 10.3.2.2/01** |
| Report | Colli, M., 2006: Effects of CYMOXANIL 33% ÷ ZOXAMIDE 33% WG on the Aphid Parasitoid, *Aphidius rhopalosiphi* De Stefani Perez (Hymenoptera, Braconidae) in Laboratory (Limit test)<br>Oxon Italia S.p.A., Italy<br>Biotecnologie BT S.r.l., Italy, Report No. BT028/06, GLP, Not published |
| Guideline(s): | Mead-Briggs *et al*, 2000<br>ESCORT I (1994)<br>ESCORT II (2001) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | No |

**Executive Summary**

The effects on the mortality and reproduction of *Aphidus rhopalosiphi* were evaluated under the worst-case exposure conditions after 48 h.
The test item was tested at dosage of 2.25 kg/ha on 10 individuals, in five replicates. Test organisms were exposed to test item on glass plates, after the spray layer was dried. The number of dead aphids and behavioural abnormalities were observed after 1, 2, 24 and 48 hours. Effects on reproduction were recorded by determining females parasitisation rate in the subsequent 24h.
'Cymoxanil 33% + Zoxamide 33% WG' didn't cause adverse effects on mortality or reproduction on *Aphidus rhopalosiphi* at application rate of 2.25 kg/ha.
The $LR_{50}$ value of test item is higher than 2.25 kg/ha

**I. MATERIALS AND METHODS**

**A. MATERIALS**

**1.Test Material:**

| | |
|---|---|
| **Description:** | Granules |
| **Lot/Batch #:** | Batch code BPL 212 |
| **Purity:** | Cymoxanil 330 g/kg; Zoxamide 330 g/kg |
| **Stability of test compound** | June 2008 (expiry date) |
| **2. Vehicle and/or positive control** | Perfekthion (dimethoate) used as positive control |

**3. Test animals**

| | |
|---|---|
| **Species:** | *Aphidius rhopalosophi* |
| **Strain** | Not relevant |
| **Age:** | Young adult |
| **Weight at dosing:** | Not relevant |
| **Source:** | In house rearing stock from BioTecnologie BT - Italy |
| **Acclimation period** | Not stated |
| **Diet:** | 1:3 (w/w) honey/water solution |
| **Water:** | Not relevant |
| **Housing:** | Cultures were maintained at 19.67-20.33 °C, 60-100 humidity conditions, 16/8 light/dark photoperiod at intensity of 1700 lux |

**4. Environmental conditions**

| | |
|---|---|
| **Temperature:** | 19.67-20.33 °C |
| **Humidity:** | 60-100 |
| **Air changes:** | Ventilation during the hole study |
| **Photoperiod:** | 16 h light, 1700 lux during mortality assessment, 12000 lux during reproduction assessment |

**B. STUDY DESIGN AND METHODS**

| | |
|---|---|
| **1. In life dates** | 26 June – 11 July 2006 |
| **2. Experimental treatment** | 'Cymoxanil 33% + Zoxamide 33% WG' was sprayed at dosage of 2.25 kg/ha on circular glass plates, assembled in cages.<br>The formulation Perfekthion (dimethoate 400 g/L) was used at 0.12 g/ha as reference test item.<br>10 individuals (including a minimum of 5 females) for each replica (5 replicates) were placed in circular glass plates when spray layer was dried. Treatment chambers were assembled to cage with the plates and aluminium frame (diameter 10 cm, height 1.5 cm, width 1 cm). Wasps were fed with honey/water solution. Insects were exposed to active ingredient residue for 48 h.<br>For the reproductive phase 15 females were chosen from treated groups and transferred in chambers in presence of wheat seedling, infected with *Sitobion avenae*. After 24 h wasps were removed and plants left under test conditions; the number of mummies and parasitised aphid was counted after 13 days. |
| **3. Observations** | Mortality and behavioural abnormalities were observed after 1, 2, 24, 48 hours<br>Number of mummies and parasitised aphid per females was counted after 13 days |
| **4. Statistics** | Statistical variations respect to the control were evaluated by Student's T-Test |

## II. RESULTS AND DISCUSSION

**A. FINDINGS**

'Cymoxanil 33% + Zoxamide 33% WG' didn't cause adverse effects on *Aphidius rhopalosophi* at dosage of 2.25 kg/ha. The corrected mortality was calculated according to the formula of Abbott (1925); it resulted 12.80 %, while the reduction of reproduction was 9.80 %. From Student's T-Test analysis no statistical differences between control and test item were found.
The reduction of beneficial capacity value was -13.48%.

**Table IIIA 10.5.1-1: Mean mortality and reproduction data**

| Nominal test item concentration (mg/L) | Corrected mortality 48 h (%) | Mean number of mummies |
|---|---|---|
| Untreated control | N.A. | 7.53 |

| | | |
|---|---|---|
| Test Item | 12.80 | 9.80 |

**III. CONCLUSIONS**

The $LR_{50}$ of Cymoxanil 33% + Zoxamide 33% is higher than 2.25 kg/ha, since no significant effect was observed. The product can be considered harmless since also the reduction of beneficial capacity is lower than 30%.

(Colli M. 2006)

## A 2.3.1.9.2 Study 2 - CYMOXANIL 33% + ZOXAMIDE 33% WG: Effects on *Typhlodromus pyri*

| | |
|---|---|
| Comments of zRMS: | Study evaluated and accepted by zRMS UK. Please refer to product fRR dated January 2012. |

**Reference:** **KCP 10.3.2.2/02**

Report: Colli, M., 2011: Effects of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' on the Predatory Mite, *Typhlodromus pyri* Scheuten (Acari Phytoseiidae) under extended laboratory conditions (Rate response test).
Oxon Italia S.p.A., Italy
Biotecnologie BT S.r.l., UK, Report No. BT031/06, GLP, Not published

Guideline(s): Blumel, S, *et al*. (2000)
IOBC, BART and EPPO Joint Initiative. Eds. Candolfi, M.P. et al.. IOBC Publication ISBN 92-9067-129-7

Deviations: No

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): No

**Executive Summary**

The effects of 'Cymoxanil 33% + Zoxamide 33% WG' on the mortality and reproduction of *Typhlodromus pyri* were tested with an extended laboratory study, spraying on broad bean plants five different concentration of the product (2.25, 0.45, 0.225, 0.1125 and 0.0563 kg/ha). Five replicates of each concentration and of the control were performed. Two different bioassays were carried out, one with treated leaves collected just after the application (bioassay 1) , and the other, at the highest concentration, using leaves aged for 7 days (bioassay 2). 20 protonymphs were introduced in each cage and exposed to active ingredient for 14 days. The survival was assessed after 3 and 7 days, while effects on reproduction were recorded at day 7, 9, 11 and 14. No statistical differences in the mortality between control and test item were shown in both bioassays. In the first bioassay a significant effect on reproduction was recorded at the highest rate of 2.25 kg product/ha (70.74%), whereas not significant effects were observed at the lower doses. The $ER_{50}$ was estimated to be 1.57 kg product/ha. In the second bioassay no reproductive decrease was observed in the predatory mites exposed to residue aged for 7 days, demonstrating a potential for re-colonization.

**I. MATERIALS AND METHODS**

**A. MATERIALS**

| | |
|---|---|
| **1.Test Material:** | |
| **Description:** | Granules |
| **Lot/Batch #:** | Batch code BPL 212 |
| **Purity:** | Cymoxanil 330 g/kg; Zoxamide 330 g/kg |
| **Stability of test compound** | June 2008 (expiry date) |
| **2. Vehicle and/or positive control** | Perfekthion (dimethoate) used as positive control |
| **3. Test animals** | |
| **Species:** | *Typhlodromus pyri* |
| **Strain** | Not relevant |
| **Age:** | Protonymph |
| **Weight at dosing:** | Not relevant |
| **Source:** | Katz Biotech. AG, Germany |
| **Acclimation period** | Not stated |
| **Diet:** | Pollen |
| **Water:** | Drinking water, *ad libitum* |
| **Housing:** | Cultures were maintained at 19.33 – 21 °C, 63.5 – 74.5% relative humidity, 16 h of light at maximum of 5000 lux |
| **4. Environmental conditions** | |
| **Temperature:** | 19.33 – 21 °C |
| **Humidity:** | 63.5 – 74.5% |
| **Air changes:** | Ventilation during the study |
| **Photoperiod:** | 16/8 light/dark at maximum of 5000 lux |

**B. STUDY DESIGN AND METHODS**

| | |
|---|---|
| **1. In life dates** | 28 August – 18 September 2006 |
| **2. Experimental treatment** | 'Cymoxanil 33% + Zoxamide 33% WG' was sprayed on bean potted plants at five concentrations: 2.25, 0.45, 0.225, 0.1125 and 0.0563 kg/ha.<br>The formulation Perfekthion 400 SC (dimethoate, 5.6 g a.i./ha) was used as reference test item.<br>Treated leaves were collected after the application, when the residues were dried. Five replicates for each group, with 20 predatory mites, were tested. An additional bioassay was performed using leaves aged for 7 days with the concentration that caused more than 50% of mortality in the first test. The test cages consisted in a Petri dish, containing a cotton wool pad and a bean leaf disc. Insects were exposed for 14 days.<br>At 7 days, a reproductive chamber with 1 male + 5 females was constituted from each treatment replicate: insects were then left in this chamber for the following 7 days. |
| **3. Observations** | The survival and behaviour of mites were assessed after 3 and 7 days.<br>The number of surviving females, laid and hatched eggs were recorded at day 7, 9, 11 and 14 during the reproduction phase. At intermediate dates eggs and juveniles were removed from chambers after counting. |
| **4. Statistics** | Statistical analysis were performed with Stat Soft (1998) software, ANOVA – Manova Analysis (Analysis of the Variance) and Post Hoc comparison of means – LSD Test |

## II. RESULTS AND DISCUSSION

**A. FINDINGS**

After 7 days from the treatment the mortality in the control and in the test item didn't present significant differences in both bioassays, with a maximum mortality recorded of 9.38%.
In the first bioassay a significant effect on reproduction was observed only at the highest concentration (2.25 kg/ha), with a reduction of 70.74%. The calculated $ER_{50}$ was 1.57 kg product/ha.
In the second bioassay no reproductive decrease was observed in the predatory mites exposed to residue aged for 7 days.

**Table IIIA 10.5.2-1: Mean mortality and reproduction data**

| | Application rate (kg/ha) | Mortality 7 days (%) | Corrected mortality 7 days (%) [+] | Mean cumulative number of eggs per female | % reduction in reproduction |
|---|---|---|---|---|---|
| Bioassay 1 | 2.250 | 4.00 | n.a. | 1.89 | 70.74* |
| | 0.450 | 9.00 | 5.21 | 5.72 | 11.46 |
| | 0.225 | 8.00 | 4.17 | 5.46 | 15.48 |
| | 0.113 | 6.00 | 2.08 | 5.02 | 22.29 |
| | 0.056 | 13.00 | 9.38 | 5.75 | 10.99 |
| | 0 (control) | 4.00 | 0.00 | 6.46 | n.a. |
| Bioassay 2 | 2.25 | 8.00 | n.a. | 3.81 | 21.12 |
| | 0 (control) | 8.00 | 0.00 | 4.83 | n.a. |

* significant difference
[+] Calculated according to Schneider-Orelli (1947) formula

## III. CONCLUSIONS

'Cymoxanil 33% + Zoxamide 33% WG' had no effect on survival of *Typhlodromus pyri* at rates of 2.25 kg product/ha or lower, immediately after the treatments. The $ER_{50}$ was estimated to be 1.57 kg product/ha. No reproductive decrease was observed in the predatory mites exposed to residue aged for 7 days, demonstrating potential for re-colonisation.

(Colli M. 2011)

### A 2.3.1.9.3 Study 3 - CYMOXANIL 33% + ZOXAMIDE 33% WG: Effects on *Orius laevigatus*

| | |
|---|---|
| Comments of zRMS: | The study follows the guideline specified by Bakker *et al.* (2000) and according to the principles of GLP.<br>No deviations were noted and all validity criteria were met. The study is acceptable for the risk assessment purposes. |

**Reference:** **KCP 10.3.2.2/03**

Report: Vinall, S., 2018: Cymoxanil 33% + Zoxamide 33% WG – A rate-response extended laboratory test to evaluate the effects of fresh residues on the predatory bug *Orius laevigatus* (Hemiptera; Anthocoridae)
Gowan Crop Protection Ltd., UK, Oxon Italia S.p.A, Italy
Mambo-Tox Ltd., UK, Report No. GOW-17-2, GLP, Not published

Guideline(s): Bakker *et al.* (2000)

Deviations: No

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) No

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | Reboot (GWN-9823) (30AL5020) |
| **Active substance content (nominal)** | 33% (w/w) cymoxanil<br>33% (w/w) zoxamide |
| **Species:** | *Orius laevigatus* |
| Number of animals: | five replicates per treatment group and control, each containing 20 predatory mites |
| Age: | The nymphs derived from batches that hatched over one day were used for the test when they were 4-5 days old |
| Source: | Biobest Belgium N.V., Belgium, via Agralan, Swindon, UK |
| Diet: | Eggs of the flour moth *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyraloidae) were supplied as food for the adult and nymphal bugs and were replenished every 1-3 days, as required. |
| **Environmental conditions** | |
| Temperature: | Mortality-assessment: 24.2-25.9°C<br>Maturation: 25.0-25.8 °C<br>Fecundity-assessment: 25.0-26.1°C |
| Photoperiod: | Mortality-assessment: 16 h (1400-2600 lux)<br>Maturation: 16 h (1500-2200 lux)<br>Fecundity-assessment: 16 h (1600-2700 lux) |
| Relative humidity | Mortality-assessment: 68-77%<br>Maturation: 68-72%<br>Fecundity-assessment: 65-72% |
| **Application rate(s)** | 1440, 720, 360, 180 and 90 g product/ha. |
| Positive control: | |
| **Post exposure observation period** | 14 DAT |
| **Remarks** | None |

The test item in this study was Cymoxanil 33% + Zoxamide 33 % WG, hereafter referred to by the product code GWN-9823, which is a water-dispersible granule formulation containing the fungicides cymoxanil (nominally 33% w/w) and zoxamide (nominally 33% w/w). The aim was to determine the effects of freshly-dried foliar residues of this test item on the predatory bug *Orius laevigatus* Fieber (Hemiptera; Anthocoridae) under extended laboratory test conditions.

GWN-9823 was evaluated at five rates, equivalent to 1440, 720, 360, 180 and 90 g product/ha. These test-item treatments were compared to a water-treated control. The active substance dimethoate (as EC formulation containing nominally 400 g a.s./L and applied at a rate of 40 mL product/ha) served as toxic reference item.

Treatments were applied at a volume rate equivalent to 200 L spray solution/ha to discs cut from leaves of the French bean plant, *Phaseolus vulgaris* L. Once spray residues had dried, the leaf discs were placed on a setting layer of an agar medium lining the base of small plastic pots. The leaf discs were of sufficient size to cover the agar surface and they were left with their treated surface uppermost. Five second-instar nymphs of *O. laevigatus* were placed on each leaf disc and were provided with untreated eggs of the flour moth, *Ephestia kuehniella* (Zeller) for food. A ventilated lid was placed on each pot. Assessments were made of the condition of the bugs for up to 9 days after treatment (DAT), when > 80% were judged to have developed into adults in the control treatment. The reproductive capacity of the surviving insects was then assessed. The reproduction assessments were made for the control and all of the test item treatments. For this assessment, the egg-laying activity of twenty females from each treatment was monitored for two 48-h periods and the viability of the eggs produced during the first period was determined.

## Results and Discussion

Environmental parameters (temperature, photoperiod and relative humidity) remained within acceptable limits throughout the study. The temperature ranged from 24.2 to 26.1°C, the light intensity ranged from 1400 to 2700 Lux and relative humidity was in the range from 65 to 77% during of the study.

At 9 days there was 11.3% mortality in the control treatment, compared with 8.8%, 8.9%, 7.5%, 16.3% and 12.5% mortality (i.e. -2.8, -2.7, -4.2, -5.6 and 1.4% corrected mortality) in the 1440, 720, 360, 180 and 90 g product/ha treatment rates of GWN-9823, respectively. None of the test-item treatments resulted in significant mortality, relative to the control (Fisher's Exact Test, $\alpha = 0.05$). In the toxic reference treatment, 100% mortality was observed at 9 days, which met the validity criterion imposed for this treatment.

The mean number of eggs produced per female (fecundity) was 7.9 in the control treatment, compared with values of 5.8, 7.1, 7.7, 8.2 and 9.0 in the 1440, 720, 360, 180 and 90 g product/ha treatment rates of GWN-9823, respectively. The mean hatching rate for eggs (fertility) was 97.8% in the control treatment, compared with values of 99.1%, 97.4% 97.6%, 97.7% and 99.5% in the 1440, 720, 360, 180 and 90 g product/ha treatment rates of GWN-9823, respectively.

None of the individual test-item treatments resulted in a significant reduction in either fecundity or fertility (Mann-Whitney *U*-test, $\alpha = 0.05$), relative to the control.

**Table A 68: Summary of bug mortality and of the reproductive performance of individually-confined female Orius over a 4-day period**

| Treatment | Product | % mortality at 9DAT[a)] | % corrected mortality [b)] | Reproduction (Fecundity)[c)] [eggs/♀/day] | % effect on fecundity [d)] | % hatching rate [e)] (Fertility) | % effect on fertility [d)] |
|---|---|---|---|---|---|---|---|
| Control | - | 11.3 | - | 7.9 | - | 97.8 | - |
| GWN-9823 | 1440 | 8.8 | -2.8 | 5.8 | 27.5 | 99.1 | -1.4 |
| | 720 | 8.9 | -2.7 | 7.1 | 10.1 | 97.4 | 0.4 |
| | 360 | 7.5 | -4.2 | 7.7 | 3.6 | 97.6 | 0.2 |
| | 180 | 16.3 | 5.6 | 8.2 | -3.0 | 97.7 | 0.1 |
| | 90 | 12.5 | 1.4 | 9.0 | -13.6 | 99.5 | -1.7 |
| Toxic ref. | | 100* | 100 | - | - | - | - |
| $LR_{50}$[f)] | > 1440 g product/ha GWN-9823 | | | | | | |

a) Treatments were compared individually to the control using Fisher's Exact Test ($\alpha = 0.05$). An asterisk (*) indicates treatments that differed significantly from the control.
b) Mortality corrected for any control treatment deaths using Abbott's formula. A negative number represents an increase and a positive number a decrease in bug survival relative to the control.

c) Results for fecundity analysed by Mann-Whitney *U*-test (α = 0.05), but none of the test-item treatments differed significantly from the control.
d) Change in numbers of eggs per female (fecundity) or hatching rate (fertility), relative to the control. A positive value indicates a decrease and a negative value indicates an increase.
e) Results for fertility analysed by Mann-Whitney *U*-test (α = 0.05), but none of the test-item treatments differed significantly from the control.
f) Median lethal rate.

The study meets all validity criteria:

- mortality in the control treatment should be < 25% (actual value = 11.3%).
- mortality in the toxic reference treatment should be > 40% (actual value = 100%).
- for the reproduction assessments, the mean number of eggs per female per day in the control treatment should be > 2.0, with no more than five individuals having zero values (actual value = 7.9 eggs/female, only one zero value).
- > 70% of the eggs sampled from the control treatment should hatch successfully (actual value = 97.8% hatch).

## Conclusion

In an extended laboratory test in which the predatory bug *Orius laevigatus* was exposed to freshly dried residues of Cymoxanil 33% + Zoxamide 33 % WG (product code GWN-9823), the 9-day LR50 was > 1440 g product/ha, the maximum rate tested. In addition, there was no statistically significant reduction of reproduction at treatment rates up to and including 1440 g product/ha, when compared to the control. Thus, the NOER for GWN-9823 was 1440 g product/ha.

(Vinall S. 2018)

### A 2.3.1.9.4 Study 4 - 'CYMOXANIL 33% + ZOXAMIDE 33% WG': Effects on *Chrysoperla carnea*

| | |
|---|---|
| Comments of zRMS: | The study follows the guideline specified by Vogt *et al.* (2000) and according to the principles of GLP.<br>No deviations were noted and all validity criteria were met. The study is acceptable for the risk assessment purposes. |

| | |
|---|---|
| **Reference:** | **KCP 10.3.2.2/04** |
| Report | Vaughan, R., 2017: Cymoxanil 33% + Zoxamide 33 % WG – A rate-response extended laboratory test to evaluate the effects of fresh residues on the green lacewing, *Chrysoperla carnea* (Neuroptera, Chrysopidae)<br>Gowan Crop Protection Ltd., UK, Oxon Italia S.p.A, Italy<br>Mambo-Tox Ltd., UK, Report No. GOW-17-1, GLP, Unpublished |
| Guideline(s): | Vogt *et al*. (2000) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication<br>(if vertebrate study) | No |

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | Reboot (GWN-9823) (30AL5020) |
| **Active substance content (nominal)** | 33% (w/w) cymoxanil<br>33% (w/w) zoxamide |
| **Species:** | *Chrysoperla carnea* Steph. (Neuroptera, Chrysopidae) |
| Number of animals: | |
| Age: | Larvae (2-3 days old) |
| Source: | Biological Crop Protection, Ashford, Kent, UK |
| Food: | UV light-killed eggs of Sitotroga cerealella (same source).<br>Three times per week adult lacewings were provided with the following for food and water during the bioassay:<br>a) an artificial diet consisting of 15 mL condensed milk, one hen's egg and one additional egg yolk, 30 g honey, 20 g fructose, 30 g brewer's yeast, 50 g wheatgerm and approximately 45 mL purified water.<br>b) a 1:2-1:3 honey/water solution on a cotton wool pad<br>c) fresh water on a cotton wool pad |
| **Environmental conditions** | |
| Temperature: | 24.5-26.4 °C |
| Photoperiod: | Photoperiod: 16h light, 8 h dark<br>Light intensity: 2200-4200 lux |
| Relative humidity | 70-80% |
| **Application rate(s)** | T = 2880, 1440, 720, 360, 180 and 90 g product/ha. |
| Positive control: | |
| **Post exposure observation period** | two 24-h periods within one week. |
| **Remarks** | None |

The test item in this study was Cymoxanil 33% + Zoxamide 33 % WG, also known by the product code GWN-9823, which is a water-dispersible granule formulation containing the fungicides cymoxanil (nominally 33% w/w) and zoxamide (nominally 33% w/w). An extended laboratory test was carried out to determine the effects of fresh, dry, foliar residues of GWN-9823 on the green lacewing, *Chrysoperla carnea* Steph. (Neuroptera, Chrysopidae). The aim was to determine any effects on both the incidence of pre-imaginal mortality and the reproductive capacity of surviving insects.

GWN-9823 was evaluated at six rates, equivalent to 2880, 1440, 720, 360, 180 and 90 g product/ha. These treatments were compared to a water-treated control and a toxic reference treatment of dimethoate (an EC formulation containing nominally 400 a.s. g/L, applied at a rate of 80 mL product/ha).

Treatments were applied to excised leaves of dwarf French bean plants (*Phaseolus vulgaris* L.) at a volume rate equivalent to 200 L spray solution/ha. Once residues had dried, the excised leaves were used to line the floor of the test arenas (n = 40 per treatment) into which individual larvae of *C. carnea* (2-3 days old) were introduced. The larvae were fed with untreated eggs of the Angoumois grain moth, *Sitotroga cerealella* (Olivier), and any pre-imaginal mortality of the lacewings was recorded.

A check was then made for sub-lethal effects on the reproductive performance of the adults surviving in the control and in all six of the test-item treatment rates. For this assessment, the egg-laying activity of grouped females from each treatment was monitored for two 24-h periods and the viability of the eggs produced was determined.

## Results and Discussion

Environmental parameters (temperature, photoperiod and relative humidity) remained within acceptable limits throughout the study. The temperature ranged from 24.5 to 26.4°C, the light intensity ranged from 2200 to 4200 Lux and relative humidity was in the range from 70 to 80% during of the study.

The results of the mortality assessments are summarised below. The *median lethal rate* ($LR_{50}$) for GWN-9823 was estimated to be > 2880 g product/ha.

**Table A 69: Mortality recorded during development of the test insects**

| Treatment | Rate (g product/ha) | % pre-imaginal mortality [a)] | Corrected % pre-imaginal mortality [b)] |
|---|---|---|---|
| Control | - | 15.0 | - |
| GWN-9823 | 2880 | 32.5 | 20.6 |
| | 1440 | 32.5 | 20.6 |
| | 720 | 27.5 | 14.7 |
| | 360 | 15.0 | 0.0 |
| | 180 | 15.0 | 0.0 |
| | 90 | 17.5 | 2.9 |
| Toxic reference | - | 100* | 100 |

a) Pre-imaginal mortality in individual treatments was compared using Fisher's Exact Test ($\alpha = 0.05$). An asterisk indicates where mortality differed significantly from the control.
b) The corrected pre-imaginal mortality was calculated using Abbott's formula.

The results of the reproduction assessments are summarised below. The mean numbers of eggs produced in all the treatments evaluated was ≥ 15eggs/female/day and the mean egg viability was ≥ 70%. These two thresholds are currently viewed as being indicative of no harmful treatment effects (Vogt *et al*., 2000).

**Table A 70: The results of the reproduction assessments**

| Treatment | Rate (mL/ha) | Meant number eggs/female/day [a)] | Mean % egg viability [b)] | Mean viable eggs/female/ day | Effects on repro-duction (%) [c)] |
|---|---|---|---|---|---|
| Control | - | 28.5 | 94.1 | 26.8 | - |
| GWN-9823 | 2880 | 28.7 | 94.9 | 27.2 | -1.5 |
| | 1440 | 35.6 | 92.7 | 33.0 | -23.1 |
| | 720 | 34.7 | 91.8 | 31.9 | -19.0 |
| | 360 | 28.3 | 94.3 | 26.7 | 0.4 |
| | 180 | 38.1 | 94.2 | 35.9 | -34.0 |
| | 90 | 33.1 | 93.9 | 31.1 | -16.0 |

a) Based on two 24-h long assessments made for each oviposition box in each treatment.
b) Based on all eggs laid on the fibrous tissue sheet lining the lid of each oviposition box.
c) Percentage change in mean number of viable eggs per female, relative to control. A positive value indicates a decrease and a negative value an increase.

All validity criteria were met:

- Pre-imaginal mortality ≤ 20% in the control treatment.
- Mean egg production in the control ≥ 15 eggs per female per day.
- Mean viability of the eggs ≥ 70%.
- Mortality ≥ 50% in the toxic reference treatment.

**Conclusion**

In an extended laboratory test to determine the effects of GWN-9823 on the green lacewing, *Chrysoperla carnea*, the $LR_{50}$ was > 2880 g product/ha (i.e. > 950 g/ha cymoxanil + 950 g/ha zoxamide), the highest rate tested. The effect on the reproductive capacity of the lacewings was lower than 50% at any treatment rate, up to and including 2880 g product/ha, indicating no unacceptable effects.

(Vaughan R. 2017)

## 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

EFSA (2017) requested: "*Further data are needed for the chronic risk assessment to earthworm of the active substance and the metabolites RH-127450, RH-24549, RH-163353 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5).*" These data are presented in the following.

##### A 2.4.1.1.1 Study 1 – Zoxamide tech./Zoxium 240 SC: Effects on the reproduction of earthworms

The following study has been performed with an SC formulation containing 240 g/L zoxamide, the representative formulation during AIR. The composition of Zoxium 240 SC (GWN-9790EU) is included in Part C. The study endpoints can be used as representative for zoxamide technical.

The following study has been provided to Latvia as RMS for zoxamide and cMSs for interzonal evaluation in July 2020.

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 222 (2016) and according to the principles of GLP. The study is considered to be reliable and suitable for the risk assessment. |

**Reference:** **KCP 10.4.1.1/01**

Report: Friedrich, S., 2020: Effects of Zoxium 240 SC on the reproduction of the earthworm *Eisenia andrei* in artificial soil with 5 % peat
Gowan Crop Protection Ltd., UK
BioChem agrar, Gerichshain, Germany, Report No. 17 48 TEC 0009, GLP, Not published

Guideline(s): OECD 222 ~~(2004)~~ (2016)

Deviations: No

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) No

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | Zoxium 240 SC (SB 2401) |
| **Active substance content or purity** | 240 g/L Zoxamide (nominal)<br>21.08 ± 0.1 % (w/w), 236.4 g/L (analysed)<br>R/S ratio of zoxamide: 50.1 : 49.9 (analysed) |
| **Species** | Earthworm *Eisenia andrei* (BOUCHÉ, 1972) |
| Age: | adult worms (approximately 4 months old, with clitellum) |
| Source: | Test facility; original breeding purchased from W. Neudorff GmbH KG, An der Mühle 3, 31860 Emmerthal, Germany |
| Weight: | 280 - 478 mg/worm |
| Acclimation period: | at least 24 hours in the artificial substrate (with food) |
| Food: | air-dried and finely ground horse manure |
| **Test system** | plastic vessel (inside dimensions: about 16.5 cm x 12 cm x 6 cm) with a lid pervious to air and light, containing 750 g wet weight corresponding to 600 g dry weight of artificial soil with a water content corresponding to 40-60 % of WHC |
| Soil: | artificial soil, 5% peat |
| Number of animals: | 10 worms/replicate<br>40 (treatment group) (80 control group) |
| Number of replicates: | 4 replicates for the test item treatments, 8 replicates for the control |
| **Environmental conditions** | |
| Temperature: | 19.8 – 20.7 °C |
| Photoperiod: | light: dark = 16 h: 8 h; 590 lux |
| Soil moisture: | guideline requirement: 40-60 % of WHC<br>test start: 24.9 – 25.1 (equivalent to 57.8 – 58.2 % of WHC) test end: 24.2 – 24.8 (equivalent to 56.1 – 57.5 % of WHC) |
| pH: | guideline requirement: 6.0 ± 0.5<br>test start: 5.97 – 6.03<br>test end: 5.69 – 5.78 |
| **Application rate(s)** | 0.392, 0.706, 1.271, 2.287, 4.117, 7.410, 13.34, 24.01 mg a.s./kg soil dry weight (nominal)<br>1.86, 3.35, 6.03, 10.9, 19.5, 35.2, 63.3, 113.9 mg test item/kg soil dry |

| | |
|---|---|
| | weight based on analysed content of a.s. in the formulated product |
| Negative control: | deionised water |
| Positive control: | Carbendazim 500 SC |
| **Post exposure observation period** | 56 days |
| **Remarks** | None |

The potential effects of the test item Zoxium 240 SC (containing nominally 240 g/L zoxamide) at application rates of 1.86, 3.35, 6.03, 10.9, 19.5, 35.2, 63.3, 113.9 mg product/kg soil dry weight on the reproduction, mortality and growth of the earthworm *Eisenia andrei* has been studies, considering dermal and alimentary uptake after mixing of the test item with an artificial soil containing 5 % organic matter.

One day before test start, the dry artificial soil was pre-moistened by adding deionised water to obtain approximately half of the final water content. Earthworms were acclimatised in a separate batch of the artificial soil (mixed with horse manure) for at least 24 hours before test start.

On the day of the test start, the test item was introduced by dispersing the quantity of test item required to obtain the desired test concentration in the volume of water required to hydrate the soil to 40-60 % of its WHC. The control substrate contained the corresponding amount of deionised water. The test solution was thoroughly mixed into the soil separately for each replicate. Each test vessel was then filled with the treated soil. After a randomising procedure according to the worm fresh weight, selected groups of 10 worms were then randomly assigned to each treatment group. The individually weighed worms (10 worms/vessel) were placed on the surface of the soil. After approximately thirty minutes, the test vessels were closed with perforated transparent lids, which allowed gas exchange between substrate and atmosphere and access of light, but prevented worms from escaping. The test vessels were then set up at random in a controlled-environment test room. One day after application, initially 5 g air-dried and finely ground horse manure was scattered on the soil surface of each test vessel, which was sprinkled with 5 mL deionised water. The feeding interval was weekly during the first four weeks of the test. The weekly amount of manure (5 g) depended on the feeding activity, which was assessed by visual estimation of the food remaining on the surface before addition of new food.

After four weeks, the adult worms were removed from the test vessels. The number of surviving worms (adult mortality) and their biomass change were determined, behaviour (including feeding activity) and pathological symptoms were recorded. The adult worms were discarded after counting and weighing. Subsequently, the soil of each vessel was mixed carefully with 5 g manure. This was the last feeding occasion of the experiment. The test was then continued for another four weeks. The final assessment included counting of juveniles per test vessel, determination of the water content and pH measurements of the artificial soil. Juveniles were counted by manual inspection of the substrate.

The endpoints were mortality, change of biomass (difference in fresh weight of surviving worms between test start and four weeks after treatment) and reproduction (the number of juveniles present). The arithmetic mean and the standard deviation per treatment and per control for reproduction, mortality and biomass were calculated. The statistical analysis was performed with the software ToxRat Professional ToxRat Professional 3.2.1 (Ratte, 2015). The ECx values (number of juveniles) were calculated by Probit analysis using linear max. likelihood regression. For identifying the NOECs, the Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm and the Williams-t-test were used to compare the control with the independent test item groups. For statistical evaluation of the biomass change, the changed mean fresh weight of surviving worms per replicate was used.

The concentration of zoxamide in the artificial soil was analysed at test start (day 0, just after test item application), middle (day 28) and test end (day 56). The nominal initial test item concentrations in the soil were analytically confirmed with a highly specific HPLC-MS/MS method fully validated according to

SANCO/3029/99 rev. 4. The active substance zoxamide was analysed in soil specimens by a method developed by Jooß (2013), using extraction with acetonitrile and separation by reverse-phase high-pressure liquid chromatography (HPLC) and tandem mass spectroscopic (MS/MS) determination of zoxamide with matrix-matched external standards. This method was adapted to the expected concentration range of this study. It was re-validated with artificial soil spiked with test item at concentrations 0.05 mg/kg (moist soil, corresponding to 0.0625 mg/kg dry weight), at 0.5 mg/kg (moist soil, corresponding to 0.625 mg/kg dry weight) and approx. 120% of the highest test concentration (23 mg/kg moist soil, corresponding to 29 mg/kg dry weight)). For analysis, 5 g (± 0.05 g) soil sample were weighed into a 100 mL Erlenmeyer flask. 1.0 mL water and 50 mL acetonitrile were added and the flasks were shaken for 45 minutes on a mechanical shaker. Then 1.0 g sodium chloride was added and the flasks again shaken for 10 minutes. The samples were transferred to centrifuge tubes and centrifuged for 3 minutes. Aliquots of the acetonitrile phase were transferred to autosampler vials and diluted. The analytes were determined after extraction with two mass transitions (zoxamide: m/z 336 -> 187 and 336 -> 159), one for quantification and one for qualification, respectively. The limit of quantification (LOQ) was defined in the context of this phase of the study as the lowest successfully validated fortification level, i.e. 0.051 mg/kg zoxamide in wet soil specimens, equivalent to 0.063 mg/kg zoxamide in dry weight soil.

## Results and discussion

Environmental conditions stayed within the recommended ranges.

The analytically verified recoveries of the active substances in the soil substrate were all greater than 80% (i.e. 96-130 % for zoxamide). Thus, confirming sufficiently high (> 80% of nominal) test item concentrations at study start.

At the start of the test, earthworm fresh weight ranged from 280 – 478 mg/worm. The weight change of adult worms ranged between 19.8 % and 23.6 % in the treated groups and was 22.5 % in the control group. The test item caused no statistically significant effects (Williams-t-test, $\alpha = 0.05$, one-sided smaller) on the change in biomass (change in fresh weight after 4 weeks relative to initial fresh weight) compared to the control group at any concentration tested.

Mortality rates of 0 % - 5.0 % were recorded in the test item treatment groups and 0 % mortality was observed in the control group. No statistically significant mortality compared to the control was observed at any concentration tested (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$, one-sided greater).

**Table A 71: Effects of the test item on mortality and reproduction of adult earthworms**

| Treatment group | Zoxium 240 SC<br>Nominal concentration (mg a.s./kg soil d.w.) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Control** | **0.392** | **0.706** | **1.271** | **2.287** | **4.117** | **7.410** | **13.34** | **24.01** |
| | **Analysed concentration on day 0 (mg a.s./kg soil d.w.)** | | | | | | | | |
| | **Control** | **0.511** | **0.714** | **1.367** | **2.453** | **4.319** | **7.105** | **13.64** | **25.38** |
| **Replicate** | **Number of surviving adult worms per replicate (4 weeks after test initiation)** | | | | | | | | |
| 1 | 10 | 10 | 10 | 10 | 10 | 10 | 9 | 10 | 9 |
| 2 | 10 | 10 | 10 | 10 | 10 | 10 | 9 | 9 | 10 |
| 3 | 10 | 10 | 9 | 9 | 10 | 10 | 10 | 10 | 10 |
| 4 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 9 |
| 5 | 10 | | | | | | | | |
| 6 | 10 | | | | | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 7 | 10 | | | | | | | | |
| 8 | 10 | | | | | | | | |
| **mean** | 10.0 | 10.0 | 9.8 | 9.8 | 10.0 | 10.0 | 9.5 | 9.8 | 9.5 |
| SD | 0.0 | 0.0 | 0.5 | 0.5 | 0.0 | 0.0 | 0.6 | 0.5 | 0.6 |
| cv % | 0.0 | 0.0 | 5.1 | 5.1 | 0.0 | 0.0 | 6.1 | 5.1 | 6.1 |
| | **Mortality (%)** | | | | | | | | |
| **mean** | **0.0** | **0.0** | **2.5** | **2.5** | **0.0** | **0.0** | **5.0** | **2.5** | **5.0** |
| | **Number of juvenile worms per replicate (8 weeks after test initiation)** | | | | | | | | |
| 1 | 117 | 130 | 125 | 111 | 103 | 87 | 13 | 8 | 0 |
| 2 | 185 | 149 | 154 | 168 | 145 | 108 | 21 | 10 | 0 |
| 3 | 160 | 137 | 119 | 131 | 157 | 91 | 38 | 23 | 0 |
| 4 | 152 | 180 | 173 | 143 | 162 | 119 | 53 | 14 | 0 |
| 5 | 110 | | | | | | | | |
| 6 | 133 | | | | | | | | |
| 7 | 146 | | | | | | | | |
| 8 | 122 | | | | | | | | |
| **mean** | **140.6** | **149.0** | **142.8** | **138.3** | **141.8** | **101.3*** | **31.3*** | **13.8*** | **0.0*** |
| SD | 25.1 | 22.1 | 25.3 | 23.8 | 26.8 | 14.9 | 17.9 | 6.7 | 0.0 |
| cv % | 17.8 | 14.8 | 17.7 | 17.2 | 18.9 | 14.7 | 57.1 | 48.4 | - |
| | **Reduction of reproduction (%)** | | | | | | | | |
| **% to control** | **-** | **-6.0** | **-1.5** | **1.7** | **-0.8** | **28.0** | **77.8** | **90.2** | **100.0** |

Not statistically significantly different compared to the control for mortality (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, α = 0.05, one-sided greater)
* statistically significantly different compared to the control (Williams-t-test for reproduction, α = 0.05, one-sided smaller)
SD: standard deviation, cv %: coefficient of variation, d.w.: dry weight
Negative % values for change of reproduction = increase, relative to control

Statistically significant effects (Williams-t-test, α = 0.05, one-sided smaller) on the number of juveniles compared to the control group were recorded at nominal concentrations of 4.117, 7.410, 13.34 and 24.01 mg a.s./kg d.w. The NOEC for mortality and biomass was determined to be 24.01 mg a.s./kg soil dry weight based on nominal zoxamide concentrations (corresponding to 25.38 mg/kg zoxamide based on analysed concentrations). The NOEC for reproduction was determined to be 2.287 mg a.s./kg soil dry weight based on nominal zoxamide concentrations (corresponding to 2.453 mg/kg zoxamide based on analysed concentrations). The $EC_{10}$, $EC_{20}$ and $EC_{50}$ values for reproduction were calculated to be 2.987, 3.655 and 5.378 mg a.s./kg soil dry weight based on nominal concentrations, corresponding to 3.304, 3.907 and 5.382 mg/kg zoxamide based on analysed concentrations, respectively.

**Table A 72: Effects of the test item on a 56-day reproduction study**

| **Endpoint** | **Treatment group** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Nominal concentration (mg a.s./kg soil d.w.)** | | | | | | | | |
| | Control | 0.392 | 0.706 | 1.271 | 2.287 | 4.117 | 7.410 | 13.34 | 24.01 |
| | **Analysed concentration on day 0 (mg a.s./kg soil d.w.)** | | | | | | | | |
| | Control | 0.511 | 0.714 | 1.367 | 2.453 | 4.319 | 7.105 | 13.64 | 25.38 |
| **Mortality of adult** | 0.0 | 0.0 | 2.5 | 2.5 | 0.0 | 0.0 | 5.0 | 2.5 | 5.0 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **worms after 4 weeks (%)** | | | | | | | | | |
| **Mean biomass change after 4 weeks (%)** | 22.5 | 23.1 | 22.5 | 23.4 | 21.9 | 23.6 | 19.8 | 20.9 | 21.2 |
| **Mean number of juveniles after 8 weeks** | 140.6 | 149.0 | 142.8 | 138.3 | 141.8 | 101.3* | 31.3* | 13.8* | 0.0* |
| **Reduction of reproduction compared to control (%)** | - | -6.0 | -1.5 | 1.7 | -0.8 | 28.0 | 77.8 | 90.2 | 100.0 |

| | Endpoint (mg a.s./kg soil dry weight) | |
|---|---|---|
| | **Nominal concentration** | **Analysed concentration on day 0** |
| **NOEC (mortality)** | 24.01 | 25.38 |
| **NOEC (biomass)** | 24.01 | 25.38 |
| **NOEC (reproduction)** | 2.287 | 2.453 |
| **$LC_{50}$ (mortality)** | > 24.01 | > 25.38 |
| **$EC_{10}$ (reproduction)[1]** | 2.987 (95 % confidence limits 2.530 - 3.528) | 3.304 (95 % confidence limits 2.802 - 3.896) |
| **$EC_{20}$ (reproduction)[1]** | 3.655 (95 % confidence limits 3.225 - 4.144) | 3.907 (95 % confidence limits 3.454 - 4.419) |
| **$EC_{50}$ (reproduction)[1]** | 5.378 (95 % confidence limits 4.954 - 5.838) | 5.382 (95 % confidence limits 4.976 - 5.821) |

* statistically significant compared to control (Williams-t-test for biomass and reproduction, α = 0.05, one-sided smaller)
1 median values with their 95 % confidence intervals, based on Probit analysis
Negative % values for change of reproduction = increase, relative to control
d.w.: dry weight (of artificial soil)

In the most recent study with Maypon Flow (BioChem project No. 17 48 TEC 0011, dated 20 January 2017) the number of juveniles was reduced by 57 and 100 % at concentrations of 5 and 10 mg product/kg soil dry weight (mean number of juveniles = 46 and 0) after 8 weeks of test duration when compared to control (mean number of juveniles = 107). Thus, confirming the sensitivity of the test system.

All validity criteria were met:

- adult mortality: ≤ 10 % (being 0 % after 4 weeks)
- number of juveniles per replicate: ≥ 30 (being 110 to 185)
- coefficient of variation of reproduction: ≤ 30 % (being 17.8 %)

## Conclusion

In a 56-day earthworm reproduction study with Zoxium 240 SC, no statistically significant adverse effects on mortality and biomass of the earthworm *Eisenia andrei* in artificial soil were determined up to and including nominally 24.01 mg a.s./kg soil dry weight, the highest nominal concentration tested (corresponding to 25.38 mg a.s./kg soil d.w. analysed). Therefore, the NOEC for mortality and biomass was determined to be 24.01 mg a.s./kg soil dry weight based on nominal zoxamide concentrations (corresponding to 25.38 mg/kg zoxamide based on analysed concentrations). The NOEC for reproduction was determined to be 2.287 mg a.s./kg soil dry weight based on nominal zoxamide concentrations (corresponding to 2.453 mg/kg zoxamide based on analysed concentrations). The $EC_{10}$, $EC_{20}$ and $EC_{50}$ values for reproduction were calculated to be 2.987, 3.655 and 5.378 mg a.s./kg soil dry weight based on nominal concentrations, corresponding to 3.304, 3.907and 5.382 mg/kg zoxamide based on analysed concentrations, respectively.

For the formulated product Zoxium 240 SC the NOEC for mortality and biomass was determined to be 112.2 mg test item/kg soil dry weight based on nominal test item concentration, corresponding to 118.6

mg test item/kg soil dry weight based on analysed a.s. concentrations in the soil. The NOEC for reproduction was determined to be 10.7 mg test item/kg soil dry weight based on nominal test item concentration, corresponding to 11.46 mg test item/kg soil dry weight based on analysed a.s. concentrations in the soil. The $EC_{10}$, $EC_{20}$ and $EC_{50}$ values for reproduction were 14.0, 17.1 and 25.1 mg test item/kg soil dry weight based on nominal test item concentration, corresponding to 15.4, 18.3 and 25.2 mg test item/kg soil dry weight based on analysed a.s. concentrations in the soil, respectively.

The study is valid.

(Friedrich S. 2020)

### A 2.4.1.1.2 Study 2 - RH-127450: Effect on the reproduction of earthworms

The following study has been provided to Latvia as RMS for zoxamide and cMSs for interzonal evaluation in July 2020.

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 222 (2016) and according to the principles of GLP. The study is considered to be reliable and suitable for the risk assessment. |

| | |
|---|---|
| **Reference:** | **KCP 10.4.1.1/02** |
| Report | Gray, J., 2020: RH-127450: Effect on reproduction in the earthworm *Eisenia fetida*<br>Gowan Crop Protection Ltd., UK<br>Smithers ERS Limited, UK, Report No.3202376, GLP, Not published |
| Guideline(s): | OECD 222 (2016) |
| Deviations: | On one occasion the minimum temperature recorded was 17.8°C, below the guideline minimum of 18°C.<br>The number of juveniles in replicates E-H inclusive of the solvent control was not assessed until day 57 as the assessment took longer than expected due to the high numbers of juveniles recorded. This did not affect the number of juveniles present as the adults had been removed at day 28.<br>However, as all the validity criteria were met, these deviations were not considered to have had any impact on the integrity or outcome of the study. |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | No |

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | RH-127450 (HHGCP002-00-1) |
| **Active substance content or purity** | 99.51 % (w/w) |
| **Species** | Earthworm *Eisenia fetida* |
| Age: | adult worms, sexually mature (i.e. between 2 and 12 months old with clitellum), weighing between 300 and 600 mg (wet mass) |

| | |
|---|---|
| Source: | Bias Labs Ltd., UK |
| Weight: | 443 – 486 mg/worm (mean, day 0) |
| Acclimation period: | at least 24 hours in the artificial substrate with feed |
| Food: | finely ground animal faeces *ad libitum* |
| **Test system** | 1 L glass test vessel containing ten earthworms in the equivalent of approximately 500 g dry weight of artificial soil substrate maintained at nominally 55% maximum water holding capacity (MWHC) |
| Soil: | artificial soil, 10% peat |
| Number of animals: | 80 (control)<br>40 (test item treatment) |
| Number of replicates: | 4 replicates per treatment rate, 8 per controls |
| **Environmental conditions** | |
| Temperature: | 17.8 – 21.4 °C |
| Photoperiod: | light: dark = 16 h: 8 h; 484-774 lux |
| Soil moisture: | guideline requirement: 40-60 % of max. WHC<br>during the study: 43-47 % of max. WHC |
| pH: | guideline requirement: 6.0 ± 0.5<br>during the study: 6.13 – 6.4 |
| **Application rate(s)** | 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.i./kg dry substrate |
| Negative control: | reverse osmosis (RO) water, solvent (acetone) using a sand carrier |
| Positive control: | Carbendazim |
| **Post exposure observation period** | 56 days |
| **Remarks** | None |

The potential effects of the test item RH-127450 at application rates of 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.i./kg soil dry weight on the reproduction, mortality and growth of the earthworm *Eisenia fetida* has been studies, considering dermal and alimentary uptake after mixing of the test item with an artificial soil containing 10 % organic matter.

The test item was solved in acetone. After addition of the solvent solution to the sand carrier, the acetone was allowed to evaporate off before the treated sand was added to the bulk test substrate and mixed in. Reverse osmosis (RO) water was added to achieve the final weight required at 55% MWHC. After mixing, eight 5 g soil samples were taken from each treatment for chemical analysis (four samples to analyse and four to retain). Two samples were also taken from each treatment and the controls for analysis of water content to enable calculation of the test substance concentration in terms of substrate dry weight. One sample was taken from each treatment for determination of pH. The remaining soil was then weighed (equivalent to 500 g dry weight) into the test vessels

Earthworms were acclimatised in a separate batch of the artificial soil (mixed with horse manure) for at least 24 hours before test start. After test substrate preparation, pots of 10 worms were randomised using an excel spreadsheet and allocated to the replicate test vessels. The earthworms placed onto the substrate surface. The vessels were re-covered with a perforated clear plastic film to allow for gaseous exchange and penetration of light. After approximately 15 minutes, each vessel was examined to confirm that no worms were remaining on the surface of the substrate. Food in the form of finely ground animal faeces,

and RO water (5 g and 5 mL, respectively), were added on day 1 of the test and weekly thereafter up to and including day 28. Uneaten food remaining in the vessel at each feeding interval was removed and replaced with fresh food. Moisture loss due to evaporation was compensated for on a weekly basis. The test was carried out in a temperature-controlled room set at 20 ± 2°C and a 16:8 hour light:dark cycle, with light intensity of 400 – 800 Lux, measured at least weekly throughout the test.

Survival of the worms was assessed 28 days after treatment by removing the soil from the test vessels, spreading it out on a plastic sheet and locating the earthworms, which were classified as dead if they did not respond to gentle mechanical stimulus to the anterior end. Missing earthworms were also considered dead due to rapid decomposition under test conditions. Following observations on day 28 and the removal of samples for chemical analysis and determination of water content, the test substrate was carefully replaced in the test vessels. The adult worms were rinsed in RO water, blotted dry and weighed in replicate groups before being humanely discarded. On day 56, the vessels were placed in a water bath set between 40 and 60ºC, leaving approximately the top 2 cm of substrate above the water level. The vessels were then left for approximately 20 to 30 minutes to allow the juvenile worms to move to the surface of the substrate and away from the heat. The juvenile worms were then removed from the surface of the substrate and counted. When all visible worms had been removed, the substrate was spread out over a tray covered with plastic. Any juvenile worms and cocoons remaining in the substrate were removed and counted, and the total number of juveniles per vessel determined. After removal of the juveniles, soil samples were taken for chemical analysis and determination of water content and pH.

The endpoints were mortality, change of biomass (difference in fresh weight of surviving worms between test start and four weeks after treatment) and reproduction (the number of juveniles present). Percentage mortality of the worms in each vessel was calculated and a treatment mean presented. A no observed effect concentration (NOEC) and $LC_{50}$ were determined for adult mortality. The percentage weight change, based on mean surviving worm weight at day 28 was calculated for each vessel and a treatment no observed effect concentration (NOEC) and $EC_{50}$ were determined empirically as there were no adverse effects. Juvenile worm numbers were determined for each vessel and a treatment mean presented. A treatment no observed effect concentration (NOEC) and $EC_{10}$, $EC_{20}$ and $EC_{50}$ values were determined empirically as there were no adverse effects on reproduction. Statistical analysis of the survival and reproduction data was not undertaken as there was no effect on survival, adult earthworm weight or the reproductive output.

The analytical procedure (SMV 3202376-03V) was used to confirm the test substance concentration in the test samples. On the day of test item application, after 28 days and at the end of the test samples of 5.0 g soil were dispensed into 50 mL Falcon tubes. They were fortified as required and shaken well by hand to mix. 20 mL of MeCN was added and the sample were extracted by shaking on a rotary shaker at 200 rpm for 10 minutes. They were then sonicated for five minutes before being centrifuged at 2500 rpm for 15 minutes. A portion of the supernatant was transferred to a suitable vial for time-of-flight mass spectrometry (LC-TOF/MS) analysis. If required, samples were diluted with unfortified control extract. Aliquots of the samples were injected onto the 5600 TOF-MS system. The method was validated according to SANCO 3029/99 rev. 4 with an LOQ of 0.016 mg/kg mg/kg.

## Results and discussion

Environmental conditions stayed within the recommended ranges.

Post application the mean test item concentrations were 0.1287, 0.2455, 0.4599, 0.8628, 1.6655, 3.4432, 5.54763 and 10.7197 mg a.i./kg dry substrate at application rates of 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.i./kg dry substrate respectively. This is equivalent to 80.43, 84.66, 86.78, 90.82, 96.83, 111.43, 98.49 and 107.20% of nominal at 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.i./kg dry substrate respectively. Thus, the analytically verified recoveries of the test item in the soil substrate were all greater than 80% (i.e. 80-111 %), confirming sufficiently high (> 80% of nominal) test item concentra-

tions at study start.

At Day 28, there was a mean gain in weight 68.0, 68.1, 72.0, 71.6, 63.5, 67.9, 63.8 and 58.1% in the 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.i./kg dry substrate groups, respectively in comparison to 66.3 and 63.4% in the water and solvent controls respectively. This was equivalent to increases of 2.6, 2.7, 8.6, 8.0 and 2.6% in the 0.16, 0.29, 0.53, 0.95 and 3.09 mg a.i./kg dry substrate groups, respectively in comparison to the water control with decreases of 4.2, 3.6 and 12.4% in the 1.71, 5.56 and 10 mg a.i./kg dry substrate groups, respectively. This was equivalent to increases of 7.3, 7.4, 13.6, 12.9, 0.2, 7.1 and 0.6% in the 0.16, 0.29, 0.53, 0.95, 1.72, 3.09 and 5.56 mg a.i./kg dry substrate groups, respectively in comparison to the solvent controls with a decrease of 8.4% in the 10 mg a.i./kg dry substrate group. The NOEC and $EC_{50}$ for overall weight change were determined to be 10 mg a.i./kg dry substrate and >10 mg a.i./kg dry substrate, respectively.

**Table A 73: Mean live weight of individual *E. fetida* per treatment and percentage weight change from days 0 to 28**

| Nominal concentration (mg a.i./kg dry substrate | Live weight (mg) | | Change (% increase) | % Effect in comparison to the water control | % Effect in comparison to the solvent control |
|---|---|---|---|---|---|
| | Day 0 | Day 28 | | | |
| Water control | 448.6 | 745.5 | 66.3 | N/A | +4.4 |
| Solvent control | 457.6 | 747.8 | 63.4 | -4.4 | N/A |
| 0.16 | 442.8 | 743.9 | 68.0 | +2.6 | +7.3 |
| 0.29 | 459.0 | 771.3 | 68.1 | +2.7 | +7.4 |
| 0.53 | 454.4 | 781.6 | 72.0 | +8.6 | +13.6 |
| 0.95 | 436.7 | 749.3 | 71.6 | +8.0 | +12.9 |
| 1.72 | 464.6 | 759.8 | 63.5 | -4.2 | +0.2 |
| 3.09 | 465.4 | 781.3 | 67.9 | +2.4 | +7.1 |
| 5.56 | 473.0 | 774.9 | 63.8 | -3.8 | +0.6 |
| 10 | 485.5 | 767.6 | 58.1 | -12.4 | -8.4 |

N/A = not applicable

After 28 days of exposure, 1.25% mortality was recorded in the water control and 2.5% mortality was recorded at 0.53 and 3.09 mg a.i./kg dry substrate. Based on these results, the NOEC for 28-day adult survival was determined to be 10 mg a.i./kg dry substrate, and the $LC_{50}$ for 28-day survival was empirically determined to be >10 mg a.i./kg dry substrate. The NOEC and $EC_{50}$ for overall weight change were determined to be 10 mg a.i./kg dry substrate and >10 mg a.i./kg dry substrate, respectively.

**Table A 74: Mean treatment mortality for adult *E. fetida***

| Nominal concentration (mg a.i./kg dry substrate | Mortality | | |
|---|---|---|---|
| | Number of *E. fetida* exposed | Day 28 Number of mortalities | Total (%) |
| Water control | 80 | 1 | 1.25 |
| Solvent control | 80 | 0 | 0 |
| 0.16 | 40 | 0 | 0 |
| 0.29 | 40 | 0 | 0 |
| 0.53 | 40 | 1 | 2.50 |
| 0.95 | 40 | 0 | 0 |

| | | | |
|---|---|---|---|
| 1.72 | 40 | 0 | 0 |
| 3.09 | 40 | 1 | 2.50 |
| 5.56 | 40 | 0 | 0 |
| 10 | 40 | 0 | 0 |

The mean number of juveniles per vessel was 463, 476, 434, 470, 475, 503, 466 and 517 in the 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.i./kg dry substrate groups respectively in comparison to 408 and 444, in the water and solvent controls respectively. This corresponded to increases in juvenile production of 13, 17, 6, 15, 16, 23, 14 and 27%, in the 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.i./kg dry substrate groups, respectively, when compared to the water control. In comparison to the solvent control there were increases of 4, 7, 6, 7, 13, 5 and 16% 0.16, 0.29, 0.95, 1.72, 3.09, 5.56 and 10 mg a.i./kg dry substrate groups, respectively, and a reduction of 2% at 0.53 mg a.i./kg dry substrate.

The number of cocoons was not included in the calculations as the mean number of cocoons per treatment rate ranged from 0 – 5.3 and there was no evidence of a dose response. The mean number of cocoons in the water and solvent control replicates was 1.0 and 0.5, respectively.

No other observations were noted.

Based on these results, the NOEC for reproduction was empirically determined to be 10 mg a.i./kg dry substrate. The $EC_{10}$, $EC_{20}$ and $EC_{50}$ values for reproduction were empirically determined to be >10 mg a.i./kg dry substrate.

**Table A 75: Mean number of juvenile worms at day 56**

| Nominal concen-tration (mg a.i./kg dry substrate | Mean number of juveniles | % Difference when compared to the water control | % Difference when compared to the solvent control | Mean Number of cocoons |
|---|---|---|---|---|
| Water control | 408 | N/A | -8.1 | 1.0 |
| Solvent control | 444 | +8.8 | N/A | 0.5 |
| 0.16 | 463 | +13.5 | +4.3 | 0.0 |
| 0.29 | 476 | +16.7 | +7.2 | 0.8 |
| 0.53 | 434 | +6.4 | -2.3 | 0.3 |
| 0.95 | 470 | +15.2 | +5.9 | 0.5 |
| 1.72 | 475 | +16.4 | +7.0 | 1.0 |
| 3.09 | 503 | +23.3 | +13.3 | 0.0 |
| 5.56 | 466 | +14.2 | +5.0 | 5.3 |
| 10 | 517 | +26.7 | +16.4 | 0.3 |

N/A = not applicable
Coefficient of variance for water and solvent controls = 12.05 and 8.8% respectively

Earthworms from the same source culture as those used in the reproductive test were used in a reference toxicity test with Carbendazim, a known toxic substance (performed under in-house GLP Smithers Viscient Study Number 3202329 from December 2018 to February 2019). The $EC_{50}$ value was estimated to be 2.05 mg a.i./kg dry soil. This is within the given toxicity range of 1 to 5 mg a.i./kg dry soil, indicating that the worms were responding as expected in the test system.

All validity criteria were met:

- The mortality of adults in the control group was ≤ 10% over the initial 28 days (actual adult mortality

= 1.3 and 0% in the water and solvent controls respectively).

- The rate of production of juveniles was ≥ 30 per control container containing 10 adults by the end of test (mean actual rate of production of juveniles = 408 and 444 in the water and solvent controls respectively).
- The coefficient of variance of reproduction in the control was 30% or less (actual coefficient of variance = 12.05 and 8.8% in the water and solvent controls respectively).

**Table A 76: Effects of the test item: summary of statistical analysis**

| Endpoint | mg test item/kg soil dry weight |
|---|---|
| 28-day NOEC for adult survival[b] | 10 mg a.i./kg dry substrate |
| 28-day $LC_{50}$ adult survival | >10 mg a.i./kg dry substrate |
| 28-day NOEC for adult weight change[b] | 10 mg a.i./kg dry substrate |
| 28-day $LC_{50}$ for adult weight change[b] | >10 mg a.i./kg dry substrate |
| 56-day NOEC for reproduction[b] | 10 mg a.i./kg dry substrate |
| 56-day $EC_{10}$ reproduction[b] | >10 mg a.i./kg dry substrate |
| 56-day $EC_{20}$ reproduction[b] | >10 mg a.i./kg dry substrate |
| 56-day $EC_{50}$ reproduction[b] | >10 mg a.i./kg dry substrate |

[a] Rounded figure
[b] Empirically determined
N/A = not applicable

## Conclusion

A laboratory test was conducted in which mature *E. fetida* were exposed to RH-127450 for a period of 28 days, after which the adult worms were removed. Any cocoons produced were then allowed to hatch and the juveniles given time to mature for a further 28 days. There were no adverse effects on adult worm survival or weight over the 28-day exposure period. There were no adverse effects on reproductive capacity at day 56. The study endpoints were determined as follows:

- 28-day NOEC value for adult *E. fetida* survival = 10 mg a.i./kg dry substrate

- 28-day $EC_{50}$ value for adult *E. fetida* survival = >10 mg a.i./kg dry substrate

- NOEC value based on reproduction = 10 mg a.i./kg dry substrate

- $EC_{10}$ value based on reproduction = >10 mg a.i./kg dry substrate

- $EC_{20}$ value based on reproduction = >10 mg a.i./kg dry substrate

- $EC_{50}$ value based on reproduction = >10 mg a.i./kg dry substrate

The study is valid.

(Gray J. 2020)

### A 2.4.1.1.3 Study 3 - RH-24549: Effect on the reproduction of earthworms

The following study has been provided to Latvia as RMS for zoxamide and cMSs for interzonal evaluation in July 2020.

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 222 (2016) and according to the principles of GLP. The study is considered to be reliable and suitable for the risk |

| | |
|---|---|
| | assessment.<br>$EC_{10}$ value based on reproduction = 7.449 mg a.i./kg dry substrate will be used in the risk assessment. |

**Reference:** **KCP 10.4.1.1/03**

Report: Gray, J., 2020: RH-24549 - Effect on Reproduction in the Earthworm *Eisenia fetida*
Gowan Crop Protection Ltd., UK
Smithers ERS Ltd., UK, Report No. 3202395, GLP, Not published

Guideline(s): The study was designed in accordance with OECD Guideline for Testing of Chemicals, Earthworm Reproduction Test, No. 222, Adopted 29 July 2016

Deviations: The light intensity deviated from the guideline times of 16:8 hours light:dark on one occasion during the reproduction phase of the test, when the timer was still recording a dark period after 15.05 hours.
The soil used in the study had a peat content of 8.1% and not 10% as stated in the protocol.
The pipette used to adjust the soil moisture content on day 35 (06 August), day 42 (13 August 2019) and day 49 (20 August 2019) was found to have failed to meet the required criteria when calibrated on 27 August 2019.
These deviations were not considered to have had an adverse impact on the study as all the validity criteria were met.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): No

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | RH-24549 (FCC25806) |
| **Active substance content or purity** | 99.59 % (w/w) |
| **Species** | Earthworm *Eisenia fetida* |
| Age: | adult worms, sexually mature (i.e. between 2 and 12 months old with clitellum), weighing between 300 and 600 mg (wet mass) |
| Source: | Bias Labs Ltd., UK |
| Weight: | 522-624 mg/worm (mean, day 0) |
| Acclimation period: | at least 24 hours in the artificial substrate with feed |
| Food: | finely ground animal faeces *ad libitum* |
| **Test system** | 1 L glass test vessel containing ten earthworms in the equivalent of approximately 500 g dry weight of artificial soil substrate maintained at nominally 55% maximum water holding capacity (MWHC) |
| Soil: | artificial soil, 10% peat |
| Number of animals: | 80 (control) |

| | |
|---|---|
| | 40 (test item treatment) |
| Number of replicates: | 4 replicates per treatment rate, 8 per controls |
| **Environmental conditions** | |
| Temperature: | 18.6 - 22°C |
| Photoperiod: | light: dark = 16 h: 8 h; 427 to 778 lux |
| Soil moisture: | guideline requirement: 40-60 % of max. WHC<br>during the study: 40.5-53 % of max. WHC |
| pH: | guideline requirement: 6.0 ± 0.5<br>during the study: 6.5 – 7.66 |
| **Application rate(s)** | 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.i./kg dry substrate |
| Negative control: | reverse osmosis (RO) water, sand carrier<br>(The test substance was applied dry using a sand carrier.) |
| Positive control: | Carbendazim |
| **Post exposure observation period** | 56 days |
| **Remarks** | None |

The potential effects of the test item RH-24549 at application rates of 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.i./kg soil dry weight on the reproduction, mortality and growth of the earthworm *Eisenia fetida* has been studies, considering dermal and alimentary uptake after mixing of the test item with an artificial soil containing 10 % organic matter.

The test substance was applied dry using a sand carrier before the treated sand was added to the bulk test substrate and mixed in. Reverse osmosis (RO) water was added to achieve the final weight required at 55% MWHC. After mixing, eight 5 g soil samples were taken from each treatment for chemical analysis (four samples to analyse and four to retain). Two samples were also taken from each treatment and the controls for analysis of water content to enable calculation of the test substance concentration in terms of substrate dry weight. One sample was taken from each treatment for determination of pH. The remaining soil was then weighed (equivalent to 500 g dry weight) into the test vessels

Earthworms were acclimatised in a separate batch of the artificial soil (mixed with horse manure) for at least 24 hours before test start. After test substrate preparation, pots of 10 worms were randomised using an excel spreadsheet and allocated to the replicate test vessels. The earthworms placed onto the substrate surface. The vessels were re-covered with a perforated clear plastic film to allow for gaseous exchange and penetration of light. After approximately 15 minutes, each vessel was examined to confirm that no worms were remaining on the surface of the substrate. Food in the form of finely ground animal faeces, and RO water (5 g and 5 mL, respectively), were added on day 1 of the test and weekly thereafter up to and including day 28. Uneaten food remaining in the vessel at each feeding interval was removed and replaced with fresh food. Moisture loss due to evaporation was compensated for on a weekly basis. The test was carried out in a temperature-controlled room set at 20 ± 2°C and a 16:8 hour light:dark cycle, with light intensity of 400 – 800 Lux, measured at least weekly throughout the test.

Survival of the worms was assessed 28 days after treatment by removing the soil from the test vessels, spreading it out on a plastic sheet and locating the earthworms, which were classified as dead if they did not respond to gentle mechanical stimulus to the anterior end. Missing earthworms were also considered dead due to rapid decomposition under test conditions. Following observations on day 28 and the removal of samples for chemical analysis and determination of water content, the test substrate was carefully replaced in the test vessels. The adult worms were rinsed in RO water, blotted dry and weighed in replicate

groups before being humanely discarded. On day 56, the vessels were placed in a water bath set between 40 and 60ºC, leaving approximately the top 2 cm of substrate above the water level. The vessels were then left for approximately 20 to 30 minutes to allow the juvenile worms to move to the surface of the substrate and away from the heat. The juvenile worms were then removed from the surface of the substrate and counted. When all visible worms had been removed, the substrate was spread out over a tray covered with plastic. Any juvenile worms and cocoons remaining in the substrate were removed and counted, and the total number of juveniles per vessel determined. After removal of the juveniles, soil samples were taken for chemical analysis and determination of water content and pH.

The endpoints were mortality, change of biomass (difference in fresh weight of surviving worms between test start and four weeks after treatment) and reproduction (the number of juveniles present). Percentage mortality of the worms in each vessel was calculated and a treatment mean presented. A no observed effect concentration (NOEC) and $LC_{50}$ were determined for adult mortality. The percentage weight change, based on mean surviving worm weight at day 28 was calculated for each vessel and a treatment no observed effect concentration (NOEC) and $EC_x$ were determined. Due to the low effect of the test substance on adult survival and weight change, the endpoints for these data have been empirically determined. Juvenile worm numbers were determined for each vessel and a treatment mean presented. A treatment no observed effect concentration (NOEC) and $EC_{10}$, $EC_{20}$ and $EC_{50}$ values were determined. Statistical analysis of the reproduction data was undertaken using CETIS version 1.8.6.8, based on the nominal test concentrations. The NOEC for number of juveniles was determined using a Bonferroni Adj t Test and the ECx values were determined using Linear Interpolation (ICPIN).

The analytical procedure (SMV 3202395-05V) was used to confirm the test substance concentration in the test samples on the day of test item application, after 28 days and at the end of the test. Concentrations of RH-24549 were determined by extracting soil samples with acetonitrile containing 1% formic acid, then diluting further with unfortified control sample extract to bring the response within the calibration range. Samples were analysed by liquid chromatography time-of-flight mass spectrometry (LC-TOF/MS) system or liquid chromatography triple quadrupole mass spectrometry (LC-TQMS). The method was validated according to SANCO 3029/99 rev. 4 with an LOQ of 0.016 mg/kg mg/kg.

## Results and discussion

Environmental conditions stayed within the recommended ranges.

Post application the mean soil concentrations were 0.1728, 0.3006, 0.4737, 0.8424, 1.5507, 2.8239, 4.0618 and 10.0142 mg a.i./kg dry substrate at application rates of 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.i./kg dry substrate respectively. This is equivalent to 107.99, 103.65, 89.37, 88.68, 90.16, 91.39, 73.05 and 100.14% of nominal at 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.i./kg dry substrate respectively. RH-24549 was not detected in the water control samples. Thus, the analytically verified recoveries of the test item in the soil substrate were 73.05-108 %, confirming sufficiently high (> 80% of nominal) test item concentrations at study start (besides 1 treatment, for which only 73.05 % of nominal could be recovered).

At day 28, there was a mean gain in weight of 38.4, 38.8, 44.3, 39.4, 36.5, 33.8, 37.2, 35.8 and 34.2% in the 0 (water control), 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.i./kg dry substrate groups, respectively. This was equivalent to changes of +1.0, +15.4, +2.6, -5.0, -12.0, -3.1, -6.8 and -10.9% in comparison to the water control. The NOEC and $EC_{50}$ for overall weight change were determined empirically to be 10 mg a.i./kg dry substrate and >10 mg a.i./kg dry substrate, respectively.

**Table A 77: Mean live weight of individual *E. fetida* per treatment and percentage weight change from days 0 to 28**

| Nominal concentration | Live weight (mg) | Increase in mean | % Effect in com- |
|---|---|---|---|

| (mg a.i./kg dry sub-strate) | Day 0 | Day 28 | weight (%) | parison to the water control |
|---|---|---|---|---|
| Water control | 416.8 | 576.9 | 38.4 | - |
| 0.16 | 403.9 | 560.7 | 38.8 | +1.0 |
| 0.29 | 408.9 | 590.1 | 44.3 | +15.4 |
| 0.53 | 406.3 | 566.5 | 39.4 | +2.6 |
| 0.95 | 392.1 | 535.1 | 36.5 | -5.0 |
| 1.72 | 420.1 | 562.2 | 33.8 | -12.0 |
| 3.09 | 420.0 | 576.3 | 37.2 | -3.1 |
| 5.56 | 420.0 | 570.4 | 35.8 | -6.8 |
| 10 | 413.8 | 555.5 | 34.2 | -10.9 |

N/A = not applicable

After 28 days of exposure, no adult mortality or sub-lethal effects were recorded in the controls or in the 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 or 10 mg a.i./kg dry substrate groups. Based on these results, the NOEC for 28-day adult survival was determined to be 10 mg a.i./kg dry substrate, and the $LC_{50}$ for 28-day survival was empirically determined to be >10 mg a.i./kg dry substrate.

**Table A 78: Mean treatment mortality for adult *E. fetida***

| Nominal concentration (mg a.i./kg dry substrate) | Mortality | | |
|---|---|---|---|
| | Number of *E. fetida* Exposed | Day 28 Number of mortalities | Total (%) |
| Water control | 80 | 0 | 0 |
| 0.16 | 40 | 0 | 0 |
| 0.29 | 40 | 0 | 0 |
| 0.53 | 40 | 0 | 0 |
| 0.95 | 40 | 0 | 0 |
| 1.72 | 40 | 0 | 0 |
| 3.09 | 40 | 0 | 0 |
| 5.56 | 40 | 0 | 0 |
| 10 | 40 | 0 | 0 |

The mean number of juveniles per vessel was 165, 173, 155, 168, 158, 165, 185 and 131 in the 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.i./kg dry substrate groups respectively in comparison to 149 in the water control. This corresponded to an increase in juveniles of 10.7, 16.1, 4.0, 12.8, 6.0, 10.7 and 24.2%, in the 0.16, 0.29, 0.53, 0.95, 1.72, 3.09 and 5.56 mg a.i./kg dry substrate groups, respectively, when compared to the water control. There was a reduction of 12.1% in the number of juveniles at 10 mg a.i./kg dry substrate in comparison to the water control.

The number of cocoons was not included in the calculations as the mean number of cocoons per treatment rate ranged from 0.3 – 3.3 and there was no evidence of a dose response. The mean number of cocoons in the water control replicates was 1.3.

No adverse behavioural observations or morphological symptoms were recorded.

**Table A 79: Mean number of juvenile worms at day 56**

| Nominal concentration | Mean number of juve- | % Difference when | Mean Number of co- |
|---|---|---|---|

| (mg a.i./kg dry substrate | niles | compared to the control | coons |
|---|---|---|---|
| Water control | 149 | - | 1.8 |
| 0.16 | 165 | +10.7 | 0.3 |
| 0.29 | 173 | +16.1 | 2.0 |
| 0.53 | 155 | +4.0 | 1.5 |
| 0.95 | 168 | +12.8 | 1.5 |
| 1.72 | 158 | +6.0 | 3.3 |
| 3.09 | 165 | +10.7 | 1.0 |
| 5.56 | 185 | +24.2 | 2.5 |
| 10 | 131 | -12.1 | 1.5 |

N/A = not applicable
Coefficient of variance for controls = 28.6%

Based on these results, the NOEC for reproduction was statistically determined to be 10 mg a.i./kg dry substrate. Based on these results, the NOEC for reproduction was statistically determined to be 10 mg a.i./kg dry substrate. The ECx values for reproductive performance were determined as follows:

**Table A 80: $EC_{10}$ $EC_{20}$ and $EC_{50}$ values for reproduction**

| Parameter | Value (mg a.i./kg dry substrate) | 95% confidence limits (mg a.i./kg dry substrate) |
|---|---|---|
| $EC_{10}$ | 7.449 | 4.664– 9.113 |
| $EC_{20}$ | 9.882 | 6.752 – N/A |
| $EC_{50}$ | >10 | N/A |

N/A= not available

Earthworms from the same source culture as those used in the reproductive test were used in a reference toxicity test with Carbendazim, a known toxic substance (performed under in-house GLP Smithers Viscient Study Number 3202329 from December 2018 to February 2019). The $EC_{50}$ value was estimated to be 2.05 mg a.i./kg dry soil. This is within the given toxicity range of 1 to 5 mg a.i./kg dry soil, indicating that the worms were responding as expected in the test system.

All validity criteria were met:

- The mortality of adults in the control group was ≤ 10% over the initial 28 days (actual adult mortality = 0%).
- The rate of production of juveniles was ≥ 30 per control container containing 10 adults by the end of test (mean actual rate of production of juveniles = 149).
- The coefficient of variance of reproduction in the control was 30% or less (actual coefficient of variance = 28.6%).

## Conclusion

A laboratory test was conducted in which mature *E. fetida* were exposed to RH-24549 for a period of 28 days, after which the adult worms were removed. Any cocoons produced were then allowed to hatch and the juveniles given time to mature for a further 28 days. The study endpoints were determined as follows:

- 28-day NOEC value for adult *E. fetida* survival = 10 mg a.i./kg dry substrate

- 28-day $LC_{50}$ value for adult *E. fetida* survival = >10 mg a.i./kg dry substrate

- NOEC value based on adult weight change = 10 mg a.i./kg dry substrate
- $EC_{50}$ value based on adult weight change = >10 mg a.i./kg dry substrate
- NOEC value based on reproduction = 10 mg a.i./kg dry substrate
- $EC_{10}$ value based on reproduction = 7.449 mg a.i./kg dry substrate
- $EC_{20}$ value based on reproduction = 9.882 mg a.i./kg dry substrate
- $EC_{50}$ value based on reproduction = >10 mg a.i./kg dry substrate

The study is valid.

(Gray J. 2020)

### A 2.4.1.1.4 Study 4 - RH-163353: Effect on the reproduction of earthworms

The following study has been provided to Latvia as RMS for zoxamide and cMSs for interzonal evaluation in July 2020.

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 222 (2016) and according to the principles of GLP. The study is considered to be reliable and suitable for the risk assessment. |

| | |
|---|---|
| **Reference:** | **KCP 10.4.1.1/04** |
| Report | Gray, J., 2020: RH-163353: Effect on reproduction in the earthworm *Eisenia fetida*<br>Gowan Crop Protection Ltd., UK<br>Smithers ERS Ltd., UK, Report No. 3202389, GLP, Not published |
| Guideline(s): | OECD 222 (2016) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | No |

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | RH-163353 (HHGCP001-00-2) |
| **Active substance content or purity** | 99.48 % (w/w); enantiomeric ratio 48.6:51.4 (racemate) |
| **Species** | Earthworm *Eisenia fetida* |
| Age: | adult worms, sexually mature (i.e. between 2 and 12 months old with clitellum), weighing between 300 and 600 mg (wet mass) |
| Source: | Bias Labs Ltd., UK |
| Weight: | 404-447 mg/worm (mean, day 0) |
| Acclimation period: | at least 24 hours in the artificial substrate with feed |
| Food: | finely ground animal faeces *ad libitum* |

| | |
|---|---|
| **Test system** | 1 L glass test vessel containing ten earthworms in the equivalent of approximately 500 g dry weight of artificial soil substrate maintained at nominally 45% maximum water holding capacity (MWHC) |
| Soil: | artificial soil, 10% peat |
| Number of animals: | 80 (control)<br>40 (test item treatment) |
| Number of replicates: | 4 replicates per treatment rate, 8 per controls |
| **Environmental conditions** | |
| Temperature: | 18 – 21.5°C |
| Photoperiod: | light: dark = 16 h: 8 h; 441 to 790 lux |
| Soil moisture: | guideline requirement: 40-60 % of max. WHC<br>during the study: 36-47 % of max. WHC |
| pH: | guideline requirement: 6.0 ± 0.5<br>during the study: 6.54 – 6.93 |
| **Application rate(s)** | 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.i./kg soil dry weight |
| Negative control: | reverse osmosis (RO) water, solvent (acetone) using a sand carrier |
| Positive control: | Carbendazim |
| **Post exposure observation period** | 56 days |
| **Remarks** | None |

The potential effects of the test item RH-163353 at application rates of 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.i./kg soil dry weight on the reproduction, mortality and growth of the earthworm *Eisenia fetida* has been studies, considering dermal and alimentary uptake after mixing of the test item with an artificial soil containing 10 % organic matter.

The test substance was diluted in acetone and applied over a sand carrier. The soil for the water control vessels was mixed with the same ratio of untreated sand and the solvent control was dosed with the same sand: solvent: soil ratio as the RH-163353 treatments. Reverse osmosis (RO) water was added to achieve the final weight required at 45% MWHC. After mixing, eight 5 g soil samples were taken from each treatment for chemical analysis (four samples to analyse and four to retain). Two samples were also taken from each treatment and the controls for analysis of water content to enable calculation of the test substance concentration in terms of substrate dry weight. One sample was taken from each treatment for determination of pH. The remaining soil was then weighed (equivalent to 500 g dry weight) into the test vessels.

Earthworms were acclimatised in a separate batch of the artificial soil (mixed with horse manure) for at least 24 hours before test start. After test substrate preparation, pots of 10 worms were randomised using an excel spreadsheet and allocated to the replicate test vessels. The earthworms placed onto the substrate surface. The vessels were re-covered with a perforated clear plastic film to allow for gaseous exchange and penetration of light. After approximately 15 minutes, each vessel was examined to confirm that no worms were remaining on the surface of the substrate. Food in the form of finely ground animal faeces, and RO water (5 g and 5 mL, respectively), were added on day 1 of the test and weekly thereafter up to and including day 28. Uneaten food remaining in the vessel at each feeding interval was removed and replaced with fresh food. Moisture loss due to evaporation was compensated for on a weekly basis. The

test was carried out in a temperature-controlled room set at 20 ± 2°C and a 16:8 hour light:dark cycle, with light intensity of 400 – 800 Lux, measured at least weekly throughout the test.

Survival of the worms was assessed 28 days after treatment by removing the soil from the test vessels, spreading it out on a plastic sheet and locating the earthworms, which were classified as dead if they did not respond to gentle mechanical stimulus to the anterior end. Missing earthworms were also considered dead due to rapid decomposition under test conditions. Following observations on day 28 and the removal of samples for chemical analysis and determination of water content, the test substrate was carefully replaced in the test vessels. The adult worms were rinsed in RO water, blotted dry and weighed in replicate groups before being humanely discarded. On day 56, the vessels were placed in a water bath set between 40 and 60ºC, leaving approximately the top 2 cm of substrate above the water level. The vessels were then left for approximately 20 to 30 minutes to allow the juvenile worms to move to the surface of the substrate and away from the heat. The juvenile worms were then removed from the surface of the substrate and counted. When all visible worms had been removed, the substrate was spread out over a tray covered with plastic. Any juvenile worms and cocoons remaining in the substrate were removed and counted, and the total number of juveniles per vessel determined. After removal of the juveniles, soil samples were taken for chemical analysis and determination of water content and pH.

The endpoints were mortality, change of biomass (difference in fresh weight of surviving worms between test start and four weeks after treatment) and reproduction (the number of juveniles present). Percentage mortality of the worms in each vessel was calculated and a treatment mean presented. A no observed effect concentration (NOEC) and $LC_{50}$ were determined for adult mortality. The percentage weight change, based on mean surviving worm weight at day 28 was calculated for each vessel and a treatment no observed effect concentration (NOEC) and $EC_x$ were determined. Juvenile worm numbers were determined for each vessel and a treatment mean presented. A treatment no observed effect concentration (NOEC) and $EC_{10}$, $EC_{20}$ and $EC_{50}$ values were determined. Statistical analysis of the reproduction data was undertaken using CETIS version 1.8.6.8, based on the nominal test concentrations. The NOEC for survival was determined using a Wilcoxon/ Bonferroni Adj Test and the LCx values were determined using Linear Interpolation (ICPIN). The NOEC for number of juveniles was determined using a Bonferroni Adj t Test for comparison to the water control and a Wilcoxon Bonferroni Adj Test for comparison with the solvent control. The ECx values were determined using Linear Interpolation (ICPIN).

The analytical procedure (SMV 3202389-02V) was used to confirm the test substance concentration in the test samples on the day of test item application, after 28 days and at the end of the test. Concentrations of RH-163353 were determined by extracting soil samples with acetonitrile/acetonitrile 3:1 (v/v) containing 1% formic acid, then diluting further with unfortified control sample extract as required to bring the response within the calibration range. Samples were analysed by injection onto a liquid chromatography-time of flight mass spectrometry (LC-TOF/MS). The method was validated according to SANCO 3029/99 rev. 4 with an LOQ of 0.016 mg/kg mg/kg.

The enantiomeric ratio of RH-163353 was assessed on day 56 soil samples. The analytical method validation for the enantiomeric ratio analysis for RH-163353 was conducted under Smithers ERS Study Number 3202586 (established analytical procedure SMV 3202586-01V). A combination of the analytical procedures, SMV 3202389-02V and SMV 3202586-01V were used to assess the enantiomeric ratio of the test substance

## Results and discussion

Environmental conditions stayed within the recommended ranges.

On Day 28 the mean soil concentrations were 0.0918, 0.1951, 0.3716, 0.6788, 1.2634, 2.3723, 4.1252 and 6.9682 mg a.i./kg dry substrate at application rates of 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.i./kg dry substrate respectively. This is equivalent to 57.38, 67.27, 70.11, 71.45, 73.46, 76.77, 74.19 and

69.68% of nominal at 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.i./kg dry substrate respectively. RH-163353 was not detected in the water or solvent control samples.

At Day 28, there was a mean gain in weight of 61.1, 67.5, 64.7, 66.0, 67.2, 72.4, 72.8 and 68.2% in the 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.i./kg dry substrate groups, respectively in comparison to 63.3 and 74.2% in the water and solvent controls respectively. This was equivalent to increases of 6.6, 2.2, 4.3, 6.2, 14.4, 15.0 and 7.7% in the 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.i./kg dry substrate groups, respectively in comparison to the water control with a reduction of 3.5% at 0.16 mg a.i./kg dry substrate. This was equivalent to reductions of 17.7, 9.0, 12.8, 11.1, 9.4, 2.5, 1.9 and 8.1% the 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.i./kg dry substrate groups, respectively in comparison to the solvent control. The NOEC and $EC_{50}$ for overall weight change were determined empirically to be 10 mg a.i./kg dry substrate and >10 mg a.i./kg dry substrate, respectively.

**Table A 81: Mean live weight of individual *E. fetida* per treatment and percentage weight change from days 0 to 28**

| Nominal concentration (mg a.i./kg dry substrate) | Live weight (mg) | | Increase in mean weight (%) | % Effect in comparison to the water control | % Effect in comparison to the solvent control |
|---|---|---|---|---|---|
| | Day 0 | Day 28 | | | |
| Water control | 438.8 | 716.5 | 63.3 | - | -14.7 |
| Solvent control | 409.6 | 713.3 | 74.2 | +17.2 | - |
| 0.16 | 446.7 | 719.6 | 61.1 | -3.5 | -17.7 |
| 0.29 | 436.0 | 730.1 | 67.5 | +6.6 | -9.0 |
| 0.53 | 440.3 | 724.9 | 64.7 | +2.2 | -12.8 |
| 0.95 | 532.4 | 717.9 | 66.0 | +4.3 | -11.1 |
| 1.72 | 415.8 | 695.0 | 67.2 | +6.2 | -9.4 |
| 3.09 | 436.2 | 752.2 | 72.4 | +14.4 | -2.5 |
| 5.56 | 404.2 | 698.4 | 72.8 | +15.0 | -1.9 |
| 10 | 425.4 | 715.3 | 68.2 | +7.7 | -8.1 |

N/A = not applicable

After 28 days of exposure, no adult mortality or sub-lethal effects were recorded in the controls or in the 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 or 10 mg a.i./kg dry substrate groups. Based on these results, the NOEC for 28-day adult survival was determined to be 10 mg a.i./kg dry substrate, and the $LC_{10}$, $LC_{20}$ and $LC_{50}$ for 28-day survival was empirically determined to be >10 mg a.i./kg dry substrate.

**Table A 82: Mean treatment mortality for adult *E. fetida***

| Nominal concentration (mg a.i./kg dry substrate) | Mortality | | |
|---|---|---|---|
| | Number of *E. fetida* Exposed | Day 28 Number of mortalities | Total (%) |
| Water control | 80 | 0 | 0 |
| Solvent control | 80 | 0 | 0 |
| 0.16 | 40 | 0 | 0 |
| 0.29 | 40 | 0 | 0 |
| 0.53 | 40 | 0 | 0 |
| 0.95 | 40 | 0 | 0 |

| | | | |
|---|---|---|---|
| 1.72 | 40 | 0 | 0 |
| 3.09 | 40 | 0 | 0 |
| 5.56 | 40 | 0 | 0 |
| 10 | 40 | 0 | 0 |

The mean number of juveniles per vessel was 211, 266, 199, 160, 219, 185, 190 and 276 in the 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.i./kg dry substrate groups respectively in comparison to 257 in the water control and 188 in the solvent control. This corresponded to a decrease in the number of juveniles of 10.6, 17.9, 22.6, 38.0, 15.1, 28.2 and 26.1%, in the 0.16, 0.53, 0.95, 1.72, 3.09 and 5.56 mg a.i./kg dry substrate groups, respectively, when compared to the water control. There were increases of 3.2 and 7.0% in the number of juveniles at 0.29 and 10 mg a.i./kg dry substrate respectively in comparison to the water control. When compared to the solvent control there were increases of 12.4, 41.3, 6.1, 16.3, 1.3 and 46.6% at 0.16, 0.29, 0.53, 1.72, 5.56 and 10 mg a.i./kg dry substrate groups respectively and decreases of 15.1 and 1.7% at 0.95 and 3.09 mg a.i./kg dry substrate groups respectively.

As the mean number of cocoons per treatment rate ranged from 24.8 – 43.5 with no evidence of a dose response, the effects on reproduction have been evaluated below using the mean number of juveniles plus cocoons in each treatment. The mean number of cocoons in the water and solvent control replicates was 30.0 and 44.3 respectively.

The mean number of juveniles plus cocoons per vessel was 255, 294, 234, 199, 246, 215, 215 and 300 in the 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.i./kg dry substrate groups respectively in comparison to 285 in the water control and 232 in the solvent control. This corresponded to a decrease in the number of juveniles plus cocoons of 10.6, 17.9, 30.1, 13.6, 24.5 and 24.4%, in the 0.16, 0.53, 0.95, 1.72, 3.09 and 5.56 mg a.i./kg dry substrate groups, respectively, when compared to the water control. There were increases of 3.2 and 5.3% in the number of juveniles at 0.29 and 10 mg a.i./kg dry substrate respectively in comparison to the water control. When compared to the solvent control there were increases of 9.9, 26.8, 0.9, 6.1 and 29.3% at 0.16, 0.29, 0.53, 1.72 and 10 mg a.i./kg dry substrate groups respectively and decreases of 14.1, 7.3 and 7.3% at 0.95, 3.09 and 5.56 mg a.i./kg dry substrate groups respectively. Each cocoon has been included as representative of one juvenile worm.

**Table A 83: Mean number of juvenile worms at day 56**

| Nominal concentration (mg a.i./kg dry substrate | Mean number of juveniles including cocoons | % Difference when compared to the control water | % Difference when compared to the solvent control | Mean Number of cocoons |
|---|---|---|---|---|
| Water control | 285 | - | +22.8 | 30.0 |
| Solvent control | 232 | -18.6 | - | 44.3 |
| 0.16 | 255 | -10.6 | +9.9 | 43.5 |
| 0.29 | 294 | +3.2 | +26.8 | 28.5 |
| 0.53 | 234 | -17.9 | +0.8 | 34.5 |
| 0.95 | 199 | -30.1 | +14.1 | 39.8 |
| 1.72 | 246 | -13.6 | 6.1 | 27.5 |
| 3.09 | 215 | -24.5 | -7.3 | 30.3 |
| 5.56 | 215 | -24.4 | -7.2 | 25.0 |
| 10 | 300 | +29.5 | +29.5 | 24.8 |

N/A = not applicable
Coefficient of variance for controls = 18.88 and 18.68% for the water and solvent controls respectively

Based on these results, the NOEC for reproduction was statistically determined to be 10 mg a.i./kg dry substrate. The $EC_{10}$, $EC_{20}$ and $EC_{50}$ values for reproduction in comparison to both water and solvent controls were statistically determined to be >10 mg a.i./kg dry substrate:

No adverse behavioural observations or morphological symptoms were recorded.

Earthworms from the same source culture as those used in the reproductive test were used in a reference toxicity test with Carbendazim, a known toxic substance (performed under in-house GLP Smithers Viscient Study Number 3202329 from December 2018 to February 2019). The $EC_{50}$ value was estimated to be 2.05 mg a.i./kg dry soil. This is within the given toxicity range of 1 to 5 mg a.i./kg dry soil, indicating that the worms were responding as expected in the test system.

All validity criteria were met:

- The mortality of adults in the control group was ≤ 10% over the initial 28 days (actual adult mortality = 0% in both water and solvent controls).
- The rate of production of juveniles was ≥ 30 per control container containing 10 adults by the end of test (mean actual rate of production of juveniles including cocoons = 285 and 232 in the water and solvent controls respectively).
- The coefficient of variance of reproduction in the control was 30% or less (actual coefficient of variance = 18.88 and 18.68% in the water and solvent controls respectively).

## Conclusion

A laboratory test was conducted in which mature *E. fetida* were exposed to RH-163353 for a period of 28 days, after which the adult worms were removed. Any cocoons produced were then allowed to hatch and the juveniles given time to mature for a further 28 days. There were no adverse effects on adult worm weight over the 28-day exposure period. The study endpoints were determined as follows:

**Table A 84: Effects of the test item: summary of statistical analysis**

| Endpoint | mg test item/kg soil dry weight |
|---|---|
| 28-day NOEC for adult survival[b] | 10 mg a.i./kg dry substrate |
| 28-day $LC_{50}$ adult survival | >10 mg a.i./kg dry substrate |
| 28-day NOEC for adult weight change[b] | 10 mg a.i./kg dry substrate |
| 28-day $LC_{50}$ for adult weight change[b] | >10 mg a.i./kg dry substrate |
| 56-day NOEC for reproduction[b] | 10 mg a.i./kg dry substrate |
| 56-day $EC_{10}$ reproduction[b] | >10 mg a.i./kg dry substrate |
| 56-day $EC_{20}$ reproduction[b] | >10 mg a.i./kg dry substrate |
| 56-day $EC_{50}$ reproduction[b] | >10 mg a.i./kg dry substrate |

[a] Rounded figure
[b] Empirically determined
N/A = not applicable

All validity criteria were met.

(Gray J. 2020)

### A 2.4.1.1.5 Study 5 - CYMOXANIL 33% + ZOXAMIDE 33% WG: Effects on the reproduction of earthworms

| | |
|---|---|
| Comments of zRMS: | Study evaluated and accepted by zRMS UK. Please refer to product fRR dated January 2012.<br>Moreover, the acute toxicity test is no longer the data requirement. |

**Reference:** **KCP 10.4.1.1/05**

Report: Colli, M., 2006: Acute toxicity of 'CYMOXANIL 33% + ZOXAMIDE 33% WG' on Earthworms, *Eisenia fetida*, using an artificial soil test.
Oxon Italia S.p.A., Italy
Biotecnologie BT S.r.l., Italy, Report No. BT027/06, GLP, Not published

Guideline(s): OECD 207 (1984)

Deviations: No

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): No

**Executive Summary**

The acute toxicity of 'Cymoxanil 33% + Zoxamide 33% WG' to *Eisenia foetida* was determined by exposing earthworms to different concentrations of test item mixed with an artificial soil with peat content 5%.
The test was carried out at five different concentrations: 100, 178, 316, 562, 1000 mg formulation/kg soil. Mortality, behavioural abnormalities and pathological symptoms were recorded after 7 and 14 days. Weights of earthworms were measured at the beginning and at the end of the test.
2-chloroacetamide was used as reference toxic item.
No relevant lethal effects to *Eisenia foetida* were found for all concentrations, so $LC_{50}$ value of 'Cymoxanil 33% + Zoxamide 33% WG' is > 1000 mg/kg of soil.
Decreasing of body weights was recorded for concentrations of 178 mg/kg and higher. Differences with the control were statistically different starting from 316 mg/kg.
The 14d $LC_{50}$ of 'Cymoxanil 33% + Zoxamide 33% WG' is > 1000 mg formulation/kg artificial soil. NOEC was set at 178 mg/kg.

## I. MATERIALS AND METHODS

### A. MATERIALS

**1.Test Material:**

| | |
|---|---|
| **Description:** | Granules |
| **Lot/Batch #:** | Batch code BPL 212 |
| **Purity:** | Nominal: Cymoxanil 330 g/kg, Zoxamide 330 g/kg<br>Measured: Cymoxanil 322.9 g/kg, Zoxamide 333.2 g/kg |
| **Stability of test compound** | June 2008 (expiry date) |
| **2. Vehicle and/or positive control** | 2-chloroacetamide used as positive control |
| **3. Test animals** | |
| **Species:** | *Eisenia fetida* |
| **Strain** | Not relevant |
| **Age:** | Adult (at least two months) |
| **Weight at dosing:** | 300 - 600 mg |
| **Source:** | In house rearing stock of testing facility |
| **Acclimation period** | Not stated |

| | |
|---|---|
| **Diet:** | Carrots, potatoes and apples |
| **Water:** | Not relevant |
| **Housing:** | In laboratory, in rearing medium. |
| **4. Environmental conditions** | |
| **Temperature:** | 19.33 – 20 °C |
| **Humidity:** | 40 -60 % soil water content |
| **Air changes:** | Not relevant |
| **Photoperiod:** | Continuos illumination (475 - 750 lux) |

**B. STUDY DESIGN AND METHODS**

| | |
|---|---|
| **1. In life dates** | 2 August – 21 September 2006 |
| **2. Experimental treatment** | On the basis of a range test finding, the assay was carried out at concentration of 100, 178, 316, 562, 1000 mg/kg soil dry weight. 2-chloroacetamide was used as reference toxic item and control test in deionised water were also made. Four replicates of each concentration and of the control were performed placing 10 worms in test containers with water or artificial soil mixed with the test substance. The peat content in the artificial soil was 5%. Worms were incubated under controlled conditions for 14 days. |
| **3. Observations** | Mortality, behavioural abnormalities and pathological symptoms were recorded after 7 and 14 days. Weights of earthworms were measured at the beginning and at the end of the test. |
| **4. Statistics** | Probit analysis, Anova – Manova – Post Hoc comparison. |

## II. RESULTS AND DISCUSSION

**A. FINDINGS**

No significant differences between the mortality in the control and the test item were found. The body weight increased in the test item at 100 mg formulation/kg, while it decreased in the others. Differences were statistical significant at 316, 562, 1000 mg/kg.

**Table IIIA 10.6.2-1: Mortality at 7 and 14 days and body weight changes in the earthworms.**

| Dosage (mg/kg artificial soil) | Mean mortality (%) and relative standard deviations | | | | Mean changes and increasing in body weight (%) | |
|---|---|---|---|---|---|---|
| | 7 days | | 14 days | | 14 days | |
| | % | s.d. | % | s.d. | Mean of body weight differences | Body weight increase |
| 0 (control) | 0 | 0 | 0 | 0 | 0.018 | 3.83 |
| 100 mg/ kg | 0 | 0 | 2.50 | 5 | 0.025 | 5.96 |
| 178 mg/kg | 0 | 0 | 0 | 0 | -0.006 | -1.29 |
| 316 mg/kg | 0 | 0 | 0 | 0 | -0.098 | -22.65* |
| 562 mg/kg | 0 | 0 | 5.00 | 5.77 | -0.138 | -31.39* |
| 1000 mg/kg | 0 | 0 | 2.50 | 5.00 | -0.140 | -30.18* |

* significant difference

## III. CONCLUSIONS

The 14d $LC_{50}$ of 'Cymoxanil 33% + Zoxamide 33% WG' is higher than 1000 mg formulation/kg artificial soil. NOEC was set at 178 mg/kg based on effects on bodyweight at 316 mg/kg.

(Colli M. 2006)

### A 2.4.1.1.6 Study 6 - Cymoxanil 33% + Zoxamide 33% WG: Effects on the reproduction of earthworms

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 222 (2016) and according to the principles of GLP. The study is considered to be reliable and suitable for the risk assessment. |

**Reference:** **KCP 10.4.1.1/06**

Report: Friedrich, S., 2019: Effects of Cymoxanil 33% + Zoxamide 33% on the reproduction of the earthworm *Eisenia andrei* in artificial soil with 5 % peat
Gowan Crop Protection Ltd., UK, SIPCAM OXON S.p.A, Italy
BioChem Agrar, Germany, Report No. 17 48 TEC 0008, GLP, Not published

Guideline(s): OECD 222 (2016)

Deviations: No

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): No

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | Cymoxanil 33% + Zoxamide 33% WG (30AL5020) |
| **Active substance content (measured)** | Cymoxanil: 33.2% (analysed)<br>Zoxamide: 32.6% (analysed) |
| **Species:** | Earthworm *Eisenia andrei* (BOUCHÉ, 1972) |
| Age: | adult worms, between 2 months and 1 year old with clitellum (individuals will not differ in age by more than 4 weeks) |
| Source: | W. Neudorff GmbH KG, Germany |
| Weight: | 250-600 mg/worm (individuals will not differ in weight by more than 200 mg) |
| Acclimation period: | at least one day in artificial soil (with food) |
| Food: | the worms will be fed with air-dried and finely ground horse manure (up to 5 g, weekly amount of manure depends on feeding activity) |
| **Test system** | plastic vessel (inside dimensions: about 16.5 cm x 12 cm x 6 cm) with a lid pervious to air and light, containing 810 g wet weight corresponding to 600 g dry weight of artificial soil with a water content corresponding to 40-60 % of WHC |
| Soil: | artificial soil, 10% peat |
| Number of animals: | 10/test vessel (=replicate)<br>40 (control group: 80)/treatment group |
| Number of replicates: | Control group: 8 (+1 replicate without earthworm for soil analysis)<br>Treated group: 4 (+1 replicate without earthworm for soil analysis) |

| | |
|---|---|
| **Environmental conditions** | |
| Temperature: | 20 ± 2 °C |
| Photoperiod: | light: dark = 16 h: 8 h; 400-800 lux |
| Water content: | 40-60 % WHC<br>test start: 34.9 – 35.1 (equivalent to 56.1 – 56.4 % of WHC)<br>test end: 34.1 – 34.9 (equivalent to 54.8 – 56.1 % of WHC) |
| pH: | 6.0 ± 0.5<br>test start: 6.03 – 6.09<br>test end: 5.71 – 5.86 |
| **Application rate(s)** | 4.55, 8.18, 14.7, 26.5, 47.7, 85.9 and 154.6 mg product/kg soil d.w. |
| **Post exposure observation period** | 56 days |
| **Remarks** | None |

The potential effects of the test item Cymoxanil 33% + Zoxamide 33% WG at ap-plication rates of 4.55, 8.18, 14.7, 26.5, 47.7, 85.9 and 154.6 mg product/kg soil dry weight on the reproduction, mortality and growth of the earthworm Eisenia andrei has been studies, considering der-mal and alimentary uptake after mixing of the test item with an artificial soil containing 10 % organic matter.

One day before test start, the dry artificial soil was pre-moistened by adding deionised water to obtain approximately half of the final water content. Earthworms were acclimatised in a separate batch of the artificial soil (mixed with horse manure) for at least 24 hours before test start.

On the day of the test start, the test item was introduced by dispersing the quantity of test item required to obtain the desired test concentration in the volume of water required to hydrate the soil to 40-60 % of its WHC (Appendix 1). The control substrate contained the corresponding amount of deionised water. The test solution was thoroughly mixed into the soil separately for each replicate. Each test vessel was then filled with the treated soil. After a randomising procedure according to the worm fresh weight, selected groups of 10 worms were then randomly assigned to each treatment group (Appendix 2). The individually weighed worms (10 worms/vessel) were placed on the surface of the soil. After approximately thirty minutes, the test vessels were closed with perforated transparent lids, which allowed gas exchange between substrate and atmosphere and access of light, but prevented worms from escaping. The test vessels were then set up at random in a controlled-environment test room. One day after application, initially 5 g air-dried and finely ground horse manure was scattered on the soil surface of each test vessel, which was sprinkled with 5 mL deionised water. The feeding interval was weekly during the first four weeks of the test. The weekly amount of manure (5 g) depended on the feeding activity, which was assessed by visual estimation of the food remaining on the surface before addition of new food.

After four weeks, the adult worms were removed from the test vessels. The number of surviving worms (adult mortality) and their biomass change were determined, behaviour (including feeding activity) and pathological symptoms were recorded. The adult worms were discarded after counting and weighing. Subsequently, the soil of each vessel was mixed carefully with 5 g manure. This was the last feeding occasion of the experiment. The test was then continued for another four weeks. The final assessment included counting of juveniles per test vessel, determination of the water content and pH measurements of the artificial soil. Juveniles were counted by manual inspection of the substrate.

The concentration of zoxamide in the artificial soil was analysed at test start (day 0, just after test item application), middle (day 28) and test end (day 56). Untreated soil (control) served for method validation.

The nominal initial test item concentrations in the soil were analytically confirmed with a highly specific HPLC-MS/MS method fully validated according to SANCO/3029/99 rev. 4. The active ingredients Cymoxanil and Zoxamide were analysed by a validated method (report no. 18 35 CRX 0033) using extraction in acidic acetonitrile and determination by reverse-phase high pressure liquid chromatography with tandem mass spectrometric detection (LC-MS/MS). The LOQ of the original method is 0.01 mg/kg soil d.w. for Cymoxanil and Zoxamide, respectively. The limit of quantification (LOQ) was defined in the context of this phase of the study as the lowest successfully validated fortification level, i.e. 0.56 mg/kg Cymoxanil and 0.55 mg/kg Zoxamide in moist soil specimens, equivalent to 0.75 mg/kg Cymoxanil and 0.74 mg/kg Zoxamide corrected to dry weight and 14 µg/L extracts. The LOQ was already validate in a previous study (report no. 18 35 CRX 0033) at 0.0125 mg/kg dry weight for Cymoxanil and Zoxamide, but a higher dilution of the soil extracts was used in the present study (factor 20 vs. factor 2). The method was adapted to the expected concentration range of analytes in this study and was revalidated according to SANCO/3029/99 rev. 4 with artificial soil at concentrations of approximately 50% of the lowest test concentration (0.55 mg a.i./kg moist soil or 0.74 mg a.i./kg soil d.w.) and approximately 120% of the highest test concentration (38 mg a.i./kg moist soil or 51 mg a.i./kg soil d.w.). 10 g soil wee wetted with 2 mL water and extracted with 20 mL acetonitrile and 0.2 mL acetic acid for 15 minutes on a vortex shaker. The acetonitrile phase was separated by the addition of about 2.5 g of a 4:1 mixture of dried magnesium sulfate and sodium chloride and centrifugation. Aliquots of the acetonitrile phase were diluted and analysed. Two mass transitions for each analyte were used for detection (Zoxamide: m/z 336 → 187 and 336 → 159; Cymoxanil: m/z 199 → 128 and 199 → 111), one for quantification and one for qualification, respectively.

## Results and discussion

Environmental conditions stayed within the recommended ranges.

The analytically verified recoveries of the active substances in the soil substrate were all greater than 80% (i.e. 87-94 % for cymoxanil and 81%-94% for zoxamide). After 28 days, the Cymoxanil concentrations had declined to 22 – 35 % of nominal, the Zoxamide concentrations to 27 – 41 % of nominal. After 56 days, the Cymoxanil concentrations had declined to 7.2 – 15 % of nominal, the Zoxamide concentrations to 10 – 18 % of nominal. Thus, confirming sufficiently high (> 80% of nominal) test item concentrations at study start.

At the start of the test, earthworm fresh weight ranged from 250 – 600 mg/worm. The physico-chemical parameters measured at the start and at the end of the tests met the guideline requirements.

The weight change of adult worms ranged between 26.7 % and 32.0 % in the treated groups and was 28.8 % in the control group (Table 2). The test item caused no statistically significant effects (Williams-test, α = 0.05, one-sided smaller) on the change in biomass (change in fresh weight after 4 weeks relative to initial fresh weight) compared to the control group at any concentration tested.

No mortality was recorded in the test item treatment groups and in the control. No pathological symptoms and no effects on behaviour (including feeding activity) of the worms were observed during the test.

**Table A 85: Effects of the test item on mortality and reproduction of adult earthworms**

| Treatment group | Cymoxanil 33 % + Zoxamide 33 % WG mg a.s./kg soil d.w. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | 4.55 | 8.18 | 14.7 | 26.5 | 47.7 | 85.9 | 154.6 |
| Replicate | Number of surviving adult worms per replicate (4 weeks after test initiation) | | | | | | | |
| 1 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 2 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 3 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 4 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 5 | 10 | | | | | | | |
| 6 | 10 | | | | | | | |
| 7 | 10 | | | | | | | |
| 8 | 10 | | | | | | | |
| **Mean** | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 |
| SD | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| cv % | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | **Mortality (%)** | | | | | | | |
| **Mean** | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** |
| | **Number of juvenile worms per replicate (8 weeks after test initiation)** | | | | | | | |
| 1 | 135 | 106 | 77 | 51 | 46 | 3 | 0 | 0 |
| 2 | 146 | 135 | 108 | 69 | 35 | 5 | 1 | 0 |
| 3 | 109 | 152 | 114 | 34 | 22 | 7 | 0 | 0 |
| 4 | 127 | 133 | 89 | 43 | 28 | 6 | 0 | 0 |
| 5 | 97 | | | | | | | |
| 6 | 90 | | | | | | | |
| 7 | 154 | | | | | | | |
| 8 | 138 | | | | | | | |
| **Mean** | **124.5** | **131.5** | **97.0*** | **49.3*** | **32.8*** | **5.3*** | **0.3*** | **0.0*** |
| SD | 23.4 | 19.0 | 17.1 | 14.9 | 10.3 | 1.7 | 0.5 | 0.0 |
| cv % | 18.8 | 14.5 | 17.6 | 30.2 | 31.5 | 32.5 | 200.0 | - |
| | **Reduction of reproduction (%)** | | | | | | | |
| **% to control** | - | -5.6 | 22.1 | 60.4 | 73.7 | 95.8 | 99.8 | 100.0 |

* statistically significantly different compared to the control (Williams-t-test for reproduction, α = 0.05, one-sided smaller)
SD: standard deviation, cv %: coefficient of variation, d.w.: dry weight
Negative % values for change of reproduction = increase, relative to control

Statistically significant effects (Williams-t-test, α = 0.05, one-sided smaller) on the number of juveniles compared to the control group were recorded at nominal concentrations of 8.18, 14.7, 26.5, 47.7, 85.9 and 154.6 mg test item/kg d.w. The NOEC for mortality and biomass was determined to be 154.6 mg test item/kg soil dry weight (corresponding to 51.02 mg/kg cymoxanil and zoxamide, respectively, based on their nominal test item concentration). The NOEC for reproduction was determined to be 4.55 mg test item/kg soil dry weight corresponding to 1.50 mg/kg cymoxanil and zoxamide, respectively, based on their nominal test item concentration). The nominal median EC10, EC20 and EC50 values for reproduction were calculated to be 5.47, 7.54 and 14.0 mg test item/kg soil dry weight, respectively (Table 5), corresponding to 1.80, 2.49 and 4.61 mg/kg cymoxanil and zoxamide, respectively, based on their nominal test item concentration.

**Table A 86: Effects of Cymoxanil 33 % + Zoxamide 33 % WG on Eisenia andrei in a 56-day reproduction study**

| ~~Endpoint~~ | ~~Treatment group~~ |
|---|---|
| | ~~Nominal concentration (mg a.s./kg soil d.w.)~~ |

| | Control | 0.392 | 0.706 | 1.271 | 2.287 | 4.117 | 7.410 | 13.34 | 24.01 |
|---|---|---|---|---|---|---|---|---|---|
| | Analysed concentration on day 0 (mg a.s./kg soil d.w.) | | | | | | | | |
| | Control | 0.511 | 0.714 | 1.367 | 2.453 | 4.319 | 7.105 | 13.64 | 25.38 |
| Mortality of adult worms after 4 weeks (%) | 0.0 | 0.0 | 2.5 | 2.5 | 0.0 | 0.0 | 5.0 | 2.5 | 5.0 |
| Mean biomass change after 4 weeks (%) | 22.5 | 23.1 | 22.5 | 23.4 | 21.9 | 23.6 | 19.8 | 20.9 | 21.2 |
| Mean number of juveniles after 8 weeks | 140.6 | 149.0 | 142.8 | 138.3 | 141.8 | 101.3* | 31.3* | 13.8* | 0.0* |
| Reduction of reproduction compared to control (%) | - | -6.0 | -1.5 | 1.7 | -0.8 | 28.0 | 77.8 | 90.2 | 100.0 |

| | Endpoint (mg a.s./kg soil dry weight) | | |
|---|---|---|---|
| | mg test item/kg soil d.w. | mg zoxamide/kg soil d.w. | mg cymoxanil /kg soil d.w. |
| NOEC (mortality) | 154.6 | 51.02 | 51.02 |
| NOEC (biomass) | 154.6 | 51.02 | 51.02 |
| NOEC (reproduction) | 4.55 | 1.50 | 1.50 |
| $LC_{50}$ (mortality) | > 154.6 | > 51.02 | > 51.02 |
| $EC_{10}$ (reproduction)[1] | 5.47 (3.80 – 7.87) | 1.80 (1.26 – 2.60) | 1.80 (1.26 – 2.60) |
| $EC_{20}$ (reproduction)[1] | 7.54 (5.74 – 9.91) | 2.49 (1.90 – 3.27) | 2.49 (1.90 – 3.27) |
| $EC_{50}$ (reproduction)[1] | 14.0 (11.7 – 16.6) | 4.61 (3.88 – 5.49) | 4.61 (3.88 – 5.49) |

* = statistically significant compared to control (Williams-t-test for biomass and reproduction, α = 0.05, one-sided smaller)
[1] = median values with their 95 % confidence intervals, based on Probit analysis
Negative % values for change of reproduction = increase, relative to control
d.w.: dry weight (of artificial soil)

Effects of Cymoxanil 33 % + Zoxamide 33 % WG on *Eisenia andrei* in a 56-day reproduction study

| Endpoint | Treatment group (mg test item/kg soil dry weight) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | 4.55 | 8.18 | 14.7 | 26.5 | 47.7 | 85.9 | 154.6 |
| Mortality of adult worms after 4 weeks (%) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Mean biomass change after 4 weeks (%) | 28.8 | 27.1 | 28.5 | 30.5 | 29.8 | 32.0 | 28.4 | 26.7 |
| Mean number of juveniles after 8 weeks | 124.5 | 131.5 | 97.0* | 49.3* | 32.8* | 5.3* | 0.3* | 0.0* |
| Reduction of reproduction compared to control (%) | - | -5.6 | 22.1 | 60.4 | 73.7 | 95.8 | 99.8 | 100.0 |

| | Endpoint | | |
|---|---|---|---|
| | mg test item/kg soil d.w. | mg zoxamide/kg soil d.w. | mg cymoxanil /kg soil d.w. |
| NOEC (mortality) | 154.6 | 51.02 | 51.02 |
| NOEC (biomass) | 154.6 | 51.02 | 51.02 |
| NOEC (reproduction) | 4.55 | 1.50 | 1.50 |
| $LC_{50}$ (mortality) | > 154.6 | > 51.02 | > 51.02 |
| $EC_{10}$ (reproduction) [1]<br>confidence limits (lower - upper) | 5.47<br>(3.80 – 7.87) | 1.80<br>(1.26 – 2.60) | 1.80<br>(1.26 – 2.60) |
| $EC_{20}$ (reproduction) [1]<br>confidence limits (lower - upper) | 7.54<br>(5.74 – 9.91) | 2.49<br>(1.90 – 3.27) | 2.49<br>(1.90 – 3.27) |
| $EC_{50}$ (reproduction) [1]<br>confidence limits (lower - upper) | 14.0<br>(11.7 – 16.6) | 4.61<br>(3.88 – 5.49) | 4.61<br>(3.88 – 5.49) |

* statistically significant compared to control (Williams-t-test reproduction, $\alpha = 0.05$, one-sided smaller)
[1] median values with their 95 % confidence intervals, based on Probit analysis
Negative % values for change of reproduction = increase, relative to control, d.w.: dry weight (of artificial soil)

All validity criteria were met:

- adult mortality: ≤ 10 % (being 0 % after 4 weeks)
- number of juveniles per replicate: ≥ 30 (being 90 to 154)
- coefficient of variation of reproduction: ≤ 30 % (being 18.8 %)

**Conclusion**

In a 56-day earthworm reproduction study with Cymoxanil 33 % + Zoxamide 33 % WG, no statistically significant adverse effects on mortality and biomass of the earthworm *Eisenia fetida* in artificial soil were determined up to and including 154.6 mg test item/kg soil dry weight, i.e. the highest concentration tested.

The NOEC for mortality and biomass was determined to be 154.6 mg test item/kg soil dry weight. The NOEC for reproduction was determined to be 4.55 mg test item/kg soil dry weight. The $E_{10}$, $EC_{20}$ and $EC_{50}$ value for reproduction was calculated to be 5.47, 7.54 and 14.0 mg test item/kg soil dry weight, respectively.

(Friedrich S. 2020)

## A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

### A 2.4.1.2.1 Study 1 - GWN-10392: Effects on earthworms under field conditions

The following study has been performed with an SC formulation containing 240 g/L zoxamide, the repre-

sentative formulation during AIR. The composition of Zoxium 240 SC (GWN-9790EU) is included in Part C. The study endpoints can be used as representative for zoxamide technical.

| Comments of zRMS: | Study not evaluated by zRMS. |
|---|---|

**Reference:** **KCP 10.4.1.2/01**

Report: Schulz, L., 2020: Effects of Zoxium 240 SC on earthworms under field conditions Gowan Crop Protection Ltd., UK
BioChem agrar, Germany, Report No. 18 48 FEW 0001, GLP, Not published

Guideline(s): ISO 11268-3 (2014)
Technical recommendations to ISO 11268-3 (Kula et al. 2006)

Deviations: No

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): No

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | Zoxium 240 SC (18011201-72-52) |
| **Active substance content or purity** | zoxamide 240 g/L (nominal), 21.7 % w/w (analysed)<br>isomeric ratio of R-/S-zoxamide = 49.8 / 50.2 |
| **Test organisms** | Naturally occurring earthworm (Annelida: *Oligochaeta*) population with an abundance greater 60 ind./m² and two dominant species representing different life forms (*Aporrectodea caliginosa* and *Lumbricus terrestris*) present at 10 % or at least 10 to 15 ind./m², with a relative homogenous distribution on a typical arable field. Climatic and soil conditions within the test area (e.g. altitude, slope, drainage system, and wind exposure) sufficiently homogeneous. |
| **Test system** | 20 plots, each 10 m x 10 m, were arranged in a randomised block design.<br>The test item applications were performed on bare soil.<br>About 1 month after the last application, the test field was seeded with the fodder crop "Landsberger Gemenge" (clover grass mixture) which stayed on the field until the end of the study. |
| Field: | Typical arable field near Dornreichenbach in Saxony, Germany.<br>Soil textural class: sandy-loamy silt (DIN 4220) / silt loam (USDA), mean pH ($CaCl_2$) 6.0, mean total organic carbon content 1.3 % and mean maximum water holding capacity 42.8 g/100 g soil dry weight. |
| Number of replicates: | Earthworms were sampled from four 0.125 m² sampling areas per plot per sampling occasion. |
| Surface monitoring: | Assessment of alive, moribund and dead earthworms on the soil surface in a monitoring area of 20.0 m² per plot in all control and test item replicates from day 1 to day 3 after each application. |
| Earthworm sampling: | Hand sorting combined with formalin extraction. |

| | |
|---|---|
| **Environmental conditions** | |
| Air temperature during application(s): | 12.3 – 27.8 °C |
| Soil moisture during applications: | 8.6 – 19.3 % w/w |
| **Application rate(s)** | 5 applications at each:<br>1) 140 g a.s./ha (0.5833 L test item/ha) = total of 700 g a.s./ha/season<br>2) 180 g a.s./ha (0.75 L test item/ha) = total of 900 g a.s./ha/season<br>3) 280 g a.s./ha (1.1667 L test item/ha) = total of 1400 g a.s./ha/season |
| Reference item: | 20 L Maypon Flow/ha in 600 L water/ha<br>(equivalent to nominally 10 kg carbendazim/ha) |
| **Post exposure observation period** | 1st sampling about 1 month after 1st application.<br>2nd sampling about 6 months after 1st application.<br>3rd sampling about 12 months after 1st application. |
| **Remarks** | None |

Potential effects and potential recovery of field populations of earthworms after the spray application of Zoxium 240 (containing nominally 240 g/L zoxamide) to bare soil (silt loam according to USDA) at a pattern of 5x 140 g a.s./ha (0.5833 L test item/ha) = total of 700 g a.s./ha/season, 5x 180 g a.s./ha (0.75 L test item/ha) = total of 900 g a.s./ha/season and 5x 280 g a.s./ha (1.1667 L test item/ha) = total of 1400 g a.s./ha/season with an interval of 7-8 days were investigated on a typical arable field located near Dornreichenbach in Saxony, Germany. The results with regard to earthworm species composition, biomass and abundance were compared to an untreated control and a reference item (Maypon Flow, containing nominally 50 % w/v carbendazim) applied at a rate of 20 L/ha, corresponding to 10 kg a.s./ha, in parallel to the 1st test item application. The correct test item application has been verified by analysis of soil samples taken immediately after applications with a method validated according to SANCO/3029/99 rev. 4 (11/07/2000).

Twenty plots, each 10 m x 10 m, were arranged in a 5 x 4 formation, each plot surrounded by a 2 m wide path - also between the plots. The set-up was a randomised block design. The assignment of the treatment groups to the plots was based on the results of a pre-sampling. The pre-sampling was conducted to determine the density, diversity and homogeneity of earthworm populations at the site.

Defined areas were sampled to assess earthworm populations before application and three times after application, i.e. about 1, 6 and 12 months after the 1st test item application. Earthworms were sampled from four 0.125 m² sampling areas per plot per sampling occasion by combining hand sorting with formalin extraction in the excavated hole (according to DIN ISO 23611-1, 2006).

Directly after digging out the soil, 2.5 L of a 0.2 % formaldehyde solution were uniformly poured into the hole in 2 or 3 portions according to seepage capacity. The total duration of the formaldehyde extraction was at least 30 minutes. All earthworms coming to the surface were collected and placed into a vessel containing water, one for each sample. Earthworms found by hand sorting were collected in separate vessels containing water, one for each sample. Vessels with earthworms were stored in a cold room in the dark for approximately 48 hours. The abundance of earthworms was recorded and the weight (biomass) determined with a precision balance for each sample separately. Before the worms were weighed each single worm was placed on a dry filter paper to free the worm body from excess water. Animals were counted, weighed, and species identified in a period of approximately 48 hours after sampling.

Environmental conditions (air temperature, relative air humidity, soil temperature (10 cm height), wind speed, wind direction, cloudiness, rainfall, and soil moisture) were assessed during the test item applica-

tions. Furthermore, soil moisture, soil temperature, vegetation coverage (in % covered soil surface) and vegetation height were evaluated during each sampling occasion.

Statistical analysis was carried out with the statistical software package ToxRat Professional. Data were analysed for normal distribution with the Shapiro-Wilk's-test or Kolmogorov-Smirnoff-test and for homogeneity in variance with the Levene's test. Afterwards, pre-sampling data were analysed with a two-factorial analysis of variance (ANOVA, 5 % significance level) with treatment as fixed factor and block as random factor. Post-treatment sampling data were analysed for monotone dose-response using trend analysis by contrasts. Afterwards, the data were analysed by a one-sided Williams-t-test, Dunnett's-t-test or Welch-t-test after Bonferroni-Holm with test item treatment group < control as well as Student-t-test or Welch-t-test with reference item treatment group < control at the 5 % significance level. Test item and reference item effects were analysed in separate analyses.

## Results and discussion

The test item has been applied on days with low wind speed and no rain during and after application. Due to warm and dry weather conditions during 2018, irrigation of the test field was required to support the exposure of the test organisms. The test field was irrigated on day 2 after the 1st application, day 1 after 2nd application, day 1 after 3rd application, day 2 after 4th application and day 1 after 5th application with each 10 mm.

No measurable residues (< LOQ) of zoxamide were determined in any of the soil samples of the control plots taken immediately after each application.

The calculated zoxamide concentrations in a 5 cm deep soil layer based on a soil bulk density of 1.5 g/cm³ amount to 0.187, 0.241 and 0.374 mg/kg soil dry weight for a single application at the low, middle and high test rate, respectively. The residue levels analysed in the field soil samples just after each application (sampling depth: of 0 - 5 cm) of the low test item treatment group (5 x 140 g a.s./ha) were 0.247, 0.434, 0.254, 0.424 and 0.342 mg a.s./kg soil d.w. after 1st, 2nd, 3rd, 4th and 5th application – and thus always above the nominal soil concentration of 0.187 mg/kg just after application. The residue levels analysed in the field soil samples just after each application (sampling depth: of 0 - 5 cm) of the middle test item treatment group (5 x 180 g a.s./ha) were 0.264, 0.472, 0.367, 0.564, and 0.454 mg a.s./kg soil d.w. after 1st, 2nd, 3rd, 4th and 5th application – and thus always above the nominal soil concentration of 0.241 mg/kg just after application. The residue levels analysed in the field soil samples just after each application (sampling depth: of 0 - 5 cm) of the high test item treatment group (5 x 280 g a.s./ha) were 0.403, 0.641, 0.687, 1.065 and 0.793 mg a.s./kg soil d.w. after 1st, 2nd, 3rd, 4th and 5th application – and thus always above the nominal soil concentration of 0.374 mg/kg just after application. After each test item application to the soil, zoxamide degrades with a geometric mean $DT_{50}$ value of 5.5 days (20°C, pF2; see EFSA, 2017).

Earthworm species found in the plots of the field site at pre-sampling were the endogeic species *Aporrectodea chlorotica* (2.7 % of total earthworms), *Aporrectodea caliginosa* (80.3 % of total earthworms) and *Aporrectodea rosea* (5.3 % of total earthworms) as well as the anecic species *Aporrectodea longa* (<0.1 % of total earthworms) and *Lumbricus terrestris* (11.0 % of total earthworms). The presence of the dominant species *Aporrectodea caliginosa* and *Lumbricus terrestris*, representing different ecological groups, indicated the suitability of the field site.

The mean earthworm abundance in the control plots was 337.0 ind./m² at pre-sampling, 49.5 ind./m² at 1st sampling, 89.5 ind./m² at 2nd sampling and 271.0 ind./m² at 3rd sampling.

The surface monitoring on days 1 - 3 after each application showed that there were no acute primary effects on earthworms by Zoxium 240 SC. No alive, moribund or dead earthworms were found on the soil surface, neither in the test item treated nor in the untreated control areas.

No statistically significant reductions in total earthworm abundance and biomass could be observed for the tested application rates of 5 x 140 g a.s./ha (nominal), 5 x 180 g a.s./ha (nominal) and 5 x 280 g a.s./ha (nominal) about 1, 6 and 12 months after 1st application. Furthermore, no statistically significant reductions in abundance and biomass of the different earthworm species (*Aporrectodea chlorotica*, *Aporrectodea caliginosa*, *Aporrectodea rosea* and *Lumbricus terrestris*) and ecological groups (endogeic and anecic earthworms) could be observed for the tested application rates about 1, 6 and 12 months after the 1st application.

The toxic reference item reduced the total earthworm abundance by 58.6 % at 1st sampling, 37.4 % at 2nd sampling and 29.9 % at 3rd sampling. *Lumbricus terrestris* was the most sensitive species and was reduced in total abundance by 87.5 % at 1st sampling, 100 % at 2nd sampling and 63.6 % at 3rd sampling. The total earthworm biomass was reduced by the reference item by 66.6 % at 1st sampling, 47.7 % at 2nd sampling and 41.3 % at 3rd sampling. *Lumbricus terrestris* was the most sensitive species and was reduced in total biomass by 96.2 % at 1st sampling, 100 % at 2nd sampling and 98.8 % at 3rd sampling. These results clearly indicated the effect of the toxic reference item and thus the suitability of the test system and the validity of the field study.

**Table A 87: Mean abundance of the earthworm populations**

| | **Treatment group** | **Abundance (ind./m²)** | | | |
|---|---|---|---|---|---|
| | | **pre-sampling** | **1st sampling** | **2nd sampling** | **3rd sampling** |
| **Total abundance** | Control | 337.0 | 49.5 | 89.5 | 271.0 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 363.5 | 50.0 | 121.0 | 302.0 |
| | | (107.9%) | (101.0%) | (135.2%) | (111.4%) |
| | Test item (middle rate) | 359.5 | 28.5 | 81.0 | 306.5 |
| | | (106.7%) | (57.6%) | (90.5%) | (113.1%) |
| | Test item (high rate) | 347.0 | 36.5 | 90.0 | 294.0 |
| | | (103.0%) | (73.7%) | (100.6%) | (108.5%) |
| | Reference item | 333.0 | **20.5** | **56.0** | 190.0 |
| | | (98.8%) | **(41.4%)** | **(62.6%)** | (70.1%) |
| **Total adult abundance** | Control | 155.5 | 11.0 | 16.0 | 75.0 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 150.5 | 6.5 | 17.0 | 87.0 |
| | | (96.8%) | (59.1%) | (106.3%) | (116.0%) |
| | Test item (middle rate) | 157.5 | 6.0 | 11.5 | 91.0 |
| | | (101.3%) | (54.5%) | (71.9%) | (121.3%) |
| | Test item (high rate) | 134.5 | 5.0 | 10.0 | 82.5 |
| | | (86.5%) | (45.5%) | (62.5%) | (110.0%) |
| | Reference item | 132.0 | 4.5 | 0.5 | **34.0** |
| | | (84.9%) | (40.9%) | (3.1%) | **(45.3%)** |
| **Total juvenile abundance** | Control | 170.0 | 38.5 | 73.5 | 177.5 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 201.5 | 43.0 | 104.0 | 192.5 |
| | | (118.5%) | (111.7%) | (141.5%) | (108.5%) |
| | Test item (middle rate) | 182.5 | 22.0 | 69.5 | 193.5 |
| | | (107.4%) | (57.1%) | (94.6%) | (109.0%) |
| | Test item (high rate) | 192.0 | 30.0 | 79.5 | 190.0 |
| | | (112.9%) | (77.9%) | (108.2%) | (107.0%) |
| | Reference item | 182.5 | **16.0** | 55.5 | 141.5 |

| | | | | | |
|---|---|---|---|---|---|
| | | (107.4%) | **(41.6%)** | (75.5%) | (79.7%) |
| ***Allolobophora chlorotica* (total)** | Control | 19.0 | 12.0 | 8.0 | 13.5 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 19.5 | 9.0 | 10.5 | 9.5 |
| | | (102.6%) | (75.0%) | (131.3%) | (70.4%) |
| | Test item (middle rate) | 9.0 | 1.5 | 1.0 | 5.5 |
| | | (47.4%) | (12.5%) | (12.5%) | (40.7%) |
| | Test item (high rate) | 0.0 | 0.0 | 0.0 | 0.0 |
| | | - | - | - | - |
| | Reference item | 0.0 | 0.0 | 0.0 | 0.5 |
| | | - | - | - | (3.7%) |
| ***Allolobophora chlorotica* (adults)** | Control | 16.5 | 5.0 | 0.0 | 0.0 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 14.0 | 2.5 | 0.5 | 0.0 |
| | | (84.8%) | (50.0%) | - | - |
| | Test item (middle rate) | 8.5 | 1.0 | 0.0 | 0.0 |
| | | (51.5%) | (20.0%) | - | - |
| | Test item (high rate) | 0.0 | 0.0 | 0.0 | 0.0 |
| | | - | - | - | - |
| | Reference item | 0.0 | 0.0 | 0.0 | 0.0 |
| | | - | - | - | - |
| ***Allolobophora chlorotica* (juveniles)** | Control | 2.5 | 7.0 | 8.0 | 13.5 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 5.5 | 6.5 | 10.0 | 9.5 |
| | | (220.0%) | (92.9%) | (125.0%) | (70.4%) |
| | Test item (middle rate) | 0.5 | 0.5 | 1.0 | 5.5 |
| | | (20.0%) | (7.1%) | (12.5%) | (40.7%) |
| | Test item (high rate) | 0.0 | 0.0 | 0.0 | 0.0 |
| | | - | - | - | - |
| | Reference item | 0.0 | 0.0 | 0.0 | 0.5 |
| | | - | - | - | (3.7%) |
| ***Aporrectodea caliginosa* (total)** | Control | 255.0 | 30.0 | 78.0 | 238.0 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 299.0 | 35.5 | 102.5 | 266.0 |
| | | (117.3%) | (118.3%) | (131.4%) | (111.8%) |
| | Test item (middle rate) | 279.5 | 20.5 | 74.0 | 272.5 |
| | | (109.6%) | (68.3%) | (94.9%) | (114.5%) |
| | Test item (high rate) | 282.5 | 29.0 | 87.0 | 271.5 |
| | | (110.8%) | (96.7%) | (111.5%) | (114.1%) |
| | Reference item | 280.5 | 19.0 | 55.5 | 178.0 |
| | | (110.0%) | (63.3%) | (71.2%) | (74.8%) |
| ***Aporrectodea caliginosa* (adults)** | Control | 113.5 | 4.0 | 14.5 | 64.0 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 118.5 | 2.5 | 13.5 | 76.0 |
| | | (104.4%) | (62.5%) | (93.1%) | (118.8%) |
| | Test item (middle rate) | 133.0 | 3.0 | 9.5 | 79.0 |
| | | (117.2%) | (75.0%) | (65.5%) | (123.4%) |
| | Test item | 115.0 | 3.5 | 9.0 | 78.0 |

| | | | | | |
|---|---|---|---|---|---|
| | (high rate) | (101.3%) | (87.5%) | (62.1%) | (121.9%) |
| | Reference item | 117.5 | 4.0 | 0.0 | **31.0** |
| | | (103.5%) | (100.0%) | - | **(48.4%)** |
| ***Aporrectodea caliginosa* (juveniles)** | Control | 138.0 | 26.0 | 63.5 | 155.5 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 172.5 | 32.5 | 89.0 | 170.5 |
| | | (125.0%) | (125.0%) | (140.2%) | (109.6%) |
| | Test item (middle rate) | 139.5 | 17.0 | 64.5 | 173.0 |
| | | (101.1%) | (65.4%) | (101.6%) | (111.3%) |
| | Test item (high rate) | 155.5 | 24.5 | 78.0 | 173.0 |
| | | (112.7%) | (94.2%) | (122.8%) | (111.3%) |
| | Reference item | 154.5 | 15.0 | 55.5 | 133.0 |
| | | (112.0%) | (57.7%) | (87.4%) | (85.5%) |
| ***Aporrectodea rosea* (total)** | Control | 20.5 | 2.5 | 0.5 | 13.5 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 17.0 | 3.5 | 1.0 | 15.0 |
| | | (82.9%) | (140.0%) | (200.0%) | (111.1%) |
| | Test item (middle rate) | 21.0 | 1.5 | 2.5 | 16.0 |
| | | (102.4%) | (60.0%) | (500.0%) | (118.5%) |
| | Test item (high rate) | 20.0 | 1.5 | 1.0 | 12.5 |
| | | (97.6%) | (60.0%) | (200.0%) | (92.6%) |
| | Reference item | 14.5 | 1.0 | 0.0 | 8.0 |
| | | (70.7%) | (40.0%) | - | (59.3%) |
| ***Aporrectodea rosea* (adults)** | Control | 11.5 | 0.5 | 0.0 | 9.5 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 8.5 | 1.0 | 0.0 | 8.5 |
| | | (73.9%) | (200.0%) | - | (89.5%) |
| | Test item (middle rate) | 10.5 | 0.0 | 1.0 | 7.5 |
| | | (91.3%) | - | - | (78.9%) |
| | Test item (high rate) | 9.5 | 0.0 | 0.5 | 2.5 |
| | | (82.6%) | - | - | (26.3%) |
| | Reference item | 7.0 | 0.5 | 0.0 | **3.0** |
| | | (60.9%) | (100.0%) | - | **(31.6%)** |
| ***Aporrectodea rosea* (juveniles)** | Control | 9.0 | 2.0 | 0.5 | 4.0 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 8.5 | 2.5 | 1.0 | 6.5 |
| | | (94.4%) | (125.0%) | (200.0%) | (162.5%) |
| | Test item (middle rate) | 10.5 | 1.5 | 1.5 | 8.5 |
| | | (116.7%) | (75.0%) | (300.0%) | (212.5%) |
| | Test item (high rate) | 10.5 | 1.5 | 0.5 | 10.0 |
| | | (116.7%) | (75.0%) | (100.0%) | (250.0%) |
| | Reference item | 7.5 | 0.5 | 0.0 | 4.5 |
| | | (83.3%) | (25.0%) | - | (112.5%) |
| ***Aporrectodea longa* (total)** | Control | 0.0 | 0.0 | 1.0 | 0.0 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 0.0 | 0.0 | 1.5 | 0.0 |
| | | - | - | (150.0%) | - |
| | Test item | 0.0 | 0.0 | 0.5 | 1.5 |

| | | | | | |
|---|---|---|---|---|---|
| | (middle rate) | - | - | (50.0%) | - |
| | Test item (high rate) | 0.5 | 0.0 | 0.0 | 0.5 |
| | | - | - | - | - |
| | Reference item | 0.0 | 0.0 | 0.5 | 0.0 |
| | | - | - | (50.0%) | - |
| ***Aporrectodea longa* (adults)** | Control | 0.0 | 0.0 | 1.0 | 0.0 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 0.0 | 0.0 | 0.5 | 0.0 |
| | | - | - | (50.0%) | - |
| | Test item (middle rate) | 0.0 | 0.0 | 0.5 | 1.0 |
| | | - | - | (50.0%) | - |
| | Test item (high rate) | 0.0 | 0.0 | 0.0 | 0.0 |
| | | - | - | - | - |
| | Reference item | 0.0 | 0.0 | 0.5 | 0.0 |
| | | - | - | (50.0%) | - |
| ***Aporrectodea longa* (juveniles)** | Control | 0.0 | 0.0 | 0.0 | 0.0 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 0.0 | 0.0 | 1.0 | 0.0 |
| | | - | - | - | - |
| | Test item (middle rate) | 0.0 | 0.0 | 0.0 | 0.0 |
| | | - | - | - | - |
| | Test item (high rate) | 0.5 | 0.0 | 0.0 | 0.0 |
| | | - | - | - | - |
| | Reference item | 0.0 | 0.0 | 0.0 | 0.0 |
| | | - | - | - | - |
| ***Lumbricus terrestris* (total)** | Control | 42.5 | 4.0 | 2.0 | 5.5 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 26.0 | 1.5 | 5.5 | 8.5 |
| | | (61.2%) | (37.5%) | (275.0%) | (154.5%) |
| | Test item (middle rate) | 45.5 | 4.5 | 2.5 | 11.0 |
| | | (107.1%) | (112.5%) | (125.0%) | (200.0%) |
| | Test item (high rate) | 41.0 | 5.0 | 1.5 | 8.5 |
| | | (96.5%) | (125.0%) | (75.0%) | (154.5%) |
| | Reference item | 36.0 | **0.5** | **0.0** | 2.0 |
| | | (84.7%) | **(12.5%)** | - | (36.4%) |
| ***Lumbricus terrestris* (adults)** | Control | 14.0 | 1.5 | 0.5 | 1.5 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 9.5 | 0.5 | 2.5 | 2.5 |
| | | (67.9%) | (33.3%) | (500.0%) | (166.7%) |
| | Test item (middle rate) | 5.5 | 2.0 | 0.5 | 3.5 |
| | | (39.3%) | (133.3%) | (100.0%) | (233.3%) |
| | Test item (high rate) | 10.0 | 1.5 | 0.5 | 2.0 |
| | | (71.4%) | (100.0%) | (100.0%) | (133.3%) |
| | Reference item | 7.5 | 0.0 | 0.0 | **0.0** |
| | | (53.6%) | - | - | - |
| ***Lumbricus terrestris* (juveniles)** | Control | 20.5 | 2.5 | 1.5 | 4.0 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item | 13.0 | 1.0 | 3.0 | 5.5 |

| | | | | | |
|---|---|---|---|---|---|
| | (low rate) | (63.4%) | (40.0%) | (200.0%) | (137.5%) |
| | Test item | 31.0 | 2.5 | 2.0 | 6.5 |
| | (middle rate) | (151.2%) | (100.0%) | (133.3%) | (162.5%) |
| | Test item | 22.5 | 3.5 | 1.0 | 6.5 |
| | (high rate) | (109.8%) | (140.0%) | (66.7%) | (162.5%) |
| | Reference item | 18.5 | 0.5 | **0.0** | 2.0 |
| | | (90.2%) | (20.0%) | - | (50.0%) |

pre-sampling on 03.05.2018 (1 weeks before 1st application)
1st sampling on 04.06.2019 (about 1 month after 1st application)
2nd sampling on 12.11.2018 (about 6 months after 1st application)
3rd sampling on 08.05.2019 (about 12 months after 1st application)
Statistic: comparisons of test item treatments *vs.* control and reference *vs*. control: one-sided t-test
**Bold values** indicate statistically significant differences to control ($p \leq 0.05$)
In brackets: the percentages from control.

## Table A 88: Mean biomass of the total earthworm populations

| | **Treatment group** | **Biomass (g/m²)** | | | |
|---|---|---|---|---|---|
| | | **pre-sampling** | **1st sampling** | **2nd sampling** | **3rd sampling** |
| **Total bio-mass** | Control | 239.39 | 14.60 | 44.12 | 140.34 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item | 244.83 | 8.45 | 67.96 | 161.94 |
| | (low rate) | (102.3%) | (57.9%) | (154.0%) | (115.4%) |
| | Test item | 244.29 | 14.67 | 41.37 | 178.45 |
| | (middle rate) | (102.0%) | (100.5%) | (93.8%) | (127.2%) |
| | Test item | 255.88 | 12.00 | 45.29 | 168.66 |
| | (high rate) | (106.9%) | (82.2%) | (102.6%) | (120.2%) |
| | Reference item | 224.51 | **4.87** | **23.07** | **82.37** |
| | | (93.8%) | **(33.4%)** | **(52.3%)** | **(58.7%)** |
| **Total adult biomass** | Control | 160.07 | 8.90 | 13.36 | 60.22 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item | 165.30 | 3.45 | 19.17 | 76.94 |
| | (low rate) | (103.3%) | (38.7%) | (143.5%) | (127.8%) |
| | Test item | 162.14 | 9.06 | 9.09 | 88.36 |
| | (middle rate) | (101.3%) | (101.8%) | (68.0%) | (146.7%) |
| | Test item | 156.97 | 6.33 | 9.28 | 75.58 |
| | (high rate) | (98.1%) | (71.1%) | (69.5%) | (125.5%) |
| | Reference item | 144.63 | 1.84 | 0.54 | **25.34** |
| | | (90.4%) | (20.7%) | (4.0%) | **(42.1%)** |
| **Total juvenile biomass** | Control | 76.55 | 5.69 | 30.77 | 75.78 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item | 76.27 | 4.90 | 48.79 | 81.39 |
| | (low rate) | (99.6%) | (86.0%) | (158.6%) | (107.4%) |
| | Test item | 75.66 | 5.55 | 32.28 | 83.61 |
| | (middle rate) | (98.8%) | (97.5%) | (104.9%) | (110.3%) |
| | Test item | 91.95 | 5.38 | 35.96 | 87.70 |
| | (high rate) | (120.1%) | (94.5%) | (116.9%) | (115.7%) |
| | Reference item | 75.56 | 3.03 | 22.53 | 54.54 |
| | | (98.7%) | (53.2%) | (73.2%) | (72.0%) |
| ***Allolobophora chlorotica*** | Control | 7.06 | 2.44 | 1.73 | 2.59 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |

| | | | | | |
|---|---|---|---|---|---|
| **(total)** | Test item (low rate) | 6.43 | 1.63 | 2.43 | 2.34 |
| | | (91.0%) | (66.7%) | (140.6%) | (90.3%) |
| | Test item (middle rate) | 3.86 | 0.39 | 0.19 | 1.30 |
| | | (54.6%) | (15.9%) | (11.0%) | (50.0%) |
| | Test item (high rate) | 0.00 | 0.00 | 0.00 | 0.00 |
| | | - | - | - | - |
| | Reference item | 0.00 | 0.00 | 0.00 | 0.06 |
| | | - | - | - | (2.2%) |
| ***Allolobophora chlorotica* (adults)** | Control | 6.43 | 1.37 | 0.00 | 0.00 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 5.23 | 0.68 | 0.12 | 0.00 |
| | | (81.3%) | (49.6%) | - | - |
| | Test item (middle rate) | 3.75 | 0.34 | 0.00 | 0.00 |
| | | (58.3%) | (24.5%) | - | - |
| | Test item (high rate) | 0.00 | 0.00 | 0.00 | 0.00 |
| | | - | - | - | - |
| | Reference item | 0.00 | 0.00 | 0.00 | 0.00 |
| | | - | - | - | - |
| ***Allolobophora chlorotica* (juveniles)** | Control | 0.64 | 1.07 | 1.73 | 2.59 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 1.20 | 0.95 | 2.31 | 2.34 |
| | | (188.9%) | (88.6%) | (133.5%) | (90.3%) |
| | Test item (middle rate) | 0.11 | 0.05 | 0.19 | 1.30 |
| | | (16.6%) | (5.0%) | (11.0%) | (50.0%) |
| | Test item (high rate) | 0.00 | 0.00 | 0.00 | 0.00 |
| | | - | - | - | - |
| | Reference item | 0.00 | 0.00 | 0.00 | 0.06 |
| | | - | - | - | (2.2%) |
| ***Aporrectodea caliginosa* (total)** | Control | 167.34 | 4.43 | 36.74 | 125.03 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 185.67 | 4.61 | 53.20 | 145.46 |
| | | (111.0%) | (104.1%) | (144.8%) | (116.3%) |
| | Test item (middle rate) | 182.67 | 2.93 | 36.62 | 152.67 |
| | | (109.2%) | (66.1%) | (99.7%) | (122.1%) |
| | Test item (high rate) | 188.76 | 3.54 | 43.29 | 153.86 |
| | | (112.8%) | (79.9%) | (117.8%) | (123.1%) |
| | Reference item | 186.11 | 4.52 | 22.53 | **80.25** |
| | | (111.2%) | (102.0%) | (61.3%) | **(64.2%)** |
| ***Aporrectodea caliginosa* (adults)** | Control | 111.08 | 2.26 | 10.19 | 51.38 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 115.41 | 1.09 | 10.58 | 65.70 |
| | | (103.9%) | (48.1%) | (103.8%) | (127.9%) |
| | Test item (middle rate) | 126.83 | 1.13 | 6.63 | 70.09 |
| | | (114.2%) | (50.2%) | (65.0%) | (136.4%) |
| | Test item (high rate) | 113.87 | 1.37 | 8.10 | 65.61 |
| | | (102.5%) | (60.9%) | (79.5%) | (127.7%) |
| | Reference item | 116.58 | 1.78 | 0.00 | **24.40** |
| | | (105.0%) | (78.9%) | - | **(47.5%)** |

| | | | | | |
|---|---|---|---|---|---|
| ***Aporrectodea caliginosa* (juveniles)** | Control | 55.47 | 2.17 | 26.55 | 69.31 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 67.42 | 3.41 | 42.62 | 76.41 |
| | | (121.5%) | (157.4%) | (160.5%) | (110.2%) |
| | Test item (middle rate) | 54.10 | 1.74 | 29.99 | 77.35 |
| | | (97.5%) | (80.1%) | (113.0%) | (111.6%) |
| | Test item (high rate) | 71.23 | 1.92 | 35.19 | 83.44 |
| | | (128.4%) | (88.8%) | (132.6%) | (120.4%) |
| | Reference item | 67.33 | 2.73 | 22.53 | 53.42 |
| | | (121.4%) | (126.1%) | (84.9%) | (77.1%) |
| ***Aporrectodea rosea* (total)** | Control | 4.62 | 0.22 | 0.06 | 2.99 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 3.83 | 0.74 | 0.05 | 3.10 |
| | | (82.9%) | (340.7%) | (84.4%) | (103.8%) |
| | Test item (middle rate) | 4.79 | 0.05 | 0.36 | 3.45 |
| | | (103.7%) | (22.7%) | (591.8%) | (115.5%) |
| | Test item (high rate) | 4.10 | 0.05 | 0.15 | 2.11 |
| | | (88.7%) | (21.1%) | (245.9%) | (70.6%) |
| | Reference item | 3.23 | 0.07 | 0.00 | 1.91 |
| | | (69.9%) | (33.9%) | - | (64.0%) |
| ***Aporrectodea rosea* (adults)** | Control | 3.52 | 0.11 | 0.00 | 2.41 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 2.81 | 0.38 | 0.00 | 2.17 |
| | | (79.9%) | (329.3%) | - | (89.8%) |
| | Test item (middle rate) | 3.73 | 0.00 | 0.25 | 2.24 |
| | | (106.0%) | - | - | (92.9%) |
| | Test item (high rate) | 2.89 | 0.00 | 0.10 | 0.77 |
| | | (82.2%) | - | - | (32.1%) |
| | Reference item | 2.30 | 0.06 | 0.00 | 0.94 |
| | | (65.4%) | (52.4%) | - | (38.8%) |
| ***Aporrectodea rosea* (juveniles)** | Control | 1.10 | 0.10 | 0.06 | 0.57 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 1.02 | 0.37 | 0.05 | 0.94 |
| | | (92.4%) | (353.4%) | (84.4%) | (162.9%) |
| | Test item (middle rate) | 1.07 | 0.05 | 0.11 | 1.21 |
| | | (96.6%) | (47.6%) | (182.8%) | (210.6%) |
| | Test item (high rate) | 1.21 | 0.05 | 0.05 | 1.33 |
| | | (109.4%) | (44.2%) | (82.0%) | (232.1%) |
| | Reference item | 0.93 | 0.01 | 0.00 | 0.91 |
| | | (84.1%) | (13.5%) | - | (159.1%) |
| ***Aporrectodea longa* (total)** | Control | 0.00 | 0.00 | 2.18 | 0.00 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 0.00 | 0.00 | 2.16 | 0.00 |
| | | - | - | (99.0%) | - |
| | Test item (middle rate) | 0.00 | 0.00 | 0.97 | 3.07 |
| | | - | - | (44.6%) | - |
| | Test item (high rate) | 0.84 | 0.00 | 0.00 | 0.53 |
| | | - | - | - | - |

| | | | | | |
|---|---|---|---|---|---|
| | Reference item | 0.00 | 0.00 | 0.54 | 0.00 |
| | | - | - | (24.7%) | - |
| ***Aporrectodea longa* (adults)** | Control | 0.00 | 0.00 | 2.18 | 0.00 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 0.00 | 0.00 | 1.41 | 0.00 |
| | | - | - | (64.6%) | - |
| | Test item (middle rate) | 0.00 | 0.00 | 0.97 | 2.69 |
| | | - | - | (44.6%) | - |
| | Test item (high rate) | 0.00 | 0.00 | 0.00 | 0.00 |
| | | - | - | - | - |
| | Reference item | 0.00 | 0.00 | 0.54 | 0.00 |
| | | - | - | (24.7%) | - |
| ***Aporrectodea longa* (juveniles)** | Control | 0.00 | 0.00 | 0.00 | 0.00 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 0.00 | 0.00 | 0.75 | 0.00 |
| | | - | - | - | - |
| | Test item (middle rate) | 0.00 | 0.00 | 0.00 | 0.00 |
| | | - | - | - | - |
| | Test item (high rate) | 0.84 | 0.00 | 0.00 | 0.00 |
| | | - | - | - | - |
| | Reference item | 0.00 | 0.00 | 0.00 | 0.00 |
| | | - | - | - | - |
| ***Lumbricus terrestris* (total)** | Control | 60.36 | 7.47 | 3.41 | 9.71 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 48.80 | 1.46 | 10.12 | 10.85 |
| | | (80.9%) | (19.5%) | (296.5%) | (111.7%) |
| | Test item (middle rate) | 52.46 | 11.30 | 3.21 | 17.97 |
| | | (86.9%) | (151.3%) | (94.1%) | (185.1%) |
| | Test item (high rate) | 61.75 | 8.35 | 1.80 | 12.13 |
| | | (102.3%) | (111.8%) | (52.7%) | (124.9%) |
| | Reference item | 35.06 | **0.28** | **0.00** | **0.12** |
| | | (58.1%) | **(3.8%)** | **-** | **(1.2%)** |
| ***Lumbricus terrestris* (adults)** | Control | 39.05 | 5.16 | 0.98 | 6.43 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 41.85 | 1.30 | 7.06 | 9.08 |
| | | (107.2%) | (25.3%) | (717.3%) | (141.2%) |
| | Test item (middle rate) | 27.83 | 7.59 | 1.24 | 13.35 |
| | | (71.3%) | (147.2%) | (125.7%) | (207.6%) |
| | Test item (high rate) | 40.21 | 4.95 | 1.09 | 9.20 |
| | | (103.0%) | (96.1%) | (110.4%) | (143.2%) |
| | Reference item | 25.74 | 0.00 | 0.00 | **0.00** |
| | | (65.9%) | - | - | - |
| ***Lumbricus terrestris* (juveniles)** | Control | 19.34 | 2.31 | 2.43 | 3.28 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 6.52 | 0.15 | 3.06 | 1.69 |
| | | (33.7%) | (6.6%) | (125.9%) | (51.4%) |
| | Test item (middle rate) | 20.36 | 3.71 | 1.97 | 3.75 |
| | | (105.2%) | (160.3%) | (81.2%) | (114.3%) |

| | | | | | |
|---|---|---|---|---|---|
| | Test item (high rate) | 18.29 | 3.40 | 0.71 | 2.93 |
| | | (94.5%) | (146.9%) | (29.3%) | (89.2%) |
| | Reference item | 7.18 | 0.28 | **0.00** | 0.12 |
| | | (37.1%) | (12.1%) | - | (3.7%) |

pre-sampling on 03.05.2018 (1 weeks before 1st application)
1st sampling on 04.06.2019 (about 1 month after 1st application)
2nd sampling on 12.11.2018 (about 6 months after 1st application)
3rd sampling on 08.05.2019 (about 12 months after 1st application)
Statistic: comparisons of test item treatments *vs.* control and reference *vs.* control: one-sided t-test
**Bold values** indicate statistically significant differences to control ($p \leq 0.05$).
In brackets: the percentages from control.

All validity criteria were met:

- The mean abundance of earthworms of the test field at trial start was 348 ind./m², thus fulfilling the guideline recommendation (60 ind./m² for arable soils).
- At least one representative of endogeic and anecic earthworms was present at the field site in a sufficient number (>10 % of total earthworms or 10 - 15 ind./m²), with abundances of 279.3 ind./m² for *Aporrectodea caliginosa* (endogeic) and 38.2 ind./m² for *Lumbricus terrestris* (anecic; pre-sampling values).
- In the reference item treatment group total earthworm abundance and biomass were reduced by 58.6 % and 66.6 % at 1st sampling (about 1 month after 1st application), respectively, fulfilling the guideline recommendation (reduction of the earthworm abundance and / or biomass of > 50 % compared to the control).

## Conclusion

Zoxium 240 SC (containing 240 g/L zoxamide) tested at application rates of 5 x 140 g a.s./ha (nominal), 5 x 180 g a.s./ha (nominal) and 5 x 280 g a.s./ha had no adverse effects on single species, ecological groups and total earthworm abundance and biomass about one year after application.

The study meets all validity criteria required by the available guidance for earthworm field studies (ISO 11268-3, 1999; KULA et al., 2006).

(Schulz L. 2020)

### A 2.4.1.2.2 Study 2 - CYMOXANIL 33% + ZOXAMIDE 33% WG: Effects on earthworms under field conditions

| | |
|---|---|
| Comments of zRMS: | In general, the study was well design. All validity criteria for earthworm field studies required by ISO 11268-3;2014 and Kula et al 2006 were met. Thus, the study is valid. Nevertheless, it should be highlighted that earthworm communities was very poor. *L. terrestris* was the only anecic species present on the test field. Therefore, data for *L. terrestris* data corresponds to data for anecic earthworms. *A. caliginosa* and *A. rosea* were the two endogeic species present on the test field.<br><br>Due to low abundance no statistically analyses were carried out for *A. rosea* which comprised 0.5 %, 0.8 %, 0.5 % and 1.3 % of the total abundance in the control at the pre-, 1st, 2nd and 3rd sampling, respectively. Therefore, results of this test concern the effects on only two species of earthworms. For this reason, in zRMS opinion, the study should be use only as supplementary data.<br><br>In conclusion, Cymoxanil 33% + Zoxamide 33% at application rates of 4 x 0.45 kg form./ha and 5 x 0.9 kg form./ha had no adverse effects on population of *L. terrestris* and *A. caliginosa*. |

| | |
|---|---|
| **Reference:** | **KCP 10.4.1.2/02** |
| Report | Schulz, L., 2020: Effects of Cymoxanil 33% + Zoxamide 33% on earthworms under field conditions<br>Gowan Crop Protection Ltd., UK, SIPCAM OXON S.p.A., Italy<br>BioChem Agrar, Germany, Report No.19 48 FEW 0003, GLP, Not published |
| Guideline(s): | ISO 11268-3 (2014)<br>Technical Recommendations to ISO 11268-3 (KULA *et al*. 2006) |
| Deviations: | 2nd application on 03.05.2019: Due to unfavourable weather conditions, the 2nd application was conducted 9 days after 1st application sampling instead of 7 ± 1 day after 1st application. There was no impact on the outcome of the study. |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | No |

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | Cymoxanil 33% + Zoxamide 33% WG (GSOL7019) |
| **Active substance content or purity** | Zoxamide : 33% (nominal), 32.75 ± 0.15% w/w (analysed)<br>isomeric ratio of R-/S-zoxamide = 50.15 / 49.85<br>Cymoxanil : 33% (nominal), 32.91 ± 0.17% w/w (analysed) |
| **Test organisms** | Naturally occurring earthworm (Annelida: Oligochaeta) population with an abundance greater 60 ind./m² and two dominant species representing different life forms (*Aporrectodea caliginosa* and *Lumbricus terrestris*) present at 10 % or at least 10 to 15 ind./m², with a relative homogenous distribution on a typical arable field. Climatic and soil conditions within the test area (e.g. altitude, slope, drainage system, and wind exposure) sufficiently homogeneous. |
| **Test system** | 16 plots, each 10 m x 10 m, arranged in a randomised block design.<br>The test item applications were performed on bare soil.<br>About 2 weeks after the last application, the test field was seeded with the fodder crop “Landsberger Gemenge” (clover grass mixture) which stayed on the field until the end of the study. |
| Field: | Typical arable field near Dornreichenbach in Saxony, Germany.<br>Soil textural class: sandy-loamy silt (DIN 4220) / silt loam (USDA), mean pH (CaCl2) 6.3, mean total organic carbon content 1.76 % and mean maximum water holding capacity 54.9 g/100 g soil dry weight |
| Number of replicates: | Earthworms were sampled from four 0.125 m² sampling areas per plot per sampling occasion. |
| Surface monitoring: | Assessment of alive, moribund and dead earthworms on the soil surface in a monitoring area of 20.0 m² per plot in all control and test item replicates from day 1 to day 3 after each application. |
| Earthworm sampling: | Hand sorting combined with formalin extraction. |

| | |
|---|---|
| **Environmental conditions** | |
| Air temperature during application: | 7.3 – 15.9 °C |
| Soil moisture during applications: | 18.36 – 21.21% (w/w) |
| **Application rate(s)** | 2 applications at each:<br>1) 5 x 0.45 kg test item/ha<br>2) 5 x 0.90 kg test item/ha |
| Reference item: | 20 L Maypon Flow/ha in 600 L water/ha (carbendazim 50 % w/v, nominal) |
| **Post exposure observation period** | pre-sampling on 08.04.2019 (about 2 weeks before 1st application)<br>1st sampling on 03.06.2019 (about 1 month after 1st application)<br>2nd sampling on 25.10.2019 (about 6 months after 1st application)<br>3rd sampling on 09.04.2020 (about 12 months after 1st application) |
| **Remarks** | None |

Potential effects and potential recovery of field populations of earthworms after the spray application of the test item Cymoxanil 33% + Zoxamide 33% WG (containing nominally 33 % w/w cymoxanil, nominally 33 % w/w zoxamide) was applied five times at application rates of 0.45 kg test item/ha (low rate) and 0.90 kg test item/ha (high rate) corresponding to 148.5 g cymoxanil/ha + 148.5 g zoxamide/ha (low rate) and 297 g cymoxanil/ha + 297 g zoxamide/ha (low rate). The results with regard to earthworm species composition, biomass and abundance were compared to an untreated control and a reference item (Maypon Flow, containing nominally 50 % w/v carbendazim) applied at a rate of 20 L/ha, corresponding to 10 kg a.s./ha. The control plots were left untreated. The correct test item application has been verified by analysis of soil samples taken immediately after applications with a method validated according to SANCO/3029/99 rev. 4 (11/07/2000).

The trial was placed on arable land near Dornreichenbach in Saxony/Germany.

Sixteen plots, each 10 m x 10 m, were arranged in a 4 x 4 formation, each plot surrounded by a 2 m wide path, between the plots. The set-up was a randomised block design. The assignment of the treatment groups to the plots was based on the results of a pre-sampling. The pre-sampling was conducted to determine the density, diversity and homogeneity of earthworm populations at the site. Defined areas were sampled to assess earthworm populations before application and three times after the application, i.e. about 1, 6 and 12 months after 1st application. Earthworms were sampled from four 0.125 m² sampling areas per plot per sampling occasion by combining hand sorting with formalin extraction in the excavated hole.

Environmental conditions (air temperature at a height of 2 m height, soil temperature at 10 cm depth, and daily rainfall) were assessed during the test item applications. Furthermore, soil moisture, soil temperature, vegetation coverage (in % covered soil surface) and vegetation height were evaluated during each sampling occasion.

Statistical analysis was carried out with the statistical software package ToxRat Professional 3.2.1 (2015) and ToxRat Professional 3.3.0 (2018). Data were analysed for normal distribution with the Shapiro-Wilk's-test or Kolmogorov-Smirnoff-test and for homogeneity in variance with the Levene's test. Afterwards, pre-sampling data were analysed with a two-factorial analysis of variance (ANOVA) with treatment as fixed factor and block as random factor. Post-treatment sampling data were analysed for monotone dose-response using trend analysis by contrasts. Afterwards, the data were analysed by a one-sided Williams-t-test, Dunnett's-t-test or Welch-t-test after Bonferroni-Holm with test item treatment group < control as well as Student-t-test or Welch-t-test with reference item treatment group < control at the 5 % significance level. Test item and reference item effects were analysed in separate analyses.

## Results and discussion

The test item has been applied on days with low wind speed and no rain during and after application. Due to unfavourable weather conditions, the 2nd application was conducted 9 days after 1st application sampling instead of 7 ± 1 day after 1st application.

No measurable residues (< LOD) of cymoxanil and zoxamide were determined in any of the soil samples of the control plots taken immediately after each application.

The mean recoveries of cymoxanil and zoxamide in the soil samples from the treated plots of the two test item treatment groups, taken immediately after each application, were 102 % (after application of 0.45 L test item/ha) and 84 % (after application of 0.90 L test item/ha). The mean recoveries of cymoxanil in the soil samples from the treated plots of the two test item treatment groups, taken immediately after 1st application, were 101 % (after application of 0.45 L test item/ha) and 79 % (after application of 0.90 L test item/ha).

The calculated zoxamide concentrations in a 5 cm deep soil layer based on a soil bulk density of 1.5 g/cm³ amount to 0.197 and 0.395 mg/kg soil dry weight for a single application at the low and the high-test rate, respectively. The residue levels of zoxamide analysed in the field soil samples just after each application (sampling depth: of 0 - 5 cm) of the low-test item treatment group (5 x 0.45 kg test item/ha) were 0.202, 0.394, 0.352, 0.854 and 0.863 mg a.s./kg soil d.w. after 1st, 2nd, 3rd, 4th and 5th application – and thus always above the nominal soil concentration of 0.197 mg/kg just after application. The residue levels of zoxamide analysed in the field soil samples just after each application (sampling depth: of 0 - 5 cm) of the high-test item treatment group (5 x 0.90 kg test item/ha) were 0.332, 0.572, 0.911, 0.667, and 0.499 mg a.s./kg soil d.w. after 1st, 2nd, 3rd, 4th and 5th application – and thus always above the nominal soil concentration of 0.395 mg/kg just after application.

The calculated cymoxanil concentrations in a 5 cm deep soil layer based on a soil bulk density of 1.5 g/cm³ amount to 0.197 and 0.393 mg/kg soil dry weight for a single application at the low and the high-test rate, respectively. The residue levels of cymoxanil analysed in the field soil samples just after each application (sampling depth: of 0 - 5 cm) of the low-test item treatment group (5 x 0.45 kg test item/ha) were 0.198, 0.196, 0.120, 0.241 and 0.226 mg a.s./kg soil d.w. after 1st, 2nd, 3rd, 4th and 5th application. The residue levels of cymoxanil analysed in the field soil samples just after each application (sampling depth: of 0 - 5 cm) of the high-test item treatment group (5 x 0.90 kg test item/ha) were 0.310, 0.273, 0.294, 0.107 and 0.109 mg a.s./kg soil d.w. after 1st, 2nd, 3rd, 4th and 5th application.

Since the average residue levels of the active substance in the soil samples taken immediately after each application were within the recommended range of 50 % to 150 % of nominal values, the correct applications of the test item were verified.

The isomer ratio of (R)- and (S)-Zoxamide was determined in selected samples to confirm the chiral stability of Zoxamide (racemate).

The mean earthworm abundance in the control plots was 95.5 ind./m² at pre-sampling, 61.5 ind./m² at 1st sampling, 109.5 ind./m2 at 2nd sampling and 115.0 ind./m² at 3rd sampling.

Earthworm species found in the plots of the field site at pre-sampling were the endogeic species *Aporrectodea caliginosa* (80.8 % of total earthworms) and *Aporrectodea rosea* (1.5 % of total earthworms) as well as the anecic species *Lumbricus terrestris* (17.5 % of total earthworms). The presence of the dominant species *Aporrectodea caliginosa* and *Lumbricus terrestris*, representing different ecological groups, indicated the suitability of the field site.

The surface monitoring on days 1 - 3 after each application showed that there were no acute primary effects on earthworms by Cymoxanil 33% + Zoxamide 33% WG. No alive, moribund or dead earthworms were found on the soil surface neither in the test item nor in the control monitoring areas.

No statistically significant reductions in total earthworm abundance and biomass could be observed for the tested application rates of 5 x 0.45 kg test item/ha and 5 x 0.90 kg test item/ha about 1, 6 and 12 months after 1st application. Furthermore, no statistically significant reductions in abundance and biomass of the different earthworm species (Aporrectodea caliginosa and Lumbricus terrestris) and ecological groups (endogeic and anecic earthworms) could be observed for the tested application rates about 1, 6 and 12 months after 1st application.

The toxic reference item reduced the total earthworm abundance by 65.9 % at 1st sampling, 47.0 % at 2nd sampling and 37.8 % at 3rd sampling. Lumbricus terrestris was the most sensitive species and was reduced in total abundance by 46.7 %, 61.2 % and 72.1 % on these sampling dates. The total earthworm biomass was reduced by the reference item by 64.6 % at 1st sampling, 31.5 % at 2nd sampling and 17.9 % at 3rd sampling. Aporrectodea caliginosa was the most sensitive species and was reduced in total biomass by 80.1 % at 1st sampling and 32.5 % at 2nd sampling. These results clearly indicated the effect of the toxic reference item and thus the validity of the field study.

**Table A 89: Mean abundance of the earthworm populations**

| | | Abundance (ind./m²) | | | |
|---|---|---|---|---|---|
| | **Treatment Group** | **pre-sampling** | **1st sampling** | **2nd sampling** | **3rd sampling** |
| Total abundance | Control | 95.5 | 61.5 | 109.5 | 115.0 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 98.0 | 51.5 | 97.0 | 133.0 |
| | | (102.6%) | (83.7%) | (88.6%) | (115.7%) |
| | Test item (high rate) | 99.5 | 54.5 | 110.0 | 147.5 |
| | | (104.2%) | (88.6%) | (100.5%) | (128.3%) |
| | Reference item | 98.0 | **21.0** | **58.0** | 71.5 |
| | | (102.6%) | **(34.1%)** | **(53.0%)** | (62.2%) |
| Total adult abundance | Control | 37.0 | 32.0 | 56.5 | 37.5 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 40.5 | 27.5 | 46.5 | 59.0 |
| | | (109.5%) | (85.9%) | (82.3%) | (157.3%) |
| | Test item (high rate) | 47.0 | 28.0 | 68.5 | 49.5 |
| | | (127.0%) | (87.5%) | (121.2%) | (132.0%) |
| | Reference item | 47.0 | **8.0** | **36.0** | 38.5 |
| | | (127.0%) | **(25.0%)** | **(63.7%)** | (102.7%) |
| Total juvenile abundance | Control | 46.0 | 27.0 | 51.0 | 77.0 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 47.0 | 23.5 | 47.5 | 73.0 |
| | | (102.2%) | (87.0%) | (93.1%) | (94.8%) |
| | Test item (high rate) | 38.0 | 26.0 | 39.5 | 98.0 |
| | | (82.6%) | (96.3%) | (77.5%) | (127.3%) |
| | Reference item | 43.0 | **13.0** | **19.0** | **32.5** |
| | | (93.5%) | **(48.1%)** | **(37.3%)** | **(42.2%)** |
| *Aporrectodea caliginosa* (total) | Control | 77.0 | 53.5 | 84.5 | 79.5 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 80.5 | 36.0 | 82.5 | 101.0 |
| | | (104.5%) | (67.3%) | (97.6%) | (127.0%) |
| | Test item (high rate) | 84.0 | 43.0 | 82.5 | 107.0 |
| | | (109.1%) | (80.4%) | (97.6%) | (134.6%) |
| | Reference item | 74.5 | **17.0** | **48.5** | 61.5 |

| | | | | | |
|---|---|---|---|---|---|
| | | (96.8%) | **(31.8%)** | **(57.4%)** | (77.4%) |
| *Aporrectodea caliginosa* (adults) | Control | 33.5 | 30.0 | 51.5 | 28.5 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 36.0 | 21.5 | 42.0 | 51.0 |
| | | (107.5%) | (71.7%) | (81.6%) | (178.9%) |
| | Test item (high rate) | 42.5 | 24.5 | 62.5 | 39.0 |
| | | (126.9%) | (81.7%) | (121.4%) | (136.8%) |
| | Reference item | 36.0 | **6.0** | **30.5** | 33.5 |
| | | (107.5%) | **(20.0%)** | **(59.2%)** | (117.5%) |
| *Aporrectodea caliginosa* (juveniles) | Control | 31.5 | 21.0 | 31.0 | 50.5 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 34.0 | 14.5 | 37.5 | 49.0 |
| | | (107.9%) | (69.0%) | (121.0%) | (97.0%) |
| | Test item (high rate) | 27.5 | 18.5 | 18.0 | 68.0 |
| | | (87.3%) | (88.1%) | (58.1%) | (134.7%) |
| | Reference item | 31.5 | **11.0** | **15.0** | 27.5 |
| | | (100.0%) | **(52.4%)** | **(48.4%)** | (54.5%) |
| *Lumbricus terrestris* (total) | Control | 17.5 | 7.5 | 24.5 | 34.0 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 16.5 | 14.5 | 14.5 | 28.0 |
| | | (94.3%) | (193.3%) | (59.2%) | (82.4%) |
| | Test item (high rate) | 14.0 | 11.5 | 26.5 | 36.5 |
| | | (80.0%) | (153.3%) | (108.2%) | (107.4%) |
| | Reference item | 20.5 | 4.0 | **9.5** | **9.5** |
| | | (117.1%) | (53.3%) | **(38.8%)** | **(27.9%)** |
| *Lumbricus terrestris* (adults) | Control | 3.5 | 2.0 | 5.0 | 7.5 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 3.5 | 5.5 | 4.5 | 6.5 |
| | | (100.0%) | (275.0%) | (90.0%) | (86.7%) |
| | Test item (high rate) | 3.5 | 3.5 | 6.0 | 8.0 |
| | | (100.0%) | (175.0%) | (120.0%) | (106.7%) |
| | Reference item | 9.0 | 2.0 | 5.5 | 4.5 |
| | | (257.1%) | (100.0%) | (110.0%) | (60.0%) |
| *Lumbricus terrestris (*juveniles) | Control | 14.0 | 5.5 | 19.5 | 26.5 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 13.0 | 8.5 | 10.0 | 21.5 |
| | | (92.9%) | (154.5%) | (51.3%) | (81.1%) |
| | Test item (high rate) | 10.0 | 7.5 | 20.5 | 28.5 |
| | | (71.4%) | (136.4%) | (105.1%) | (107.5%) |
| | Reference item | 10.5 | 2.0 | **4.0** | **5.0** |
| | | (75.0%) | (36.4%) | **(20.5%)** | **(18.9%)** |

pre-sampling on 08.04.2019 (about 2 weeks before 1st application)
1st sampling on 03.06.2019 (about 1 month after 1st application)
2nd sampling on 25.10.2019 (about 6 months after 1st application)
3rd sampling on 09.04.2020 (about 12 months after 1st application)
Statistic: comparisons of test item treatments *vs.* control and reference *vs*. control: one-sided t-test
**Bold values** indicate statistically significant differences to control ($p \leq 0.05$)
in brackets: the percentages from control

**Table A 90: Mean biomass of the total earthworm populations**

| | | Biomass (g/m²) | | | |
|---|---|---|---|---|---|
| | **Treatment Group** | **pre-sampling** | **1st sampling** | **2nd sampling** | **3rd sampling** |
| Total bio-mass | Control | 98.54 | 61.85 | 112.83 | 110.33 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 102.87 | 69.88 | 107.67 | 141.53 |
| | | (104.4%) | (113.0%) | (95.4%) | (128.3%) |
| | Test item (high rate) | 113.79 | 62.63 | 132.79 | 143.61 |
| | | (115.5%) | (101.3%) | (117.7%) | (130.2%) |
| | Reference item | 133.13 | **21.91** | **77.30** | 90.61 |
| | | (135.1%) | **(35.4%)** | **(68.5%)** | (82.1%) |
| Total adult biomass | Control | 59.45 | 48.34 | 87.81 | 72.84 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 63.32 | 55.38 | 80.98 | 104.90 |
| | | (106.5%) | (114.6%) | (92.2%) | (144.0%) |
| | Test item (high rate) | 71.49 | 46.33 | 115.98 | 95.00 |
| | | (120.2%) | (95.8%) | (132.1%) | (130.4%) |
| | Reference item | 91.94 | **14.79** | 67.62 | 73.80 |
| | | (154.7%) | **(30.6%)** | (77.0%) | (101.3%) |
| Total juve-nile biomass | Control | 34.17 | 12.14 | 24.14 | 37.38 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 36.92 | 13.68 | 25.82 | 36.46 |
| | | (108.1%) | (112.7%) | (106.9%) | (97.5%) |
| | Test item (high rate) | 35.63 | 15.81 | 15.53 | 48.60 |
| | | (104.3%) | (130.2%) | (64.3%) | (130.0%) |
| | Reference item | 37.84 | 7.12 | **8.68** | **16.60** |
| | | (110.7%) | (58.7%) | **(36.0%)** | **(44.4%)** |
| *Aporrectodea caliginosa* (total) | Control | 63.48 | 50.46 | 72.96 | 38.41 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 65.34 | 33.23 | 73.26 | 74.81 |
| | | (102.9%) | (65.9%) | (100.4%) | (194.8%) |
| | Test item (high rate) | 80.26 | 33.24 | 89.66 | 61.08 |
| | | (126.4%) | (65.9%) | (122.9%) | (159.0%) |
| | Reference item | 65.79 | **10.06** | 49.23 | 51.41 |
| | | (103.6%) | **(19.9%)** | (67.5%) | (133.9%) |
| *Aporrectodea caliginosa* (adults) | Control | 40.60 | 40.25 | 63.29 | 28.25 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 43.88 | 27.01 | 58.08 | 62.12 |
| | | (108.1%) | (67.1%) | (91.8%) | (219.9%) |
| | Test item (high rate) | 56.01 | 25.84 | 83.38 | 44.30 |
| | | (137.9%) | (64.2%) | (131.7%) | (156.8%) |
| | Reference item | 46.23 | **4.72** | **42.74** | 42.11 |
| | | (113.9%) | **(11.7%)** | **(67.5%)** | (149.1%) |
| *Aporrectodea caliginosa* (juveniles) | Control | 18.08 | 8.84 | 8.80 | 10.05 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 18.84 | 6.22 | 14.32 | 12.52 |
| | | (104.2%) | (70.4%) | (162.7%) | (124.6%) |

| | | | | | |
|---|---|---|---|---|---|
| | Test item (high rate) | 18.36 | 7.40 | 5.00 | 16.78 |
| | | (101.5%) | (83.8%) | (56.8%) | (167.0%) |
| | Reference item | 16.58 | 5.34 | 5.49 | 9.09 |
| | | (91.7%) | (60.4%) | (62.4%) | (90.5%) |
| *Lumbricus terrestris* (total) | Control | 34.83 | 11.29 | 39.77 | 71.43 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 37.22 | 36.42 | 34.41 | 65.93 |
| | | (106.9%) | (322.7%) | (86.5%) | (92.3%) |
| | Test item (high rate) | 33.21 | 29.39 | 42.94 | 81.51 |
| | | (95.3%) | (260.4%) | (108.0%) | (114.1%) |
| | Reference item | 66.62 | 11.86 | 28.07 | **38.91** |
| | | (191.3%) | (105.0%) | (70.6%) | **(54.5%)** |
| *Lumbricus terrestris* (adults) | Control | 18.85 | 8.09 | 24.52 | 44.10 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 19.14 | 28.19 | 22.91 | 42.27 |
| | | (101.5%) | (348.3%) | (93.4%) | (95.9%) |
| | Test item (high rate) | 15.18 | 20.49 | 32.60 | 49.93 |
| | | (80.5%) | (253.2%) | (132.9%) | (113.2%) |
| | Reference item | 45.18 | 10.08 | 24.88 | 31.41 |
| | | (239.7%) | (124.5%) | (101.5%) | (71.2%) |
| *Lumbricus terrestris (*juveniles) | Control | 15.98 | 3.20 | 15.24 | 27.33 |
| | | (100.0%) | (100.0%) | (100.0%) | (100.0%) |
| | Test item (low rate) | 18.08 | 7.42 | 11.50 | 23.66 |
| | | (113.2%) | (232.1%) | (75.4%) | (86.6%) |
| | Test item (high rate) | 17.25 | 8.40 | 10.34 | 31.58 |
| | | (108.0%) | (262.9%) | (67.8%) | (115.5%) |
| | Reference item | 21.07 | 1.78 | **3.19** | **7.50** |
| | | (131.9%) | (55.7%) | **(20.9%)** | **(27.5%)** |

pre-sampling on 08.04.2019 (about 2 weeks before 1st application)
1st sampling on 03.06.2019 (about 1 month after 1st application)
2nd sampling on 25.10.2019 (about 6 months after 1st application)
3rd sampling on 09.04.2020 (about 12 months after 1st application)
Statistic: comparisons of test item treatments *vs.* control and reference *vs*. control: one-sided t-test
**Bold values** indicate statistically significant differences to control ($p \leq 0.05$).
in brackets: the percentages from control

All validity criteria were met:

- Earthworm abundance on arable land (average): ≥ 60/m² at test initiation (pre-sampling)
- Each of two dominant species representing different life forms (anecics, endogeics) present in a sufficiently high density of 10 to 15 individuals per m² or 10 % of total earthworm population
- Significant reduction of the earthworm abundance and / or biomass by the reference item: at least 50 % on
- at least one post application sampling date

## Conclusion

The current study meets all criteria required for a valid earthworm field study as requested by the available guidance for earthworm field studies (ISO 11268-3, 1999; KULA et al., 2006). It can be concluded that Cymoxanil 33% + Zoxamide 33% WG tested at application rates of 5 x 0.45 kg/h and 5 x 0.90 kg/ha (corresponding to 5 x 148.5 g cymoxanil/ha + 5 x 148.5 g zoxamide/ha and 5 x 297 g cymoxanil/ha + 5 x

297 g zoxamide/ha) had no adverse effects on single species, ecological groups and total earthworm abundance and biomass about one year after application.

(Schulz L. 2020)

## A 2.4.2 KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

### A 2.4.2.1.1 Study 1 – Zoxamide tech. / Zoxium 240 SC: Effects on *Folsomia*

In addition to the further zoxamide metabolite studies on soil macro-organisms (other than earthworms) requested by EFSA (2017), studies on *Folsomia* and *Hypoaspis* have been performed with the parent compound zoxamide. The zoxamide studies have been conducted with a SC formulation containing 240 g/L zoxamide, the representative formulation during AIR. The composition of Zoxium 240 SC (GWN-9790 EU) is included in Part C. The studies are regarded representative to address the potential risk from the use of zoxamide technical.

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 232 and according to the principles of GLP. The study is considered to be reliable and suitable for the risk assessment. |

| | |
|---|---|
| Reference: | **KCP 10.4.2/01** |
| Report | Parsons, Ch. 2020: Zoxium 240 SC - A laboratory test to determine the effects of fresh residues on the springtail *Folsomia candida* (Collembola, Isotomidae) in artificial soil substrate<br>Gowan Crop Protection Ltd., UK<br>Mambo-Tox, UK., Report No. GOW-17-13, GLP, Not published |
| Guideline(s): | OECD 232 (2016) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | No |

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | Zoxium 240 SC (18011201-72-52) |
| **Content of a.s.:** | 240 g/L Zoxamide (nominal), 21.7 % (w/w) (analysed) |
| **Species:** | *Folsomia candida* (Collembola, Isotomidae) |
| Age: | 11-12 days old |
| Source: | In-house culture since 2015; original source: Bias Labs Ltd., UK |
| Acclimation period: | 2 days |
| Food: | 6 mg of dried granulated yeast on day 0 and 14 during the bioassay |
| **Test system** | glass jars (approximately 125 mL capacity and 4.5 cm internal |

| | |
|---|---|
| | diameter), secured with close-fitting, screw-top lids. A 5-mm-diameter hole was made in the lid for ventilation and this was covered with fine nylon netting (80 μm mesh). The arenas were filled with test substrate to a depth of 2-4 cm. |
| Soil | OECD artificial soil, 5% peat |
| **Environmental conditions** | |
| Temperature: | 19.5 – 21.5°C |
| Photoperiod: | 16-hour photoperiod (580-650 lux) |
| Soil moisture: | 50 % max. WHC |
| pH: | 6 ±0.5 |
| **Application rate(s)** | 1000 mg f.p./kg soil dry weight<br>(217 mg a.s./kg soil dry weight) |
| Negative control: | purified water |
| Positive control: | boric acid |
| **Post exposure observation period** | 28 days |
| **Remarks** | None |

The toxicity of the test item Zoxium 240 SC (product code GWN-9790EU), a suspension concentrate (SC) formulation containing the active substance zoxamide at nominally 240 g/L, has been tested under laboratory test conditions on the springtail *Folsomia candida* (Willem) (Collembola, Isotomidae). Based on the results of a non-GLP range-finder, Zoxium 240 SC was evaluated in a limit test bioassay at a single concentration of 1000 mg test item/kg soil dry weight (217 mg a.s./kg soil dry weight). This was compared to an untreated (water only) control. A toxic reference item (boric acid) was included in a separate validation study performed by the laboratory.

The control treatment and test item treatment, diluted in purified water, were thoroughly mixed into an artificial soil substrate (containing 5% w/w peat). Ten juvenile *F. candida* (11-12 days old) were introduced into small, ventilated, glass jar arenas (8 per treatment). Dry granulated yeast was provided on the soil surface as food and this was re-plenished 14 days after treatment (DAT). The soil was kept at 50% max. WHC. The bioassay was carried out under environmentally controlled conditions.

At 28 DAT, the numbers of both surviving adults and F1 progeny (i.e. juvenile springtails) in each test arena were recorded at 28 DAT. The test substrate from each arena was tipped into a tray (approximately 11 cm x 17 cm in area and 6 cm in depth). Water (approx. 200 mL) was then added to the substrate and stirred gently, so that the soil sank and the springtails floated to the surface. Any adult springtails floating on the water were counted and removed. Black ink was then added to the water and the numbers of any nymphs (smaller in size to adults) left in each arena were assessed. The ink darkened the water so that it contrasted with the light-coloured springtails floating on the water surface. A grill was held over the surface to aid with counting using a binocular microscope. It was assumed that any adult springtails that were recovered would have been those originally introduced and that any shortfall in the original number was an indication that they had died during the bioassay.

Statistical analyses were performed using validated computer software, namely SPSS (IBM Corp., 2016). The percentage mortality of the springtails originally introduced was calculated for the test item treatment, both before and after correction for any control treatment losses using Abbott's formula. The

28-day mortality data for the individual test-item treatment were compared to those for the control using Fisher's Exact Test (α = 0.05). The results were used to determine the no observed effect concentration (NOEC) with respect to mite survival. The mean number of offspring produced per replicate and the standard deviation were calculated for the control and test item treatment. In addition, the percentage difference in reproductive performance in the test-item treatment group, compared to the control group, was calculated. The data from the reproduction assessments in the individual treatments were checked for normality (Shapiro-Wilk test, α = 0.05) and for equality of variances (Levene's test, α = 0.05). As normality and homogeneity could be assumed, the numbers of F1 progeny produced in the test-item treatment was compared to numbers in the control, using t-test for independent samples (α = 0.05). A NOEC with respect to springtail reproduction was determined.

Three separate samples of approximately 25-30 g of soil were preserved from both the test-item treatment and the control treatment for analytical verification of the concentration of test item present in the treated soil. The 0 DAT samples were frozen immediately after preparation and the remaining samples were kept alongside the test arenas until 14 DAT or 28 DAT when they were made up to 50% WHC before being frozen. The samples were shipped frozen to BioChem Agrar for analytical verification of the test item concentrations in the soil with a method fully validated according to SANCO/3029/99 rev. 4 under project number 18 35 CRX 0024.

## Results and discussion

Environmental conditions stayed within the recommended ranges.

The nominal initial test item concentration in the freshly prepared soil specimen was analytically confirmed (112% recovery for the active substance). Moreover, the zoxamide concentrations stayed within the range of 98 – 112% of the nominal initial concentration throughout the whole study period.

At 28 days, there was 5% mortality in the control treatment, compared with 8% mortality (3% corrected mortality) in the 1000 mg test item/kg soil dry weight treatment concentration of Zoxium 240 SC. The result from the test item treatment was not statistically significantly different from the control (Fisher's Exact Test, α = 0.05). The NOEC value was therefore 1000 mg test item/kg soil dry weight.

**Table A 91: Percentage mortality of adult mites at 28 days**

| Treatment (mg f.p./kg soil dry weight) | Initial number of introduced collembola | Mean mortality per treatment (%) | Effect in comparison to the control (%) |
|---|---|---|---|
| Control | 80 | 5 | - |
| 1000 | 80 | 8 | 3 |

At 28 days of exposure, based on the numbers of offspring produced, the $EC_{20}$ and EC10 values for Zoxium 240 SC were >1000 mg test item/kg soil dry weight. Statistical comparisons with the control indicated that the overall NOEC with respect to both springtail survival and reproduction was 1000 mg test item/kg soil dry weight (equivalent to 217 mg a.s./kg soil dry weight, based on analysed a.s. content of 21.7% w/w in the test item).

The mean number of progeny produced per replicate was 469 in the control and 439 (6% reduction compared to the control) in the 1000 mg test item/kg soil dry weight treatment. The result from the test item treatment was not statistically significantly different from the control, the NOEC value was therefore 1000 mg test item/kg soil dry weight. The reproduction data was not appropriate for regression analysis to calculate effect concentrations (ECx) from a dose-response curve. The $EC_{20}$ and $EC_{10}$ values were therefore extrapolated from the available data as > 1000 mg test item/kg soil dry weight.

**Table A 92: Mean number of juveniles produced after 28 days**

| Treatment (mg f.p./kg soil dry weight) | Mean number of juveniles | Effect in comparison to the control (%) |
|---|---|---|
| Control | 469 | - |
| 1000 | 439 | 6 |

All validity criteria were met:

- The mortality of the parental collembola in the water control group did not exceed 20% (actual value of control in test = 5%).
- The mean number of juveniles recorded in the control treatment should exceed 100 per replicate at the end of the test (actual value of control in test = 469).
- The coefficient of variation of reproduction in the control should not exceed 30% (actual value of control in test = 15.1%).

The efficiency of the method used to extract the springtails in this test was > 95%. This was confirmed in a separate test carried out by the test facility in October 2014. Here, the efficiency of the extraction method was 100% for adult and 98.3% for juvenile springtails.

The sensitivity of the test system was confirmed in a separate study with boric acid as test item, in which the $EC_{50}$ was calculated to be 111 mg boric acid/kg artificial soil dry weight.

## Conclusion

In a laboratory bioassay with Zoxium 240 SC and the springtail *Folsomia candida*, assessments of survival and reproductive performance were made over 28 days.

For Zoxium 240 SC the lowest observed effect concentration (LOEC), the $EC_{50}$, $EC_{20}$ and $EC_{10}$ values were >1000 mg test item/kg soil dry weight. Statistical comparisons with the control indicated that the overall NOEC with respect to both springtail survival and reproduction was 1000 mg test item/kg soil dry weight (equivalent to 217 mg a.s./kg soil dry weight, based on analysed a.s. content of 21.7% w/w in the test item) – the highest test item rate investigated (limit test).

(Parsons Ch. 2020)

### *A 2.4.2.1.2* Study 2 – Zoxamide tech. / Zoxium 240 SC: Effects on *Hypoaspis*

In addition to the further zoxamide metabolite studies on soil macro-organisms (other than earthworms) requested by EFSA (2017), studies on *Folsomia* and *Hypoaspis* have been performed with the parent compound zoxamide. The zoxamide studies have been conducted with a SC formulation containing 240 g/L zoxamide, the representative formulation during AIR. The composition of Zoxium 240 SC (GWN-9790 EU) is included in Part C. The studies are regarded representative to address the potential risk from the use of zoxamide technical.

| | |
|---|---|
| Comments of zRMS: | Study not evaluated by zRMS. |

Reference: **KCP 10.4.2/02**

Report xxx., 2020: Zoxium 240 SC – A laboratory test to determine the effects of fresh residues on the predatory soil mite *Hypoaspis aculeifer* (Acari, Laelapidae) in an artificial soil substrate

| | |
|---|---|
| | Gowan Crop Protection Ltd., UK<br>xxx., Report No. GOW-17-14, GLP, Not published |
| Guideline(s): | OECD 232 (2016) |
| Deviations: | The temperature range at which the test mite culture was intended to be maintained was 18-22°C. The actual temperature range recorded was 20.2-22.6°C. A total of 76 out of 696 readings exceeded 22°C on 6 separate occasions. This deviation from the intended range was due high ambient temperatures in the laboratory, which caused a malfunction in the controlled-environment facilities. With regard to performance of the test organisms in the control, this deviation was considered to not cause adverse effects on the bioassay and to not affect the integrity of the study. |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication<br>(if vertebrate study) | No |

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | Zoxium 240 SC (18011201-72-52) |
| **Content of a.s.:** | 240 g/L Zoxamide (nominal), 21.7 % (w/w) (analysed) |
| **Species:** | *Hypoaspis aculeifer* |
| Age: | Adult mites |
| Source: | culture maintained at the test facility since 2016; original source: Bias Labs Ltd., UK |
| Acclimation period: | At least 2 days |
| Food: | Cheese mites (*Tyrophagus putrescentiae* (Schrank)) *ad libitum* and springtails (*Folsomia candida* (Willem)) |
| **Test system** | 60 mL capacity glass jars (5.5 cm tall x 5.2 cm outer diameter, 4.4 cm inner diameter), with screw-top lids. An 8-mm-diameter hole was made in the lid for ventilation and this was covered with fine nylon netting (80 µm mesh size). |
| Soil | OECD artificial soil, 5% peat |
| **Environmental conditions** | |
| Temperature: | 19.2 – 21.3°C |
| Photoperiod: | 16-hour photoperiod (600-690 lux) |
| Soil moisture: | 50 % max. WHC |
| pH: | 6 ±0.5 |
| **Application rate(s)** | 1000 mg f.p./kg soil dry weight<br>(217 mg a.s./kg soil dry weight) |
| Negative control: | purified water |

| | |
|---|---|
| Positive control: | Dimethoate |
| **Post exposure observation period** | 14 days |
| **Remarks** | None |

The test item Zoxium 240 SC (product codes GWN-9790EU and GWN-10624), a suspension concentrate (SC) formulation containing the active substance zoxamide at nominally 240 g/L, has been studied under laboratory conditions whether it has harmful effects on the predatory soil mite *Hypoaspis aculeifer* (Canestrini) (Acari, Laelapidae).

Based on the results of a range-finding study, Zoxium 240 SC was evaluated in a bioassay at a single treatment concentration, equivalent to 1000 mg test item/kg soil dry weight (limit test). This was compared to an untreated (water only) control. A toxic reference item (dimethoate) was included in a separate validation study.

The control treatment and test item treatment, diluted in purified water, were thoroughly mixed into an artificial soil substrate (containing 5% w/w peat), aliquots of which were then transferred into small, ventilated, glass jar arenas (n = 8 per treatment). Ten female soil mites (approximately 7-14 days after becoming adult) were then introduced into each arena. There were eight replicate arenas for the control treatment and for the test-item treatment concentration. Cheese mites (*Tyrophagus putrescentiae* (Schrank)) and springtails (*Folsomia candida* (Willem)) were provided as food for the predatory soil mites and were replenished *ad libitum*. The moisture content of the soil was adjusted to 50 % max. WHC. The pH of soil was measured according to ISO 10390 in abiotic replicate vessels at the start and end of the study. The test was carried out under environmentally controlled conditions.

At 14 days after treatment (DAT), both the number of surviving adult predatory soil mites and of their offspring were recorded following extraction from the soil via Tullgren funnel. Over a two-day period, the heat of a light-bulb (25 Watt, with a 24 h photoperiod) slowly dried the soil from the top, forcing the *H. aculeifer* to move downwards through the soil until they fell from the base of the funnels into collecting vials with a 70% (v/v) methyl alcohol in which they were preserved.

Statistical analyses were performed using validated computer software, namely SPSS (IBM Corp., 2016). The percentage mortality of the mites originally introduced was calculated for the test item treatment, both before and after correction for any control treatment losses using Abbott's formula. The 14-day mortality data were compared to those for the control using Fisher's Exact Test ($\alpha = 0.05$). The results were used to determine the no observed effect concentration (NOEC) with respect to mite survival. The mean number of offspring produced per replicate and the standard deviation were calculated for the control and test item treatment. In addition, the percentage difference in reproductive performance in the test-item treatment group, compared to the control group, was calculated. The data from the reproduction assessments in the individual treatments were checked for normality (Shapiro-Wilk test, $\alpha = 0.05$) and for equality of variances (Levene's test, $\alpha = 0.05$). As normality and homogeneity could be assumed, the numbers of F1 progeny produced in the test-item treatment was compared to numbers in the control, using t-test for independent samples ($\alpha = 0.05$). A NOEC with respect to springtail reproduction was determined.

Three separate samples of approximately 25-30 g of soil were preserved from both the test-item treatment and the control treatment for analytical verification of the concentration of test item present in the treated soil. The 0 DAT samples were frozen immediately after preparation and the remaining samples were kept alongside the test arenas until 14 DAT when they were made up to 50% WHC before being frozen. The samples were shipped frozen to Biochem Agrar for analytical verification of the test item concentrations in the soil with a method fully validated according to SANCO/3029/99 rev. 4 under project number 18 35 CRX 0025.

## Results and discussion

Environmental conditions stayed within the recommended ranges.

The nominal initial test item concentration in the freshly prepared soil specimen was analytically confirmed (102% recovery for the active substance). The zoxamide concentrations stayed within the range of 102 – 109% of the nominal initial concentration throughout the whole study period.

At 14 days, there was 6% mortality in the control treatment, compared with 9% mortality (3% corrected mortality) in the 1000 mg test item/kg soil dry weight treatment concentration of Zoxium 240 SC. The result from the test item treatment was not statistically significantly different from the control (Fisher's Exact Test, $\alpha = 0.05$). The NOEC value was therefore 1000 mg test item/kg soil dry weight.

**Table A 93: Mortality of adult *H. aculeifer* after 14 days**

| Treatment (mg f.p./kg soil dry weight) | Initial number of introduced *Hypoaspis* | Mean mortality per treatment (%) | Effect in comparison to the control (%) |
|---|---|---|---|
| Control | 80 | 6 | - |
| 1000 | 80 | 9 | 3 |

The mean number of progeny-produced per replicate was 235 in the control and 231 in the 1000 mg test item/kg soil dry weight treatment concentration of Zoxium 240 SC.

**Table A 94: Mean number of juveniles produced after 14 days**

| Treatment (mg f.p./kg soil dry weight) | Mean number of juveniles | Effect in comparison to the control (%) |
|---|---|---|
| Control | 235 | - |
| 1000 | 231 | 1 |

Based on this data the $EC_{20}$ and $EC_{10}$ values for Zoxium 240 SC were > 1000 mg test item/kg soil dry weight. The result from the test item treatment was not statistically significantly different from the control (t-test for independent samples, $\alpha = 0.05$). The overall NOEC with respect to both mite survival and reproduction was 1000 mg test item/kg soil dry weight (equivalent to 217 mg a.s./kg soil dry weight, based on analysed a.s. content of 21.7% w/w in the test item).

The reproduction data was not appropriate for regression analysis since only a single concentration of the test item was evaluated (limit test). Key effect concentrations ($EC_{20}$ and $EC_{10}$) were therefore extrapolated with respect to this single test item treatment.

The following validity criteria were met for the study; therefore, these data are considered to be valid.

- The mean mortality of the original parental mites in the control treatment did not exceed 20% over 14 days (actual value of control in test = 6%).
- The mean number of juveniles recorded in the control treatment should be at least 50 per replicate at the end of the test (actual value of control in test = 235).
- The coefficient of variation of reproduction in the control should not exceed 30% (actual value of control in test = 5.7%).

The efficiency of the method used to extract the mites should be ≥ 90%. In a separate test carried out by the test facility this was determined to be 98.0% (98.0% for the adult female mites and for the juvenile mites).

The sensitivity of the test system has been determined in a separate bioassay using a formulated sample of

dimethoate. As a result, an $EC_{50}$ of 6.3 mg a.s./kg soil dry weight (95% confidence limits = 5.6 and 7.0 mg a.s./kg soil dry weight) was calculated.

**Conclusion**

In a laboratory bioassay with Zoxium 240 SC and the predatory soil mite *Hypoaspis aculeifer*, assessments of survival and reproductive performance were made over 14 days.

The lowest observed effect concentration (LOEC), the $EC_{50}$, $EC_{20}$ and $EC_{10}$ values were >1000 mg test item/kg soil dry weight. Statistical comparisons with the control indicated that the overall NOEC with respect to both soil mite survival and reproduction was 1000 mg test item/kg soil dry weight (equivalent to 217 mg a.s./kg soil dry weight, based on analysed a.s. content of 21.7% w/w in the test item) – the highest test item rate investigated (limit test).

(Parsons Ch. 2020)

According to EFSA (20017): *"Further data are needed to address the risk to soil macro-organisms other than earthworms for the metabolites RH-163353 and RH-141455 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5)."* These data are provided in the following.

### A 2.4.2.1.3 Study 3 - RH-163353: Effects on Collembolan reproduction

The following study has been provided to Latvia as RMS for zoxamide and cMSs for interzonal evaluation in July 2020.

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 232 and according to the principles of GLP. In the test a lot of deviations were noted. Moreover, the solvent control did not pass the validity criteria. Reductions in reproduction in comparison to the water control were 26.5, 31.8, 22.6, 10.4, 42.7, 49.3, 47.3 and 47.6% at 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.i./kg dry substrate respectively. Thus, in opinion of zRMS, no reliable NOEC or $EC_{10}$ value can be derived.<br>The study is considered to be not suitable for the risk assessment. |

| | |
|---|---|
| Reference: | **KCP 10.4.2/03** |
| Report | xxx., 2020: RH-163353: Collembolan reproduction test in soil<br>Gowan Crop Protection Ltd., UK<br>xxx, Report No. 3202390, GLP, Not published |
| Guideline(s): | OECD 232 (2016) |
| Deviations: | Each test vessel was weighed and the weight adjusted to the Day 0 level by addition of RO water. In this way individual vessel moisture loss was made up to the correct level whereas the use of a single moisture control vessel as stated in the protocol does not account for the variation in loss between vessels. The process was repeated at Day 21 to ensure optimum environmental conditions were maintained as far as possible throughout the exposure period.<br>The light period was less than 16 hours on one occasion over the 28-day exposure period.<br>The mean % MWHC was below the minimum of 40% stated in the protocol for all treatments and the control throughout the study. As the % MWHC to obtain |

the correct soil structure had been determined to be 40%MWHC, the lowest level specified, any moisture lost during the mixing process would have resulted in the final % MWHC being below the selected level.
These deviations are considered to have no impact on the study integrity.
In addition, the OECD 232 guideline states 'The moisture content of the testing soil should be optimised to obtain a loose porous structure allowing the collembolans to enter into the pores. This is usually between 40-60 % maximum WHC.' Therefore, the % MWHC that provides the correct soil structure may be outside the 40-60 % range. It is considered that as the validity criterion of ≥100 juveniles per replicate was achieved in the controls and at all rates of application the lower % MWHC has had no impact on the reproductive output.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) No

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | RH-163353 (HHGCP001-00-2) |
| **Purity** | 99.48 % (w/w); enantiomeric ratio 48.6:51.4 (racemate) |
| **Species:** | *Folsomia candida* |
| Age: | 9-12 days old |
| Source: | Source – Bias Labs Ltd., UK |
| Acclimation period: | Not relevant |
| Food: | dried yeast (~2 -10 mg) was added to each test vessel on day 0 after introduction and again on days 14 and 21 |
| **Test system** | 50 mm diameter glass vessel with a volume of approximately 120 mL, covered with a screw lid as a minimum. Each vessel contained ten juvenile *F. candida* in 30 g dry weight equivalent of substrate. |
| Soil | OECD artificial soil, 5% peat |
| **Environmental conditions** | |
| Temperature: | 19.8 – 21.0°C |
| Photoperiod: | 16:8-hour light: dark cycle at a range of 428 to 639 Lux |
| Soil moisture: | 50 % max. WHC |
| pH: | 5.89 - 6.60 |
| **Application rate(s)** | 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.i./kg dry substrate |
| Negative control: | reverse osmosis (RO) water<br>solvent control (acetone) |
| Positive control: | boric acid |
| **Post exposure observation period** | 28 days |

| | |
|---|---|
| **Remarks** | None |

The toxicity of the zoxamide metabolite RH-163353 has been tested under laboratory test conditions on the springtail *Folsomia candida* (Willem) (Collembola, Isotomidae). Based on the results of a non-GLP range-finder, RH-163353 was evaluated in a bioassay at five concentrations of 0.08, 0.4, 2.0, 10 and 50 mg a.i./kg dry artificial soil substrate. These were compared to a solvent control and an untreated (water only) control. A toxic reference item (boric acid) was included in a separate validation study performed by the laboratory.

The test item was applied in acetone to a sand carrier. The acetone was allowed to evaporate before the treated sand was mixed with the soil and a required amount of reverse osmosis (RO) water to achieve a soil moisture of 40% max. WHC. A control and a solvent control were included in the test.

Ten juvenile 9 to 12-day old *F. candida* were introduced into eight replicate test vessels for the controls and four vessels at each RH-163353 treatment rate. The test organisms were fed with dried yeast (~2 -10 mg) added to each test vessel (containing *F. candida*) on day 0, 14 and 21 of the bioassays. The moisture content was adjusted to 40 % max. WHC. The test vessels were aerated at least twice a week by removing the lid for several seconds. The pH (in 1 M KCl) of the treated substrate, sampled on days 0 and 28 (taken from abiotic vessels) was determined. The test was carried out in a temperature-controlled incubator at 20 ± 2°C and a 16:8 hour light:dark cycle (light intensity of 400 – 800 Lux), measured at least weekly throughout the test.

After 28 days the numbers of the original springtails still surviving and the numbers of any offspring they had produced were recorded. RO water was added to the test substrate in each vessel to allow the collembola to rise to the surface. Enough water was added to achieve standing water above the test substrate and was stirred gently to allow any collembola present in air pockets to float to the surface. A small amount of liquid black dye was added to each test chamber to create a contrast between the collembola and the flotation liquid. *Ca*. 5 mL ethanol were added to anaesthetise the collembola. The number of surviving adult (parental Collembola) and juvenile *F. candida* were counted using a magnifying sheet.

Percent mortality of the introduced parental collembola was determined for each vessel and a treatment mean was calculated. A no observed effect concentration (NOEC) and $LC_{10}$, $LC_{20}$ and $LC_{50}$ values were determined. Statistical analysis of the reproduction data was undertaken using CETIS version 1.8.6.8, based on nominal test concentrations. The NOEC for adult survival was determined using a Bonferroni Adj t Test and the $LC_x$ values were determined using Linear Interpolation (ICPIN). The NOEC for number of juveniles was determined using a Bonferroni Adj t Test and the $EC_x$ values were determined using Linear Interpolation (ICPIN). Corrected mortality, in comparison to both the water and solvent controls, was calculated using Abbott's formula (Abbott, 1925) The reproductive output of the collembola is presented as the number of juveniles present in each vessel at the end of the test. A mean for each treatment was calculated from the vessel counts. No observed effect concentration (NOEC) and $EC_{10}$, $EC_{20}$ and $EC_{50}$ values were determined.

On the day of test item application and at the end of the test four 2 g substrate samples were taken from each treatment for chemical analysis (two to analyse and two to retain). The samples were stored frozen until analysis of RH-163353 (racemate) soil concentrations and the ratio of the enantiomers of exposed RH-163353. The methods were validated according to SANCO 3029/99 rev. 4 with an LOQ of 0.016 mg/kg (racemate). Concentrations of RH-163353 were determined by extracting soil samples with 1% formic acid in acetonitrile/acetone 3:1 v/v, then diluting further with unfortified control sample extract as required to bring the response within the calibration range. Samples were analysed by injection onto a liquid chromatography-time of flight mass spectrometry (LC-TOF/MS) system. The analytical procedure SMV 3202390-01V (SMV 3202390-02V to include stability data) was used to determine RH-166353 and

SMV 3202586-01V (a combination of the analytical procedures SMV 3202390-02V and SMV 3202586-01V) to assess the enantiomeric ratio the test substance.

## Results and discussion

Environmental conditions stayed within the recommended ranges.

Post application the mean test item concentrations were 0.6917, 1.1829, 2.0970, 3.9415, 6.8889, 13.3316, 23.4319 and 39.9660 mg a.i./kg dry substrate at application rates of 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.i./kg dry substrate respectively. This is equivalent to 84.36, 80.47, 79.13, 82.80, 80.38, 86.40, 84.35 and 79.99% of nominal at 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.i./kg dry substrate, respectively.
On day 28 the mean test item concentrations were 0.5624, 0.9504, 1.6736, 3.0910, 5.8986, 10.8197, 19.4087 and 35.1938 mg a.i./kg dry substrate at application rates 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.i./kg dry substrate respectively. This is equivalent to 68.59, 64.65, 63.15, 64.94, 68.83, 70.12, 69.87 and 70.39% of nominal at 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.i./kg dry substrate, respectively.
The mean enantiomeric ratio of the incubated RH-163353 in the 28 day samples was 47.93 : 52.07 % for isomer A and B, respectively. This is in the range of the enantiomeric ratio of 48.6 : 51.4 given on the Certificate of Analysis for the test item batch, confirming its chiral stability during incubation.

As the mortality in all the treatment rates was less than in the solvent control, it indicates that the mortality in the treatments was not directly attributable to the presence or action of the solvent. In addition, mortality in the treatments reached a maximum of 20% in one rate only and this was therefore not significantly higher than in the water control (16.25%) and was also within the control validity criterion. In the treatments mortality was in the range was 5-20% with five of the rates having lower mortality than the water control. In this circumstance it is considered acceptable to use the valid water control as the point of reference.

After 28 days of exposure there was no relevant mortality compared to the control. Based on mortality, the $LC_{10}$, $LC_{20}$, $LC_{50}$ and LOEC were > 50 mg a.i./kg dry substrate, the NOEC was determined at 50 mg a.i./kg dry substrate.

**Table A 95: Percentage mortality of adult mites after 28 days**

| Treatment (mg a.i./kg dry soil) | Initial number of introduced collembola | Mean mortality per treatment (%) | % reduction in comparison to the water control (%) |
|---|---|---|---|
| Water control | 80 | 16.25 | - |
| Solvent control | 80 | 35.00 | 22.39 |
| 0.82 | 40 | 12.50 | 0 |
| 1.47 | 40 | 17.50 | 1.49 |
| 2.65 | 40 | 17.50 | 1.49 |
| 4.76 | 40 | 10.00 | 0 |
| 8.57 | 40 | 10.00 | 0 |
| 15.43 | 40 | 20.00 | 4.48 |
| 27.78 | 40 | 15.00 | 0 |
| 50 | 40 | 5.00 | 0 |

For RH-163353, the $EC_{10}$ $EC_{20}$ and $EC_{50}$ values, LOEC and NOEC based on reproductive performance were:

$EC_{50}$ > 50 mg a.i./kg dry substrate

$EC_{20}$ 0.6992 mg a.i./kg dry substrate (0.2183 - 10.65)

$EC_{10}$ 0.3009 mg a.i./kg dry substrate (0.11 - 8.52)

LOEC 8.57 mg a.i./kg dry substrate

NOEC 4.76 mg a.i./kg dry substrate

The 95% confidence levels are given in parentheses.

**Table A 96: Mean number of juvenile mites produced after 28 days**

| Treatment (mg a.i./kg dry soil) | Mean number of juveniles | Reduction compared to the water control (%) |
|---|---|---|
| Water control | 223.8 | - |
| Solvent control | 129.8 | 42.0 |
| 0.82 | 164.4 | 26.5 |
| 1.47 | 152.5 | 31.8 |
| 2.65 | 173.1 | 22.6 |
| 4.76 | 200.5 | 10.4 |
| 8.57 | 128.3 | 42.7 |
| 15.43 | 113.4 | 49.3 |
| 27.78 | 118.0 | 47.3 |
| 50 | 117.3 | 47.6 |

All validity criteria were met:

- The mortality of the parental collembola in the water control group did not exceed 20% (actual adult mortality = 16.25 %). Mortality in the solvent control group was 36.25%, effects have been calculated in relation to the water control.
- The rate of production of juveniles was at least 100 per control vessel (mean actual rate of production of juveniles = 224 and 130 in the water and solvent controls, respectively).
- The coefficient of variance of reproduction in the water control treatment was 30% or less (actual coefficient of variance = 28.8% in the water control). The coefficient of variance of reproduction in the solvent control treatment was 55.1%, effects have been calculated in relation to the water control.

The sensitivity of the test system was confirmed in a separate study with boric acid as test item. The $EC_{50}$ value was calculated to be 52.1 mg boric acid/kg artificial soil dry weight (ERS Study Number 3202464).

## Conclusion

In a laboratory bioassay with zoxamide metabolite RH-163353 and the springtail *Folsomia candida*, assessments of survival and reproductive performance were made over 28 days. Included were data for a solvent control and an untreated (water only) control.

For RH-163353 the $LC_{50}/EC_{50}$, LOEC and NOEC values - based on comparison of the effect data to the (water only) control data – were determined as follow:

| Endpoint | $LC_{50}$ / $EC_{50}$ | LOEC | NOEC |
|---|---|---|---|
| | (mg a.i./kg dry soil) | | |
| Parental mortality | >50 | >50 | 50 |

| | | | |
|---|---|---|---|
| Reproductive output | >50 | 8.57 | 4.76 |

The following validity criteria as specified in OECD guideline 232 were achieved during the study and therefore the study data were considered valid: Mortality in the water control treatment was ≤ 20% and there were ≥ 100 juveniles per vessel. The co-efficient of variance for the reproductive output in the water control treatment was ≤ 30%.

(Gray J. 2020)

### A 2.4.2.1.4 Study 4 – RH-141455: Effects on Collembolan reproduction

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 232 and according to the principles of GLP. In the test deviations were noted. The actual adult mortality was 20 % in both the water and solvent controls. Moreover, the solvent control did not pass the validity criteria as coefficient of variance was 36.8%. Thus, both controls were very weak. The highest differences in %MWHC between Day 0 and Day 28 were in both controls. Additionally, more juveniles were produced at all rates of application than in either of the controls (up to 48.2 compering to water control and up to 88% in solvent control).<br>Thus, in opinion of zRMS, The study is considered to be not suitable for the risk assessment. |

| | |
|---|---|
| Reference: | **KCP 10.4.2/04** |
| Report | xxx., ~~2020~~ 2021: RH-141455: Collembola reproduction test in soil<br>Gowan Crop Protection Ltd., UK<br>xxx, Report No. 3202382, GLP, Not published |
| Guideline(s): | OECD 232 (2016) |
| Deviations: | During the main test at day 28 the mean % MWHC was below the minimum of 40% stated in the protocol in both the controls and in the 1.47 and 4.76 mg a.i./kg dry substrate treatment rates. Moisture content over the exposure period varied by >10% in both controls and at 0.82, 1.47, 2.65 and 4.75 mg a.i./kg dry substrate. However, these deviations were not considered to have had an adverse impact on the conduct or outcome of the study as the validity criteria were met.<br>In addition, the OECD 232 guideline states 'The moisture content of the testing soil should be optimised to obtain a loose porous structure allowing the collembolans to enter into the pores. This is usually between 40-60% maximum WHC.' Therefore, the % MWHC that provides the correct soil structure may be outside the 40 – 60 % range. It is considered that as the validity criterion of ≥ 100 juveniles per replicate was achieved in the controls and at all rates of application the lower % MWHC has had no impact on the reproductive output. |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication<br>(if vertebrate study) | No |

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | RH-141455 (A19X08291) |
| **Purity** | 92.77% (w/w) |
| Species: | *Folsomia candida* |
| Age: | 10-12 days old |
| Source: | Source – Bias Labs Ltd., UK |
| Acclimation period: | Not relevant |
| Food: | dried yeast (~2 -10 mg) was added to each test vessel on day 0 after introduction and again on days 14 and 21 |
| **Test system** | 50 mm diameter glass vessel with a volume of approximately 120 mL, covered with a screw lid as a minimum. Each vessel contained ten juvenile F. candida in 30 g dry weight equivalent of substrate. |
| **Soil** | OECD artificial soil, 5% peat |
| **Environmental conditions** | |
| Temperature: | 19.3 – 20.6 °C |
| Photoperiod: | 16:8-hour light: dark cycle at a range of 405 to 669 Lux |
| Soil moisture: | 50 % max. WHC |
| pH: | 7.22 - 7.41 |
| **Application rate(s)** | 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.i./kg dry substrate |
| Negative control: | reverse osmosis (RO) water<br>solvent control (acetone) |
| Positive control: | boric acid |
| **Post exposure observation period** | 28 days |
| **Remarks** | None |

The toxicity of the zoxamide metabolite RH-141455 has been tested under laboratory test conditions on the springtail *Folsomia candida* (Willem) (Collembola, Isotomidae). Based on the results of a non-GLP range-finder, RH-163353 was evaluated in a bioassay at five concentrations of 0.08, 0.4, 2.0, 10 and 50 mg a.i./kg dry artificial soil substrate. These were compared to a solvent control and an untreated (water only) control. A toxic reference item (boric acid) was included in a separate validation study performed by the laboratory.

**Table 1**
**Test Substrate Moisture Content and pH Determination**

| Treatment (mg a.i./kg dry substrate) | Moisture Content | | %MWHC | | pH | |
|---|---|---|---|---|---|---|
| | Day 0 | Day 28 | Day 0 | Day 28 | Day 0 | Day 28 |
| Water control | 26.58 | 21.67 | 47.93 | 39.07 | 7.24 | 7.22 |
| Solvent control | 25.79 | 19.86 | 46.50 | 35.81 | 7.41 | 7.30 |
| 0.82 | 26.58 | 22.48 | 47.93 | 40.53 | 7.34 | 7.28 |
| 1.47 | 26.58 | 21.88 | 47.93 | 39.45 | 7.29 | 7.34 |
| 2.65 | 25.79 | 22.51 | 46.50 | 40.58 | 7.34 | 7.34 |
| 4.76 | 25.79 | 21.29 | 46.50 | 38.39 | 7.35 | 7.38 |
| 8.57 | 26.58 | 24.85 | 47.93 | 44.80 | 7.36 | 7.41 |
| 15.43 | 25.79 | 24.21 | 46.50 | 43.65 | 7.39 | 7.39 |
| 27.78 | 25.79 | 24.69 | 46.50 | 44.52 | 7.41 | 7.38 |
| 50 | 26.58 | 24.58 | 47.93 | 44.32 | 7.36 | 7.38 |

MWHC Maximum water holding capacity
MWHC = 55.46% (1% MWHC = 0.5546 g $H_20$/g dry substrate)
To achieve 50% MWHC for 100 g dry substrate: = (100 + ((0.5 x 55.46)) = 127.73 g final weight = 27.73% moisture content
50% MWHC = 27.73% moisture content, 1% moisture content = 50/27.73 % MWHC = 1.803% MWHC
MWHC = 1.803 x water content

The test item was applied in acetone to a sand carrier. The acetone was allowed to evaporate before the treated sand was mixed with the soil and a required amount of reverse osmosis (RO) water to achieve a soil moisture of 50% max. WHC. A control and a solvent control were included in the test.

Ten juvenile 10 to 12-day old *F. candida* were introduced into eight replicate test vessels for the controls and four vessels at each RH-141455 treatment rate. The test organisms were fed with dried yeast (~2 -10 mg) added to each test vessel (containing *F. candida*) on day 0, 14 and 21 of the bioassays. The moisture content was adjusted to 50 % max. WHC. The test vessels were aerated at least twice a week by removing the lid for several seconds. The pH (in 1 M KCl) of the treated substrate, sampled on days 0 and 28 (taken from abiotic vessels) was determined. The test was carried out in a temperature-controlled incubator at 20 ± 2°C and a 16:8 hour light:dark cycle (light intensity of 400 – 800 Lux), measured at least weekly throughout the test.

After 28 days the numbers of the original springtails still surviving and the numbers of any offspring they had produced were recorded. RO water was added to the test substrate in each vessel to allow the collembola to rise to the surface. Enough water was added to achieve standing water above the test substrate and was stirred gently to allow any collembola present in air pockets to float to the surface. A small amount of liquid black dye was added to each test chamber to create a contrast between the collembola and the flotation liquid. *Ca*. 5 mL ethanol were added to anaesthetise the collembola. The number of surviving adult (parental Collembola) and juvenile *F. candida* were counted using a magnifying sheet.

Percent mortality of the introduced parental collembola was determined for each vessel and a treatment mean was calculated. A no observed effect concentration (NOEC) and $LC_{10}$, $LC_{20}$ and $LC_{50}$ values were determined. Statistical analysis of the reproduction data was undertaken using CETIS version 1.8.6.8, based on nominal test concentrations. The NOEC for adult survival was determined using a Bonferroni Adj t Test and the $LC_x$ values were determined using Linear Interpolation (ICPIN). The NOEC for number of juveniles was determined using a Bonferroni Adj t Test and the $EC_x$ values were determined using Linear Interpolation (ICPIN). Corrected mortality, in comparison to both the water and solvent controls, was calculated using Abbott's formula (Abbott, 1925) The reproductive output of the collembola is presented as the number of juveniles present in each vessel at the end of the test. A mean for each treatment was calculated from the vessel counts. No observed effect concentration (NOEC) and $EC_{10}$, $EC_{20}$ and $EC_{50}$ values were determined.

On the day of test item application and at the end of the test four 2 g substrate samples were taken from each treatment for chemical analysis (two to analyse and two to retain). The samples were stored frozen until analysis of RH-141455 soil concentrations. The methods were validated according to SANCO 3029/99 rev. 4 with an LOQ of 0.2 mg/kg. Concentrations of RH-141455 were determined by extracting soil samples with an extraction solvent (acetonitrile/acetone 2:1 v:v containing 0.1% formic acid), then diluting further with unfortified control sample extract to bring the response within the calibration range. Samples were analysed by liquid chromatography Fourier-transform mass spectrometry (LC-FT/MS). The analytical procedure (SMV 3202383-01V and its revisions) was used to determine RH-141455.

## Results and discussion

Environmental conditions stayed within the recommended ranges.

Post-application the mean test item concentrations were 0.7724, 1.3638, 2.5931, 4.4097, 7.6760, 14.2632, 24.7952 and 41.0196 mg a.i./kg dry soil at application rates of 0.82, 1.47, 2.65, 476, 8.57, 15.43, 27.78 and 50 mg a.i./kg dry soil, respectively. This is equivalent to 94.19, 92.78, 97.85, 92.64, 89.57, 92.44, 89.26 and 82.04% of nominal at 0.82, 1.47, 2.65, 476, 8.57, 15.43, 27.78 and 50 mg a.i./kg dry soil, respectively. Due to recoveries of 82.04-97.85 % of nominal (calculated) values, the correct dosing of the test item has been confirmed.

On day 28 the mean substrate concentrations were 0.6979, 1.4805, 2.5892, 5.0642, 8.8461, 15.1094, 29.1776 and 57.9505 mg a.i./kg dry soil at application rates 0.82, 1.47, 2.65, 476, 8.57, 15.43, 27.78 and 50 mg a.i./kg dry soil, respectively. This is equivalent to 85.11, 100.72, 97.71, 106.39, 103.22, 97.92, 105.03 and 115.90% of nominal at 0.82, 1.47, 2.65, 476, 8.57, 15.43, 27.78 and 50 mg a.i./kg dry soil, respectively. Recoveries of 85.11-115.9 % of nominal (calculated) values at the end of the test confirm the stability of the test item over 28 days in soil under the test conditions.

After 28 days of exposure there was no clear dose response and no relevant test item related mortality up to and inclusive the highest test rate of 50 mg a.i./kg dry soil compared to the control.

**Table A 97: Percentage mortality of adult mites after 28 days**

| Treatment (mg a.i./kg dry soil) | Initial number of introduced collembola | Mean mortality per treatment (%) | Effects in comparison to the water control (%) |
|---|---|---|---|
| Water control | 80 | 20.0 | N/A |
| Solvent control | 80 | 20.0 | 0 |
| 0.82 | 40 | 17.5 | -3.1 |
| 1.47 | 40 | 22.5 | 3.1 |
| 2.65 | 40 | 30.0 | 15.6 |
| 4.76 | 40 | 25.0 | 6.3 |
| 8.57 | 40 | 17.5 | -3.1 |
| 15.43 | 40 | 30.5 | 12.5 |
| 27.78 | 40 | 17.5 | -3.1 |
| 50 | 40 | 15.0 | -6.3 |

After 28 days of exposure there was up to and inclusive the highest rate of 50 mg a.i./kg dry soil no negative impact of the test item on the reproductive performance and no dose response notable.

As a result, the $EC_{10}$, $EC_{20}$, $EC_{50}$, LOEC and NOEC based on reproductive output were determined at:

- $EC_{10}$, $EC_{20}$ and $EC_{50}$ > 50 mg a.i./kg dry substrate

- LOEC >50 mg a.i./kg dry substrate
- NOEC 50 mg a.i./kg dry substrate

**Table A 98: Mean number of juvenile mites produced after 28 days**

| Treatment (mg a.i./kg dry substrate) | Mean number of Juveniles | Effect compared to the water control (%) | Effect in comparison to the solvent control (%) |
|---|---|---|---|
| Water control | 130.3 | -- | +26.8 |
| Solvent control | 102.7 | -21.0 | -- |
| 0.82 | 143.9 | +10.5 | +40.1 |
| 1.47 | 156.6 | +20.2 | +52.5 |
| 2.65 | 170.6 | +31.0 | +66.2 |
| 4.76 | 176.3 | +35.3 | +71.6 |
| 8.57 | 193.0 | +48.2 | +88.0 |
| 15.43 | 160.3 | +23.0 | +56.1 |
| 27.78 | 184.6 | +41.7 | +79.8 |
| 50 | 175.1 | +34.5 | +70.5 |

All validity criteria were met:

The mortality of the original parental collembola in the control group(s) did not exceed 20% (actual adult mortality = 20 % in both the water and solvent controls).

- The mean rate of production of juveniles was at least 100 per control vessel (mean actual rate of production of juveniles = 130.3 and 102.7 in the water and solvent controls respectively).
- The coefficient of variance of reproduction in the water control treatment was 30% or less (actual coefficient of variance = 27.7%).
- The coefficient of variance of reproduction in the solvent control treatment exceeded 30% (actual coefficient of variance = 36.8%), however as no treatment related effects were recorded at any rate of application of RH-141455 the study is considered valid.

The sensitivity of the test system was confirmed in a separate study with boric acid as test item. The $EC_{50}$ value was calculated to be 52.1 mg boric acid/kg artificial soil dry weight (ERS Study Number 3202464).

## Conclusion

In a laboratory bioassay with zoxamide metabolite RH-141455 and the springtail *Folsomia candida*, assessments of survival and reproductive performance were made over 28 days. Included were data for a solvent control and an untreated (water only) control.

For RH-141455 the $LC_{50}/EC_{50}$, LOEC and NOEC values - based on comparison of the effect data to the (water only) control data – were determined as follow:

| Endpoint | $LC_{50}$ / $EC_{50}$ | LOEC | NOEC |
|---|---|---|---|
| | (mg a.i./kg dry soil) | | |
| Parental mortality | >50 | >50 | 50 |
| Reproductive output | >50 | >50 | 50 |

The following validity criteria as specified in OECD guideline 232 were achieved during the study and therefore the study data were considered valid: Mortality in the water and solvent control treatment was ≤ 20% and there was a mean of ≥ 100 juveniles; per vessel. The co-efficient of variance for the reproductive output in the water control treatment was ≤ 30%.

(Gray J. 2020)

### A 2.4.2.1.5 Study 5 - RH-163353: Effect on *Hypoaspis*

The following study has been provided to Latvia as RMS for zoxamide and cMSs for interzonal evaluation in July 2020.

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 226 and according to the principles of GLP. The study is considered to be reliable and suitable for the risk assessment |

| | |
|---|---|
| Reference: | **KCP 10.4.2/05** |
| Report | Gray, J. 2020: RH-163353: Effect on reproduction of *Hypoaspis* (Geolaelaps) *aculeifer*<br>Gowan Crop Protection Ltd., UK<br>Smithers ERS Ltd., U.K., Report No. 3202391, GLP, Not published |
| Guideline(s): | OECD 226 (2016) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication<br>(if vertebrate study) | No |

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | RH-163353 (HHGCP001-00-2) |
| **Purity** | 99.48 % (w/w); enantiomeric ratio 48.6:51.4 (racemate) |
| **Species:** | *Hypoaspis aculeifer* |
| Age: | Adult mites |
| Source: | Bias Labs Ltd., UK |
| Acclimation period: | -- |
| Food: | juvenile collembola *ad-libitum* |
| **Test system** | 50 mm diameter glass vessel with a volume of approximately 120 mL, covered with a screw lid as a minimum, aerated two to three times weekly. Each replicate contained ten female *H. aculeifer* in 20 g dry weight of artificial soil. |
| Soil: | artificial soil, 5% peat |
| **Environmental conditions** | |
| Temperature: | 19.4 – 20.2°C |
| Photoperiod: | 16-hour photoperiod (432 to 609 lux) |
| Soil moisture: | 45 % max. WHC |
| pH: | 5.72 - 6.14 |

| | |
|---|---|
| **Application rate(s)** | 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.i./kg dry soil |
| Negative control: | reverse osmosis (RO) water<br>solvent control (acetone) |
| Positive control: | Dimethoat |
| **Post exposure observation period** | 14 days |
| **Remarks** | None |

The zoxamide metabolite RH-163353 has been studied under laboratory test conditions whether it has harmful effects on the predatory soil mite *Hypoaspis* (Geolaelaps) *aculeifer* (Acari, Laelapidae).

Based on the results of a range-finding study, RH-163353 was evaluated in a bioassay at application rates of 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.i./kg dry soil. These were compared to an untreated (water only) control. A toxic reference item (dimethoate) was included in a separate validation study.

The test item was applied in acetone to a sand carrier. The acetone was allowed to evaporate before the treated sand was mixed with the soil and a required amount of reverse osmosis (RO) water. A solvent control was included in the test.

Ten adult female *H. aculeifer* were introduced in seven replicate vessels per test item treatment and 11 for control. The vessels contained 20 g dry weight of an artificial soil (5% peat). The test organisms were fed with juvenile collembola *ad-libitum.* The moisture content was adjusted to 45% max. WHC. The test vessels were aerated at least twice a week by removing the lid for several seconds. The pH (in 1 M KCl) of the treated substrate, sampled on days 0 and 14 (taken from abiotic vessels) was determined. The test was carried out in a temperature-controlled incubator at 20 ± 2°C and a 16:8 hour light:dark cycle (light intensity of 400 – 800 Lux), measured at least weekly throughout the test.

After 14 days the numbers of the original springtails still surviving and the numbers of any offspring they had produced were recorded. A Tullgren extraction funnel was used; the funnels are designed to extract organisms from substrate using specialist heat lamps with an increasing temperature gradient, prompting the adult and juvenile *H. aculeifer* to burrow downwards into collection pots containing fixation fluid. The fixation fluid from each replicate was emptied into a petri-dish. The collection pots were rinsed out using ca 1–2 mL RO water to ensure all organisms were removed from the collection pots. The number of the originally introduced adult mites, and the juvenile mites produced during the 14-day test, were then counted using a microscope, and recorded per replicate. Furthermore, any observed differences between the morphology of the mites were also recorded, where necessary.

Percentage adult mortality of the *H. aculeifer* in each vessel was calculated and a treatment mean presented. A no observed effect concentration (NOEC) and $LC_{50}$ were determined. Corrected mortality, in comparison to both the water and solvent controls, was calculated using the Abbott's formula. Statistical analysis of the reproduction data, in comparison to both the water and pooled controls, was undertaken using CETIS version 1.8.6.8, based on the nominal test concentrations. The NOEC and LOEC for the effects on survival were determined using a Bonferroni Adj t Test for comparison with the water control and a Wilcoxon/Bonferroni Adj Test (pooled controls) and the ECx values were determined using Linear Interpolation (ICPIN). The NOEC and LOEC for the number of juveniles were determined using a Bonferroni Adj t Test and the $EC_x$ values were determined using Linear Interpolation (ICPIN).

On the day of test item application and at the end of the test four 2 g substrate samples were taken from each treatment for chemical analysis (two to analyse and two to retain). The samples were stored frozen until analysis of the test item soil concentrations. The methods were validated according to SANCO 3029/99 rev. 4 with an LOQ of 0.016 mg/kg. Concentrations of RH-163353 were determined by extract-

ing soil samples with an extraction solvent (acetonitrile/acetone 3:1 v/v containing 1% formic acid), then diluting further with unfortified control sample extract to bring the response within the calibration range. Samples were analysed by liquid chromatography time of flight mass spectrometry (LC-TOF/MS). The analytical procedure (SMV 3202390-01V, and its revisions) was used to determine RH-163353.

The analytical method validation for the enantiomeric ratio analysis for RH-163353 was conducted under Smithers ERS Study Number 3202586 (established analytical procedure, SMV 3202586-01V). A combination of the analytical procedures, SMV 3202390-01V and SMV 3202586-01V were used to assess the enantiomeric ratio of the test substance

## Results and discussion

Environmental conditions stayed within the recommended ranges.

Post application the mean substrate concentrations were 0.8656, 1.4456, 2.9364, 4.4112, 8.2104, 15.5855, 24.2260 and 47.2443 mg a.i./kg dry substrate at application rates of 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.i./kg dry substrate respectively. This is equivalent to 105.56, 98.34, 110.81, 92.67, 95.80, 101.01, 87.21 and 94.49% of nominal at 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.i./kg dry substrate respectively. The recoveries of 87.21-110.81 % of nominal confirm the correct dosing of the test item. On Day 14 the mean substrate concentrations were 0.6869, 1.5427, 2.4617, 3.9928, 7.8009, 12.9666, 25.2966 and 50.8642 mg a.i./kg dry substrate at application rates 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.i./kg dry substrate respectively. This is equivalent to 83.77, 104.95, 92.90, 83.88, 91.03, 84.04, 91.06 and 101.73% of nominal at 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.i./kg dry substrate respectively. The moisture content was adjusted for each individual sample. Recoveries of 83.77-101.73 % of nominal at the end of the test confirm the stability of the test item over 14 days in soil under the test conditions.

At Day 14 there was 45.43, 46.36, 47.01, 46.79, 46.84, 48.66, 48.56 and 48.82% of Isomer A present in the 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.i./kg dry substrate groups respectively in comparison to a mean of 47.66% for the calibration samples. This was equivalent to 95.32, 97.27, 98.63, 98.18, 98.29, 102.10, 101.89 and 102.44% of the calibration mean. There was 54.57, 53.64, 52.99, 53.21, 53.16, 51.34, 51.44 and 51.15% of Isomer B present in the 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.i./kg dry substrate groups respectively in comparison to a mean of 52.34% for the calibration samples. This was equivalent to 104.26, 102.49, 101.24, 101.66, 101.56, 98.09, 98.28 and 97.78% of the calibration mean. The mean ratio for the day 14 samples was 47.31% isomer A and 52.69% of isomer B of RH-163353. Compared to the enantiomeric ratio in the certificate of analysis of 48.6:51.4, this confirms the stability of the isomer ratio of RH-163353.

After 14 days of exposure, there was 12.5, 5.0, 5.0, 15.0, 5.0, 7.5, 12.5 and 5.0% mortality observed in the 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.i./kg dry substrate groups respectively, in comparison to 4.0 % mortality in both the water and solvent controls. Corrected mortality was 8.9, 1.0, 1.0, 11.5, 1.0, 3.6, 8.9 and 1.0% observed in the 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.i./kg dry substrate groups respectively. Based on these results, the NOEC and LOEC for 14-day survival were statistically determined to be 50 mg a.i./kg dry substrate and >50 mg a.i./kg dry substrate, respectively in comparison to both the water and pooled controls. The $LC_{10}$, $LC_{20}$ and $LC_{50}$ values for 14-day survival were all statistically determined to be >50 mg a.i./kg dry substrate in comparison to both the water and pooled controls.

**Table A 99: Mortality of adult *H. aculeifer* after 14 days**

| Treatment (mg a.i./kg dry substrate) | Initial number of introduced *Hypoaspis* | Mean mortality per treatment (%) | Effect in comparison to the water control (%) | Effect in comparison to the solvent control (%) |
|---|---|---|---|---|
| Water control | 50 | 4.0 | N/A | 0.0 |
| Solvent control | 50 | 4.0 | 0.0 | N/A |
| 0.82 | 40 | 12.5 | 8.9 | 8.9 |
| 1.47 | 40 | 5.0 | 1.0 | 1.0 |
| 2.65 | 40 | 5.0 | 1.0 | 1.0 |
| 4.76 | 40 | 15.0 | 11.5 | 11.5 |
| 8.57 | 40 | 5.0 | 1.0 | 1.0 |
| 15.43 | 40 | 7.5 | 3.6 | 3.6 |
| 27.78 | 40 | 12.5 | 8.9 | 8.9 |
| 50 | 40 | 5.0 | 1.0 | 1.0 |

N/A = not applicable

The mean number of juveniles per vessel was 118.0, 126.5, 122.5, 137.0, 162.5, 143.3, 130.0 and 86.3 in the 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.i./kg dry substrate groups respectively, in comparison to 118.4 and 114.8 in the water and solvent controls respectively. At 50 mg a.i./kg dry substrate this corresponded to a reduction of 27.2%, when compared to the water control and 24.9% when compared to the solvent control. Increases of 0.3, 6.8, 3.5, 15.7, 37.2, 21.0 and 9.8% were recorded at 0.82, 1.47, 2.65, 4.76, 8.57, 15.43 and 27.78 mg a.i./kg dry substrate groups respectively, in comparison to the water control. Increases of 2.8, 10.2, 6.7, 19.3, 41.6, 24.8 and 13.2% were recorded at 0.82, 1.47, 2.65, 4.76, 8.57, 15.43 and 27.78 mg a.i./kg dry substrate groups respectively, in comparison to the solvent control.

**Table A 100: Mean number of juveniles at day 14**

| Treatment (mg a.i./kg dry substrate) | Mean number of juveniles[a] | Effect in comparison to the water control (%) | Effect in comparison to the solvent control (%) | Effect in comparison to pooled controls (%) |
|---|---|---|---|---|
| Water control | 118.4 | N/A | +3.1 | +1.5 |
| Solvent control | 114.8 | -3.0 | N/A | -1.5 |
| 0.82 | 118.0 | -0.3 | +2.8 | +1.2 |
| 1.47 | 126.5 | +6.8 | +10.2 | +8.5 |
| 2.65 | 122.5 | +3.5 | +6.7 | +5.1 |
| 4.76 | 137.0 | +15.7 | +19.3 | +17.5 |
| 8.57 | 162.5 | +37.2 | +41.6 | +39.4 |
| 15.43 | 143.3 | +21.0 | +24.8 | +22.9 |
| 27.78 | 130.0 | +9.8 | +13.2 | +11.5 |
| 50 | 86.3 | -27.2 | -24.9 | -26.0 |

N/A = not applicable
[a] pooled control mean = 116.6 juveniles

There were no other observations noted.

**Validity criteria:**

The following validity criteria were met for the study; therefore, these data are considered to be valid.

- The mean mortality of the original parental mites in the control treatment did not exceed 20% over 14 days (actual mean mortality = 4.0% in both water and solvent controls)
- The mean number of juveniles was at least 50 in the controls over 14 days (actual mean number of juveniles = 118.4 and 114.8 in the water and solvent controls respectively)
- The coefficient of variance of the calculated reproduction in the control was 30% or less over 14 days (actual coefficient of variance = 6.56 and 5.86% in the water and solvent controls respectively)

*H. aculeifer* from the same source culture were used in a reference toxicity test with Dimethoate 400 g/L EC, a known toxic substance (performed under in-house GLP Smithers ERS. Study Number 3202598, December 2019). The $EC_{50}$ value was estimated to be 2.043 mg a.i/kg dry substrate.

**Table A 101: NOEC, $EC_{10}$ $EC_{20}$ and $EC_{50}$ values**

| Parameter | Parent mortality | Value on reproduction compared to water control | Value on reproduction compared to pooled controls |
|---|---|---|---|
| | mg a.i./kg dry substrate (95 % confidence limits) | | |
| NOEC | 50 | 27.78 | 27.78 |
| LOEC | >50 | >50 | >50 |
| $LC_{10}/EC_{10}$ | >50<br>(N/A) | 32.19<br>(20.29 - 39.03) | 32.19<br>(24.61 - 38.67) |
| $LC_{20}/EC_{20}$ | >50<br>(N/A) | 38.46<br>(29.08 – N/A) | 38.46<br>(30.19 – 53.4) |
| $LC_{50}/EC_{50}$ | >50<br>(N/A) | >50<br>(N/A) | >50<br>(N/A) |

N/A = not available

## Conclusion

In a laboratory bioassay with RH-163353 and the predatory soil mite *Hypoaspis aculeifer*, assessments of survival and reproductive performance were made over 14 days. After 14 days, based on adult mortality and the numbers of offspring produced, the following endpoints were noted in comparison to pooled controls:

14-day NOEC value for adult *H. aculeifer* survival = 50 mg a.i./kg dry substrate

14-day $LC_{50}$ value for adult *H. aculeifer* survival = >50 mg a.i./kg dry substrate

NOEC value based on reproduction = 27.78 mg a.i./kg dry substrate

$EC_{10}$ value based on reproduction = 32.19 mg a.i./kg dry substrate

$EC_{20}$ value based on reproduction = 38.46 mg a.i./kg dry substrate

$EC_{50}$ value based on reproduction = >50 mg a.i./kg dry substrate

The study is valid.

(Gray J. 2020)

### A 2.4.2.1.6 Study 6 - RH-141455: Effect on *Hypoaspis*

The following study has been provided to Latvia as RMS for zoxamide and cMSs for interzonal evaluation in July 2020.

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 226 and according to the principles of GLP. The study is considered to be reliable and suitable for the risk assessment. |

Reference: **KCP 10.4.2/06**

Report: Gray, J., 2020: RH-141455: Effect on reproduction of *Hypoaspis* (Geolaelaps) *aculeifer*
Gowan Crop Protection Ltd., UK
Smithers ERS Limited, UK, Report No.3202383, GLP, Not published

Guideline(s): OECD 226 (2016)

Deviations: The light period was less than the 16 hours specified.
The analytical data confirmed that the applied dose was in excess of the required 50 mg a.i./kg but showed it was also outside the accepted range of 80-100% of nominal (+29.28% at Day 0 and + 50.01% at Day 14). However, as the dose was in excess of that required it is considered that this confirms no adverse effect on survival or reproduction at the intended dose.
As all the validity criteria were met. Therefore, these deviations were not considered to have had any impact on the integrity or outcome of the study.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): No

**Materials and methods**

| | |
|---|---|
| **Test material (Lot/Batch No.)** | RH-141455 (A19X08291) |
| **Purity** | 92.77% (w/w) |
| **Species:** | *Hypoaspis aculeifer* |
| Age: | Adult mites |
| Source: | Bias Labs Ltd., UK |
| Acclimation period: | -- |
| Food: | juvenile collembola *ad-libitum* |
| **Test system** | 50 mm diameter glass vessel with a volume of approximately 120 mL, covered with a screw lid as a minimum, aerated two to three times weekly. Each replicate contained ten female *H. aculeifer* in 20 g dry weight of artificial soil. |
| Soil: | artificial soil, 5% peat |
| **Environmental conditions** | |
| Temperature: | 20.1 – 20.4°C |
| Photoperiod: | 16-hour photoperiod (642-763 lux) |

| | |
|---|---|
| Soil moisture: | 55 % max. WHC |
| pH: | 7.36 – 7.59 |
| **Application rate(s)** | 50 mg a.i./kg dry soil (limit test) |
| Negative control: | reverse osmosis (RO) water<br>solvent control (acetone) |
| Positive control: | Dimethoat |
| **Post exposure observation period** | 14 days |
| **Remarks** | None |

The zoxamide metabolite RH-141455 has been studied under laboratory test conditions whether it has harmful effects on the predatory soil mite *Hypoaspis* (Geolaelaps) *aculeifer* (Acari, Laelapidae).

Based on the results of a range-finding study, RH-141455 was evaluated in a bioassay at an application rate of 50 mg a.i./kg dry soil (limit test), compared to an untreated (water only) control. A toxic reference item (dimethoate) was included in a separate validation study.

The test item was applied in acetone to a sand carrier. The acetone was allowed to evaporate before the treated sand was mixed with the soil and a required amount of reverse osmosis (RO) water. A solvent control was included in the test.

Ten adult female *H. aculeifer* were introduced in eight replicate vessels per test item treatment and control. The vessels contained 20 g dry weight of an artificial soil (5% peat). The test organisms were fed with juvenile collembola *ad-libitum.* The moisture content was adjusted to 55% max. WHC. The test vessels were aerated at least twice a week by removing the lid for several seconds. The pH (in 1 M KCl) of the treated substrate, sampled on days 0 and 14 (taken from abiotic vessels) was determined. The test was carried out in a temperature-controlled incubator at 20 ± 2°C and a 16:8 hour light:dark cycle (light intensity of 400 – 800 Lux), measured at least weekly throughout the test.

After 14 days the numbers of the original springtails still surviving and the numbers of any offspring they had produced were recorded. A Tullgren extraction funnel was used; the funnels are designed to extract organisms from substrate using specialist heat lamps with an increasing temperature gradient, prompting the adult and juvenile *H. aculeifer* to burrow downwards into collection pots containing fixation fluid. The fixation fluid from each replicate was emptied into a petri-dish. The collection pots were rinsed out using ca 1–2 mL RO water to ensure all organisms were removed from the collection pots. The number of the originally introduced adult mites, and the juvenile mites produced during the 14-day test, were then counted using a microscope, and recorded per replicate. Furthermore, any observed differences between the morphology of the mites were also recorded, where necessary.

Percentage adult mortality of the *H. aculeifer* in each vessel was calculated and a treatment mean presented. A no observed effect concentration (NOEC) and $LC_{50}$ were determined. Corrected mortality, in comparison to both the water and solvent controls, was calculated using the Abbott's formula. Statistical analysis of the reproduction data, in comparison to both the water and pooled controls, was undertaken using CETIS version 1.8.6.8, based on the nominal test concentrations. Further statistical analysis was not undertaken as a limit test was conducted.

On the day of test item application and at the end of the test four 2 g substrate samples were taken from each treatment for chemical analysis (two to analyse and two to retain). The samples were stored frozen until analysis of RH-141455 soil concentrations. The methods were validated according to SANCO 3029/99 rev. 4 with an LOQ of 0.2 mg/kg. Concentrations of RH-141455 were determined by extracting soil samples with an extraction solvent (acetonitrile/acetone 2:1 v/v containing 1% formic acid), then

diluting further with unfortified control sample extract to bring the response within the calibration range. Samples were analysed by liquid chromatography Fourier-transform mass spectrometry (LC-FT/MS). The analytical procedure (SMV 3202383-01V, and its revisions) was used to determine RH-141455.

**Results and discussion**

Environmental conditions stayed within the recommended ranges.

Post application the mean substrate concentration was 64.6394 mg a.i./kg dry substrate at 50 mg a.i./kg dry substrate. This is equivalent to 129.28% of nominal. The moisture content of the samples was corrected using the mean moisture content of the appropriate treatment rate. On Day 14 the mean substrate concentration was 66.80 mg a.i./kg dry substrate at 50 mg a.i./kg dry substrate. This is equivalent to 133.60% of nominal. The recoveries confirm an overdosing of the test item at study start and the stability of the test item over 14 days in soil under the test conditions.

After 14 days of exposure, there was 10.00% mortality observed in the 50 mg a.i./kg dry substrate group, in comparison to 18.75 and 16.25% mortality in the water and solvent controls respectively. Based on these results, the NOEC and $LC_{50}$ for 14-day survival were empirically determined to be 50 mg a.i./kg dry substrate and >50 mg a.i./kg dry substrate, respectively.

**Table A 102: Mortality of adult *H. aculeifer* after 14 days**

| Treatment (mg a.i./kg dry substrate) | Initial number of introduced *Hypoaspis* | Mean mortality per treatment (%) | Effect in comparison to the water control (%) | Effect in comparison to the solvent control (%) |
|---|---|---|---|---|
| Water control | 80 | 18.75 | N/A | 2.99 |
| Solvent control | 80 | 16.25 | 0 | N/A |
| 50 | 80 | 10.00 | 0 | 0 |

N/A = not applicable

The mean number of juveniles per vessel was 125.9 in the 50 mg a.i./kg dry substrate test groups, in comparison to 123.4 and 120.6 in the water and solvent controls respectively. There was no reduction when compared either the water or solvent control. Based on these results, the NOEC for reproduction was empirically determined to be 50 mg a.i./kg dry substrate and the $EC_{10}$, $EC_{20}$ and $EC_{50}$ values for reproduction were empirically determined to be >50 mg a.i./kg dry substrate, respectively.

**Table A 103: Mean number of juveniles at day 14**

| Treatment (mg a.i./kg dry substrate) | Mean number of juveniles[a] | Effect in comparison to the water control (%) | Effect in comparison to the solvent control (%) |
|---|---|---|---|
| Water control | 123.4 | N/A | 0 |
| Solvent control | 120.6 | 2.27 | N/A |
| 50 | 125.9 | 0 | 0 |

N/A = not applicable
Coefficient of Variance for controls = 27.7 and 15.9% for the water and solvent controls respectively

There were no other observations noted.

**Validity criteria:**

The following validity criteria were met for the study; therefore, these data are considered to be valid.

- The mean mortality of the original parental mites in the control treatment did not exceed 20% over 14 days (actual mean mortality = 18.75 and 16.25% in the water and solvent controls respectively)

- The mean number of juveniles was at least 50 in the controls over 14 days (actual mean number of juveniles = 123.4 and 120.6 in the water and solvent controls respectively)
- The coefficient of variance of the calculated reproduction in the control was 30% or less over 14 days (actual coefficient of variance = 27.7 and 15.9% in the water and solvent controls respectively).

*H. aculeifer* from the same source culture were used in a reference toxicity test with Dimethoate 400 g/L EC, a known toxic substance (performed under in-house GLP Smithers ERS. Study Number 3202598, December 2019). The $EC_{50}$ value was estimated to be 2.043 mg a.i/kg dry substrate.

**Conclusion**

In a laboratory bioassay with RH-141455 and the predatory soil mite *Hypoaspis aculeifer,* assessments of survival and reproductive performance were made over 14 days in a limit test. After 14 days, based on adult mortality and the numbers of offspring produced, the following endpoints were noted in comparison to pooled controls:

14-day NOEC value for adult *H. aculeifer* survival = 50 mg a.i./kg dry substrate

14-day $LC_{50}$ value for adult *H. aculeifer* survival = >50 mg a.i./kg dry substrate

NOEC value based on reproduction = 50 mg a.i./kg dry substrate

$EC_{50}$ value based on reproduction = > 50 mg a.i./kg dry substrate

It was not possible to calculate $EC_{10}$ and $EC_{20}$ values since there was no significant effect at the concentration tested (limit test).

The study is valid.

(Gray J. 2020)

### A 2.4.2.1.7 Study 7 - CYMOXANIL 33% + ZOXAMIDE 33% WG: Effects on *Folsomia*

New study with the formulated product 'CYMOXANIL 33% + ZOXAMIDE 33% WG'.

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 232 and according to the principles of GLP. The study is considered to be reliable and suitable for the risk assessment. |

| | |
|---|---|
| **Reference:** | **KCP 10.4.2/07** |
| Report: | Parsons, Ch., 2020: Cymoxanil 33% + Zoxamide 33 % WG (GWN-9823) – A laboratory test to determine the effects of fresh residues on the springtail *Folsomia candida* (Collembola, Isotomidae) in an artificial soil substrate<br>Gowan Crop Protection Ltd., UK, SIPCAM OXON S.p.A, Italy<br>Mambo-Tox Ltd., UK., Report No. GOW-17-3, GLP, Not published |
| Guideline(s): | OECD 232 (2016) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | No |

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | Cymoxanil 33% + Zoxamide 33% WG / GWN-9823 (30AL5020) |
| **Content of a.s.:** | Cymoxanil 33% w/w, Zoxamide 33% w/w (nominal)<br>Cymoxanil 33.2 % w/w, Zoxamide 32.6% w/w (analysed) |
| **Species:** | *Folsomia candida* |
| Age: | 11-12 days old |
| Source: | Bias Labs Ltd., UK |
| Acclimation period: | |
| Food: | Approximately 30 mg of dried granulated baker's yeast ('Easy Bake Yeast'; Allinson, Peterborough, UK) was provided 2-3 times per week, for food. |
| **Test system** | glass jars (approximately 125 mL capacity and 4.5 cm internal diameter), secured with close-fitting, screw-top lids. A 5-mm-diameter hole was made in the lid for ventilation and this was covered with fine nylon netting (80 µm mesh). The arenas were filled with test substrate to a depth of 2-4 cm. |
| Soil | OECD artificial soil, 5% peat |
| **Environmental conditions** | |
| Temperature: | 19.5 – 21.5°C |
| Photoperiod: | 16-hour photoperiod (580-650 lux) |
| Soil moisture: | 50 % max. WHC |
| pH: | 6 ±0.5 |
| **Application rate(s)** | 1000 mg f.p./kg soil dry weight |
| Negative control: | purified water |
| Positive control: | boric acid |
| **Post exposure observation period** | 28 days |
| **Remarks** | None |

The test item Cymoxanil 33% + Zoxamide 33% WG, hereafter referred to by the product code GWN-9823, is a water dispersible granule (WG) formulation containing the active substances cymoxanil at nominally 33% w/w and zoxamide at nominally 33% w/w. The aim of this study was to determine under laboratory test conditions whether the test item has harmful effects on the springtail Folsomia candida Willem (Collembola, Isotomidae). Based on the results of a non-GLP range-finding study, GWN-9823 was evaluated in a bioassay at a single treatment concentration equivalent to 1000 mg test item/kg soil dry weight (corresponding to 332 mg/kg cymoxanil and 326 mg/kg Zoxamide per soil dry weight). This was compared to an untreated (water only) control. A toxic reference item (boric acid) was tested in a separate validation study.

The control treatment and test item treatment, diluted in purified water, were thoroughly mixed into an artificial soil substrate (containing 5% w/w peat), aliquots of which were then transferred into small, ventilated, glass jar arenas (n = 8 per treatment). Ten juvenile F. candida (11-12 days old) were introduced into each jar arena. Dry granulated yeast was provided on the soil surface as food and this was

replenished 14 days after treatment (DAT). At 28 DAT, both the numbers of surviving springtails originally introduced and the numbers of any offspring they had produced were recorded.

At 28 DAT, both the number of surviving adults and of F1 progeny (i.e. juvenile test springtails) in each test arena were assessed at 28 DAT. For this assessment, the test substrate from each arena was tipped into a tray (approximately 11 cm x 17 cm in area and 6 cm in depth). Water (approx. 200 mL) was then added to the substrate and stirred gently, so that the soil sank and the springtails floated to the surface. Any adult springtails floating on the water were counted and removed. Black ink was then added to the water and the numbers of any nymphs (smaller in size to adults) left in each arena were assessed. The ink darkened the water so that it contrasted with the light-coloured springtails floating on the water surface. A grill was held over the surface to aid with counting using a binocular microscope. It was assumed that any adult springtails that were recovered would have been those originally introduced and that any shortfall in the original number was an indication that they had died during the bioassay.

Statistical analyses were performed using validated computer software, namely SPSS (IBM Corp., 2016). The percentage mortality of the springtails originally introduced was calculated for the test item treatment, both before and after correction for any control treatment losses using Abbott's formula. The 28-day mortality data for the individual test-item treatment were compared to those for the control using Fisher's Exact Test ($\alpha = 0.05$) (Sokal and Rohlf, 1981). The results were used to determine the no observed effect concentration (NOEC) with respect to mite survival. The mean number of offspring produced per replicate and the standard deviation were calculated for the control and test item treatment. In addition, the percentage difference in reproductive performance in the test-item treatment group, compared to the control group, was calculated. The data from the reproduction assessments in the individual treatments were checked for normality (Shapiro-Wilk test, $\alpha = 0.05$) and for equality of variances (Levene's test, $\alpha = 0.05$). As normality and homogeneity could be assumed, the numbers of F1 progeny produced in the test-item treatment was compared to numbers in the control, using t-test for independent samples ($\alpha = 0.05$). A NOEC with respect to springtail reproduction was determined.

Three separate samples of approximately 25-30 g of soil were preserved from both the test-item treatment and the control treatment. The soil samples' original moisture content was restored to 50% WHC, by the addition of purified water, before transfer to labelled plastic sample pots for freezing. The samples were shipped frozen to Biochem Agrar for analytical verification of the test item concentrations in the soil with a method fully validated according to SANCO/3029/99 rev. 4 under project number 18 35 CRX 0026.

## Results and discussion

Environmental conditions stayed within the recommended ranges.

The nominal initial test item concentration in the freshly prepared soil specimen was analytically confirmed (recovery of 102% for cymoxanil and 103% for zoxamide). After 28 days, the cymoxanil concentration had declined to 73% of nominal. The zoxamide concentration stayed within the range of 96–103% of the nominal concentration, throughout the whole study period.

At 28 DAT, there was 6% mortality in the control treatment, compared with 5% mortality (-1% corrected mortality) in the 1000 mg test item/kg soil dry weight treatment concentration of GWN-9823. The test item result was not statistically significantly different from the control (Fisher's exact test, $\alpha = 0.05$). The NOEC value was therefore 1000 mg test item/kg soil dry weight.

**Table A 104: Percentage mortality of adult springtails at 28 days**

| Treatment | Test item concentration (mg/kg soil dry weight= | % mortality [a)] | Corrected % mortality [b)] |
|---|---|---|---|
| Control | - | 6 | - |
| GWN-9823 | 1000 | 5 | -1 |

[a] Mortality in the treatments was compared using Fisher's exact test (α = 0.05), but there was no statistically significant difference.
[b] Data corrected for the control mortality using Abbott's formula; a negative value indicates a decrease in mortality relative to the control.

At 28 DAT, based on the number of offspring produced, the $EC_{50}$ and $EC_{20}$ values for GWN-9823 were >1000 mg test item/kg soil dry weight and the $EC_{10}$ value was not determined. Statistical comparisons with the control indicated that the overall NOEC with respect to both springtail survival and reproduction was 1000 mg test item/kg soil dry weight (equivalent to 332 mg cymoxanil and 326 mg Zoxamide per kg soil dry weight, based on the measured a.s. content of 33.2% w/w cymoxanil and 32.6% w/w zoxamide in the test item), the only concentration tested.

The mean number of progeny produced per replicate was 351 in the control and 303 in the 1000 mg test item/kg soil dry weight treatment concentration of GWN-9823. The percentage change in progeny, relative to the control, was a decrease of 14% for the test item treatment. Therefore, the EC50 and EC20 values were > 1000 mg test item/kg soil dry weight and the EC10 value was not be determined. The test item result was not statistically significantly different from the control (t-test for independent samples, α = 0.05). Therefore, the NOEC value was 1000 mg test item/kg soil dry weight.

**Table A 105: Mean number of juvenile springtails produced after 28 days**

| Treatment | Test item concentration (mg/kg soil dry weight) | Mean progeny per replicate [a] | % change relative to the control [b] |
|---|---|---|---|
| Control | - | 351 | - |
| GWN-9823 | 1000 | 303 | 14 |

[a] Treatments were compared by t-test for independent samples (α = 0.05), but there was no statistically significant difference.
[b] A positive value indicates a decrease in reproduction, relative to the control.

All validity criteria were met:

- control treatment mortality should not exceed 20% at the end of the test (actual value of control in test = 6%).
- the mean number of juveniles recorded in the control treatment should exceed 100 per replicate at the end of the test (actual value of control in test = 351).
- the coefficient of variation of reproduction in the control should not exceed 30% (actual value of control in test = 25.3%).
- the efficiency of the method used to extract the springtails in this test should be > 95%. In a separate test, carried out by the Test Facility, this was determined to be 100% for the adult springtails and 98.3% for the juvenile springtails (Geary, 2014).

## Conclusion

In a laboratory bioassay with GWN-9823 and the springtail *Folsomia candida*, assessments of survival and reproductive performance were made over 28 days. At 28 DAT, based on the numbers of offspring produced, the EC50 and EC20 values for GWN-9823 were >1000 mg test item/kg soil dry weight. The EC10 value was not determined. The NOEC value for both survival and reproduction was 1000 mg test item/kg soil dry weight (equivalent to 332 mg cymoxanil/kg soil dry weight and 326 mg zoxamide/kg soil dry weight, based on the measured content of a.s. in the test item), the only concentration tested.

(Parsons Ch. 2020)

### A 2.4.2.1.8 Study 8 - CYMOXANIL 33% + ZOXAMIDE 33% WG: Effects on *Hy-*

***poaspis***

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 226 and according to the principles of GLP. The study is considered to be reliable and suitable for the risk assessment. |

| | |
|---|---|
| **Reference:** | **KCP 10.4.2/08** |
| Report | Parsons, Ch., 2020: Cymoxanil 33% + Zoxamide 33% – A laboratory test to determine the effects of fresh residues on the predatory mite *Hypoaspis aculeifer* (Acari: Laelapidae)<br>Gowan Crop Protection Ltd, UK, SIPCAM OXON S.p.A., Italy<br>Mambo-Tox Ltd., UK., Report No. GOW-17-4, GLP, Not published |
| Guideline(s): | OECD 226 (2008) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication<br>(if vertebrate study) | No |

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | Cymoxanil 33% + Zoxamide 33 % WG / GWN-9823 (30AL5020) |
| **Active substances content (nominal):** | Cymoxanil 33% w/w, Zoxamide 33 % w/w (nominal)<br>Cymoxanil 33.2% w/w, Zoxamide 32.6 % w/w (measured) |
| **Species:** | *Hypoaspis aculeifer* |
| Age: | Adult mites (approximately 7-14 days after becoming adult) |
| Source: | Bias Labs Ltd., UK |
| Acclimation period: | |
| Food: | cheese mites [*Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae)] were provided as food for the predatory mites |
| **Test system** | 60 mL capacity glass jars (5.5 cm tall x 5.2 cm outer diameter, 4.4 cm inner diameter), with screw-top lids. An 8-mm-diameter hole was made in the lid for ventilation and this was covered with fine nylon netting (80 µm mesh size). |
| Soil | OECD artificial soil, 5% peat |
| **Environmental conditions** | |
| Temperature: | 19.2 - 21.6 °C |
| Photoperiod: | 16-hour photoperiod (600 - 690 lux) |
| Soil moisture: | 50 % max. WHC |
| pH: | 6 ±0.5 |
| **Application rate(s)** | 1000 mg/kg soil dry weight |

| | |
|---|---|
| Negative control: | purified water |
| Positive control: | Dimethoate |
| **Post exposure observation period** | 14 days |
| **Remarks** | None |

The test item in this study was Cymoxanil 33% + Zoxamide 33 % WG, hereafter referred to by the product code GWN-9823, a water-dispersible granule formulation containing the active substances cymoxanil (nominally 33% w/w) and zoxamide (nominally 33% w/w). The aim of this study was to determine under laboratory test conditions whether the test item has harmful effects on the predatory soil mite Hypoaspis aculeifer (Canestrini) (Acari, Laelapidae). Based on the results of a non-GLP range-finding study, GWN-9823 was evaluated at a single concentration equivalent to 1000 mg product/kg soil dry weight (corresponding to 332 mg/kg cymoxanil and 326 mg/kg Zoxamide per soil dry weight). This was compared to an untreated (water only) control. A toxic reference item (dimethoate) was included in a separate validation study

The control treatment and test item treatment, diluted in purified water, were thoroughly mixed into an artificial soil substrate (containing 5% w/w peat), aliquots of which were then transferred into small, ventilated, glass jar arenas (n = 8 per treatment). Ten female soil mites (approximately 7-14 days after becoming adult) were then introduced into each arena. Cheese mites (Tyrophagus putrescentiae (Schrank)) and springtails (Folsomia candida (Willem)) were provided as food for the predatory soil mites and were replenished *ad libitum*. The moisture content of the soil was adjusted to approx. 50 g max. WHC. The pH of soil in one of these abiotic replicates was measured according to ISO 10390 (ISO, 2005) (in the presence of 1 mol/L KCl) at the start of the test, using a GPH 014 meter (Greisinger Electronic GmbH, Germany). The other additional arena was maintained alongside those used in the bioassay and the pH of the soil was measured at the end of the study. The test was carried out under environmentally controlled conditions.

At 14 days after treatment (DAT), both the number of surviving adults and of F1 progeny (i.e. juvenile test mites) in each test arena were assessed at 14 DAT. For this assessment, the soil from each arena was placed into individual Tullgren funnel apparatus. This consisted of a metal mesh (1-1.5mm) sieve suspended over a funnel. Above the funnel was fitted a light-bulb (25 Watt, with a 24 h photoperiod). Over a two-day period, the heat of the bulbs slowly dried the soil from the top, forcing the H. aculeifer to move downwards until they fell from the base of the funnels into collecting vials placed beneath. These vials contained 70% v/v methyl alcohol in which the mites drowned and were preserved.

Statistical analyses were performed using validated computer software, namely SPSS (IBM Corp., 2016). The percentage mortality of the mites originally introduced was calculated for the test item treatment, both before and after correction for any control treatment losses using Abbott's formula. The 14-day mortality data were compared to those for the control using Fisher's Exact Test ($\alpha = 0.05$) (Sokal and Rohlf, 1981). The results were used to determine the no observed effect concentration (NOEC) with respect to mite survival. The mean number of offspring produced per replicate and the standard deviation were calculated for the control and test item treatment. In addition, the percentage difference in reproductive performance in the test-item treatment group, compared to the control group, was calculated. The data from the reproduction assessments in the individual treatments were checked for normality (Shapiro-Wilk test, $\alpha = 0.05$) and for equality of variances (Levene's test, $\alpha = 0.05$). The numbers of $F_1$ progeny produced in the test-item treatment was compared to numbers in the control, using t-test for independent samples ($\alpha = 0.05$) (Fowler et al., 1998). A NOEC with respect to springtail reproduction was determined.

Three separate samples of approximately 20-25 g of soil were preserved from both the test-item treatment and the control treatment for analytical verification of the concentration of test item present in the treated soil. The 0 DAT samples were frozen immediately after preparation and the remaining samples were kept alongside the test arenas until 14 DAT when they were made up to 50% WHC before being frozen. The samples were stored in a laboratory freezer at ≤ -20ºC until transferred (in a frozen state and surrounded with dry ice) to the Test Site by courier 29 days after test sample preparation. Samples were analysed after a maximum total freezer storage period of 107 days after sampling. The samples were shipped frozen to Biochem Agrar for analytical verification of the test item concentrations in the soil with a method fully validated according to SANCO/3029/99 rev. 4 under project number 18 35 CRX 0027.

## Results and discussion

Environmental conditions stayed within the recommended ranges.

The nominal initial test item concentration in the freshly prepared soil specimen was analytically confirmed (recovery of 87% for cymoxanil and 90% for zoxamide). After 14 days the cymoxanil concentration had declined to 76% of nominal. The zoxamide concentrations stayed within the range of 90-94% of the nominal concentration throughout the whole study period.

At 14 DAT, there was 6% mortality in the control treatment, compared with 6% mortality (0% corrected mortality) in the 1000 mg test item/kg soil dry weight treatment concentration of GWN-9823. The result for the test item treatment was not statistically significantly different from the control (Fisher's exact test, $\alpha = 0.05$). The NOEC value was therefore 1000 mg test item/kg soil dry weight.

**Table A 106: Mortality of adult *H. aculeifer* after 14 days**

| Treatment | Test item concentration (mg/kg soil dry weight) | % mortality [a)] | Corrected % mortality [b)] |
|---|---|---|---|
| Control | - | 6 | - |
| GWN-9823 | 1000 | 6 | 0 |

a) Mortality in the test item treatment was compared to the control using Fisher's exact binomial test (one-sided, > control, $\alpha = 0.05$), but there was no statistically significant difference.

b) Data corrected for the control mortality using Abbott's formula; a negative value indicates a decrease in mortality relative to the control.

The mean number of progeny produced per replicate was 235 in the control and 227 in the 1000 mg test item/kg soil dry weight treatment concentration of GWN-9823. The percentage change in progeny, relative to the control, was a decrease of 3.4% for the test item treatment. Therefore, the $EC_{50}$, $EC_{20}$ and $EC_{10}$ values were all > 1000 mg test item/kg soil dry weight. The result for the test item treatment was not statistically significantly different from the control (t-test for independent samples, $\alpha = 0.05$). Therefore, the NOEC value was 1000 mg test item/kg soil dry weight.

**Table A 107: Mean number of juveniles produced after 14 days**

| Treatment | Test item concentration (mg/kg soil dry weight) | Mean progeny per replicate [a)] | % change relative to the control [b)] |
|---|---|---|---|
| Control | - | 235 | - |
| 1000 | 1000 | 227 | 3.4 |

a) Treatments were compared by Student's t-test for homogenous variances ($\alpha = 0.05$), but there was no statistically significant difference.

b) A positive value indicates a decrease in reproduction, relative to the control.

All validity criteria were met:

- control treatment mortality should not exceed 20% at the end of the test (actual value of control in test = 6%).

- the mean number of juveniles recorded in the control treatment should be at least 50 per replicate at the end of the test (actual value of control in test = 235).
- the coefficient of variation of reproduction in the control should not exceed 30% (actual value of control in test = 5.7%).
- the efficiency of the method used to extract the mites in this test should be ≥90%. In a separate test, carried out by the Test Facility, this was determined to be 96.2% (98.3% for the adult female mites and 94.0% for the juvenile mites) (Geary, 2017).

**Conclusion**

In a laboratory bioassay with GWN-9823 and the predatory soil mite *Hypoaspis aculeifer*, assessments of survival and reproductive performance were made over 14 days. At 14 DAT, based on the numbers of offspring produced, the $EC_{50}$, $EC_{20}$ and $EC_{10}$ values for GWN-9823 were all >1000 mg test item/kg soil dry weight. The NOEC value for both survival and reproduction was 1000 mg test item/kg soil dry weight (equivalent to 332 mg cymoxanil and 326 mg zoxamide per kg soil dry weight, based on the measured a.s. content in the test item), the only concentration tested.

(Parsons Ch. 2020)

### A 2.4.2.2 KCP 10.4.2.1 Species level testing

### A 2.4.2.3 KCP 10.4.2.2 Higher tier testing

## A 2.5 KCP 10.5 Effects on soil nitrogen transformation

EFSA (2017) has requested *"Further data to address the risk to soil microorganisms for metabolites RH-127450, RH-24549, RH-163353 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5)."* These studies have been performed and are presented hereafter.

The following study has been provided to Latvia as RMS for zoxamide and cMSs for interzonal evaluation in July 2020.

### A 2.5.1.1.1 Study 1 - RH-127450: Soil nitrogen transformation test

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 216 and according to the principles of GLP. The study is considered to be reliable and suitable for the risk assessment |

| | |
|---|---|
| Reference: | **KCP 10.5/01** |
| Report | Jarrom, R., 2019: RH-127450: Soil nitrogen transformation test<br>Gowan Crop Protection Ltd., UK<br>Smithers ERS Ltd., UK, Report No. 3202377, GLP, Not published |
| Guideline(s): | OECD 216 (2000) |
| Deviations: | The protocol contained an error that stated the soil would be collected in accordance with ISO 10381-6. This is a previous revision of ISO 18400-102. This had no impact as the soil was collected in accordance with the most up to date revision. |

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) No

**Materials and methods**

| | |
|---|---|
| **Test material (Lot/Batch No.)** | RH-127450 (HHGCP002-00-1) |
| Purity: | 99.22% |
| **Test species** | Micro-organisms naturally occurring in biologically active soil |
| **Test soil** | Lufa 2.3 |
| Source: | LUFA Speyer, Germany |
| Soil batch no.: | F23 1219 (Lufa 19/002) |
| Date of sampling: | 19th March 2019 |
| Fertilisers: | no during ≥ 4 years |
| Pesticides: | no during ≥ 4 years |
| Nitrogen content (% N): | 0.08 ± 0.02 |
| Organic carbon content (% C): | 0.66 ± 0.07 |
| Microbial biomass (% of organic C): | 1.5 |
| Cation exchange capacity (meq/100g): | 7.3 ± 1.1 |
| pH: | 5.6 |
| WHCmax (%): | 34.9 ± 1.8 |
| **Soil type (USDA)** | sandy loam |
| Clay (%) (< 0.002 mm): | 7.6 ± 0.5 |
| Silt (%) (0.002-0.050 mm): | 33.3 ± 0.6 |
| Sand (%) (0.050-2.0 mm): | 59.1 ± 0.4 |
| **Test vessels** | 500 mL amber glass jars |
| Filling: | 100 g soil dry weight per vessel |
| Pre-incubation: | 15 days under test environmental conditions |
| **Environmental conditions** | |
| Soil moisture: | approx. 50 % of max. WHC |
| Temperature: | 20 ± 2°C |
| Air: | After treatment with the test substance, the test vessels were sealed and the lid perforated to allow air exchange. |
| Light conditions: | Dark |
| **Application rate(s)** | 0.039 and 0.195 mg/kg dry soil |
| **Post exposure observation period** | 28 days |
| **Remarks** | None |

A fresh sample of LUFA 2.3 soil with a microbial biomass of 1.5 % of its organic carbon content was used for the study. The history of the soil batch demonstrated that no fertiliser and plant protection products were used over at least the last 4 years.

The effect of the test item RH-127450 on soil micro-organisms has been studied by investigation of the $NO_3$-N formation in a field fresh arable LUFA 2.3 soil amended with a nitrogen source, powdered Lucerne. Results for a test item treated soil were compared to the results of a non-treated soil.

Soil equivalent to 400 g dry weight was weighed out. The test substance, incorporated in sand, was mixed by means of an electric hand mixer with the sand along with water to adjust the soil moisture to 50% maximum WHC. 2.0 g of powdered Lucerne (alfalfa) was also added (concentration in soil 0.5%). Application rates were 0.039 and 0.195 mg/kg dry soil. The test vessels were sealed and the lid perforated to allow air exchange. Triplicate samples of 100 g each per test rate and control were incubated for 28 days under environmentally controlled conditions.

After test item application on day 0, and after moisture adjustment on day 7, 14 and 28 of incubation, soil samples were taken on day 0 and at 7, 14 and 28 days after application from each test vessel. The samples were extracted with potassium chloride (0.1 M KCl, approximately 35 mL) and extract aliquots of 1.0 mL mixed with each 0.20 mL of Hach LCK 339 solution to analyse their $NO_3$-N content using a Hach Lange DR 3900 spectrophotometer. Two aliquots per sample were measured. The Limit of Quantification (LOQ) for this technique was 0.23 mg/L $NO_3$-N.

The data was subjected to statistical analysis (Dunnett Multiple Comparison Test) to determine the 28-day nitrate production NOEC and LOEC, using CETIS version 1.8.6.8. As there was no effect up to and including the highest test item concentration, $EC_{10}$, $EC_{25}$ and $EC_{50}$ values could not be calculated.

**Results and discussion**

Soil temperature remained within the required range of 20 ± 2 °C during incubation.

Over a 28-day period, the test substance had no significant effects on the amount of the nitrate produced in the soil type tested.

**Table A 108: Mean amount of nitrate measured over 28 days**

| Treatment (mg/kg dry soil) | Mean Nitrate Nitrogen $NO_3$-N (mg N/kg dry soil) | | | | Mean Nitrate $NO_3^-$ (mg N/kg dry soil) | | | | Difference Compared to the Control (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day 0 | Day 7 | Day 14 | Day 28 | Day 0 | Day 7 | Day 14 | Day 28 | Day 7 | Day 14 | Day 28 |
| Control (0) | 8.93 | 1..06 | 17.30 | 26.84 | 39.3 | 4.68 | 76.14 | 118.12 | - | NA | - |
| 0.039 | 9.35 | 1.72 | 21.03 | 26.53 | 41.12 | 7.56 | 92.52 | 116.75 | 61.5 | 21.5 | -1.2 |
| 0.195 | 9.24 | 1.28 | 18.42 | 26.08 | 40.67 | 5.65 | 81.06 | 114.77 | 20.8 | 6.5 | -2.8 |

NA = not applicable
1 mg/kg nitrate nitrogen ($NO_3^-$N) = 4.4 mg/kg nitrate ($NO_3^-$)
Negative percentage difference compared to the control indicates lower nitrate production than the control.

**Table A 109: Mean amount of nitrate produced per day in the test soil**

| Treatment (mg/kg dry soil) | Mean Nitrate Nitrogen $NO_3^-$N (mg N/kg dry soil) | | | Mean Nitrate $NO_3^-$ (mg N/kg dry soil) | | | Difference Compared to the Control (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Day 7 | Day 14 | Day 28 | Day 7 | Day 14 | Day 28 | Day 7 | Day 14 | Day 28 |
| Control | -1-12 | 0-60 | 0-64 | -4-95 | 2-63 | 2-81 | - | NA | - |
| 0.039 | -1-09 | 0-83 | 0-61 | -4-80 | 3-67 | 2-70 | -3-0 | 39-5 | -4-1 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 0.195 | -1.14 | 0.66 | 0.60 | -5.00 | 2.88 | 2.65 | 1.1 | 9.6 | -6.0 |

NA = not applicable
1 mg/kg nitrate nitrogen ($NO_3^-N$) = 4.4 mg/kg nitrate ($NO_3^-$)
Negative percentage difference compared to the control indicates lower nitrate production than the control.

The validity criterion was met:

The variation in $NO_3$-N concentrations between replicate control vessel samples was <15% on day 0, 7, 14 and 28. The coefficient of variation was calculated to be 2.92, 11.45, 12.57 and 9.24% for the day 0, 7, 14 and 28 extractions, respectively.

**Conclusions**

The effect of the test substance RH-127450 on soil micro-organisms has been studied by investigation of the $NO_3$-N formation in a field fresh arable LUFA 2.3 soil amended with a nitrogen source, powdered Lucerne. Test results of the test item treated soil were compared to the results of a non-treated soil.

On day 28 after test start both treatment groups showed < 25% change in nitrate production compared to the control.

The NOEC for nitrate concentrations on day 28 was statistically determined to be 0.195 mg/kg dry soil, the LOEC for nitrate concentrations on day 28 was statistically determined to be >0.195 mg/kg dry soil.

It was not possible to calculate $EC_{10}$, $EC_{25}$ and $EC_{50}$ values since there was no significant effect at the highest concentration in the test.

The study is valid.

(Jarrom R. 2019)

### A 2.5.1.1.2 Study 2 - RH-24549: Soil nitrogen transformation test

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 216 and according to the principles of GLP. The study is considered to be reliable and suitable for the risk assessment |

| | |
|---|---|
| Reference: | **KCP 10.5/02** |
| Report | Jarrom, R., 2019: RH-24549: Soil nitrogen transformation test<br>Gowan Crop Protection Ltd., UK<br>Smithers ERS Ltd., UK, Report No. 3202396, GLP, Not published |
| Guideline(s): | OECD 216 (2000) |
| Deviations: | The protocol contained an error that stated the soil would be collected in accordance with ISO 10381-6. This is a previous revision of ISO 18400-102. This had no impact as the soil was collected in accordance with the most up to date revision. |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication<br>(if vertebrate study) | No |

**Materials and methods**

| | |
|---|---|
| **Test material (Lot/Batch No.)** | RH-24549 (FCC25806) |

| | |
|---|---|
| Purity: | 99.59 % (w/w) |
| **Test species** | Micro-organisms naturally occurring in biologically active soil |
| **Test soil** | Lufa 2.3 |
| Source: | LUFA Speyer, Germany |
| Soil batch no.: | F23 1219 (Lufa 19/002) |
| Date of sampling: | 19th March 2019 |
| Fertilisers: | no during ≥ 4 years |
| Pesticides: | no during ≥ 4 years |
| Nitrogen content (% N): | 0.08 ± 0.02 |
| Organic carbon content (% C): | 0.66 ± 0.07 |
| Microbial biomass (% of organic C): | 1.5 |
| Cation exchange capacity (meq/100g): | 7.3 ± 1.1 |
| pH: | 5.6 |
| WHCmax (%): | 34.9 ± 1.8 |
| **Soil type (USDA)** | sandy loam |
| Clay (%) (< 0.002 mm): | 7.6 ± 0.5 |
| Silt (%) (0.002-0.050 mm): | 33.3 ± 0.6 |
| Sand (%) (0.050-2.0 mm): | 59.1 ± 0.4 |
| **Test vessels** | 500 mL amber glass jars |
| Filling: | 100 g soil dry weight per vessel |
| Pre-incubation: | 8 days under test environmental conditions |
| **Environmental conditions** | |
| Soil moisture: | approx. 45 % of max. WHC |
| Temperature: | 20 ± 2°C |
| Air: | After treatment with the test substance, the test vessels were sealed and the lid perforated to allow air exchange. |
| Light conditions: | Dark |
| **Application rate(s)** | 0.070 and 0.350 mg/kg dry soil |
| **Post exposure observation period** | 28 days |
| **Remarks** | None |

A fresh sample of LUFA 2.3 soil with a microbial biomass of 1.5 % of its organic carbon content was used for the study. The history of the soil batch demonstrated that no fertiliser and plant protection products were used over at least the last 4 years.

The effect of the test item RH-24549 on soil micro-organisms has been studied by investigation of the $NO_3$-N formation in a field fresh arable LUFA 2.3 soil amended with a nitrogen source, powdered Lucerne. Results for a test item treated soil were compared to the results of a non-treated soil.

Soil equivalent to 400 g dry weight was weighed out. The test substance, incorporated in sand, was mixed by means of an electric hand mixer with the sand along with water to adjust the soil moisture to 45% maximum WHC. 2.0 g of powdered Lucerne (alfalfa) was also added (concentration in soil 0.5%). Appli-

cation rates were 0.070 and 0.350 mg/kg dry soil. The test vessels were sealed and the lid perforated to allow air exchange. Triplicate samples of 100 g each per test rate and control were incubated for 28 days under environmentally controlled conditions.

After test item application on day 0, and after moisture adjustment on day 7, 14 and 28 of incubation, soil samples were taken on day 0 and at 7, 14 and 28 days after application from each test vessel. The samples were extracted with potassium chloride (0.1 M KCl, approximately 35 mL) and extract aliquots of 1.0 mL mixed with each 0.20 mL of Hach LCK 339 solution to analyse their $NO_3$-N content using a Hach Lange DR 3900 spectrophotometer. Two aliquots per sample were measured. The Limit of Quantification (LOQ) for this technique was 0.23 mg/L $NO_3$-N.

The data was subjected to statistical analysis (Dunnett Multiple Comparison Test) to determine the 28-day nitrate production NOEC and LOEC, using CETIS version 1.8.6.8. As there was no effect up to and including the highest test item concentration, $EC_{10}$, $EC_{25}$ and $EC_{50}$ values could not be calculated.

**Results and discussion**

Soil temperature remained within the required range of 20 ± 2 °C during incubation.

Over a 28-day period, the test substance had no significant effects on the amount of the nitrate produced in the soil type tested.

**Table A 110: Mean amount of nitrate measured over 28 days**

| Treatment (mg/kg dry soil) | Mean Nitrate-Nitrogen $NO_3^-N$ (mg N/kg dry soil) | | | | Mean Nitrate $NO_3^-$ (mg N/kg dry soil) | | | | Difference compared to the Control (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day 0 | Day 7 | Day 14 | Day 28 | Day 0 | Day 7 | Day 14 | Day 28 | Day 7 | Day 14 | Day 28 |
| Control | 10.09 | 7.10 | 28.87 | 34.70 | 44.39 | 31.26 | 127.02 | 152.68 | - | NA | - |
| 0.070 | 8.82 | 6.98 | 30.24 | 35.78 | 38.79 | 30.73 | 133.08 | 157.44 | -1.7 | 4.8 | 3.1 |
| 0.350 | 10.78 | 7.12 | 27.75 | 36.20 | 47.42 | 31.33 | 122.10 | 159.26 | 0.2 | -3.9 | 4.3 |

NA = not applicable
1 mg/kg nitrate nitrogen ($NO_3^-N$) = 4.4 mg/kg nitrate ($NO_3^-$)
Negative percentage difference compared to the control indicates lower nitrate production than the control.

**Table A 111: Mean amount of nitrate produced per day in the test soil**

| Treatment (mg/kg dry soil) | Mean Nitrate-Nitrogen $NO_3^-N$ (mg N/kg dry soil) | | | Mean Nitrate $NO_3^-$ (mg N/kg dry soil) | | | Difference compared to the Control (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Day 7 | Day 14 | Day 28 | Day 7 | Day 14 | Day 28 | Day 7 | Day 14 | Day 28 |
| Control | -0.43 | 1.34 | 0.88 | -1.88 | 5.90 | 3.87 | - | NA | - |
| 0.070 | -0.26 | 1.53 | 0.96 | -1.15 | 6.73 | 4.24 | -38.6 | 14.1 | 9.6 |
| 0.350 | -0.52 | 1.21 | 0.91 | -2.30 | 5.33 | 3.99 | 22.5 | -9.6 | 3.3 |

NA = not applicable
1 mg/kg nitrate nitrogen ($NO_3^-N$) = 4.4 mg/kg nitrate ($NO_3^-$)
Negative percentage difference compared to the control indicates lower nitrate production than the control.

The validity criterion was met:

The variation in $NO_3$-N concentrations between replicate control vessel samples was <15% on day 0, 7, 14 and 28. The coefficient of variation was calculated to be 7.64, 1.92, 4.23 and 7.73% for the day 0, 7, 14 and 28 extractions, respectively.

**Conclusions**

The effect of the test substance RH-24549 on soil micro-organisms has been studied by investigation of the $NO_3$-N formation in a field fresh arable LUFA 2.3 soil amended with a nitrogen source, powdered Lucerne. Test results of the test item treated soil were compared to the results of a non-treated soil.

On day 28 after test start both treatment groups showed < 25% change in nitrate production compared to the control.

The NOEC for nitrate concentrations on day 28 was statistically determined to be at 0.350 mg/kg dry soil, the LOEC for nitrate concentrations on day 28 was statistically determined to be >0.350 mg/kg dry soil.

It was not possible to calculate $EC_{10}$, $EC_{25}$ and $EC_{50}$ values since there was no significant effect at the highest concentration in the test.

The study is valid.

(Jarrom R. 2019)

### A 2.5.1.1.3 Study 3 - RH-163353: Soil nitrogen transformation test

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 216 and according to the principles of GLP. The study is considered to be reliable and suitable for the risk assessment |

| | |
|---|---|
| Reference: | **KCP 10.5/03** |
| Report | Jarrom, R., 2020: RH-163353: Soil nitrogen transformation test<br>Gowan Crop Protection Ltd., UK<br>Smithers ERS Limited, UK, Report No.3202392, GLP, Not published |
| Guideline(s): | OECD 216 (2000) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication<br>(if vertebrate study) | No |

**Materials and methods**

| | |
|---|---|
| **Test material (Lot/Batch No.)** | RH-163353 (HHGCP001-00-2) |
| Purity: | 99.48 % (w/w); enantiomeric ratio 48.6:51.4 (racemate) |
| **Test species** | Micro-organisms naturally occurring in biologically active soil |
| **Test soil** | Lufa 2.3 |
| Source: | LUFA Speyer, Germany |
| Soil batch no.: | F23 4119 (Lufa 19/003) |
| Date of sampling: | 7th October 2019 |
| Fertilisers: | no during ≥ 4 years |
| Pesticides: | no during ≥ 4 years |
| Nitrogen content (% N): | 0.07 ± 0.02 |

| | |
|---|---|
| Organic carbon content (% C): | 0.65 ± 0.08 |
| Microbial biomass (% of organic C): | 3.8 |
| Cation exchange capacity (meq/100g): | 6.8 ± 1.4 |
| pH: | 5.99 |
| WHCmax (%): | 35.2 ± 1.8 |
| **Soil type (USDA)** | sandy loam |
| Clay (%) (< 0.002 mm): | 7.3 ± 0.9 |
| Silt (%) (0.002-0.050 mm): | 33.3 ± 0.6 |
| Sand (%) (0.050-2.0 mm): | 59.4 ± 0.7 |
| **Test vessels** | 500 mL amber glass jars |
| Filling: | 100 g soil dry weight per vessel |
| Pre-incubation: | 8 days under test environmental conditions |
| **Environmental conditions** | |
| Soil moisture: | approx. 45 % of max. WHC |
| Temperature: | 20 ± 2°C |
| Air: | After treatment with the test substance, the test vessels were sealed and the lid perforated to allow air exchange. |
| Light conditions: | Dark |
| **Application rate(s)** | 0.073 and 0.365 mg/kg dry soil |
| **Post exposure observation period** | 28 days |
| **Remarks** | None |

A fresh sample of LUFA 2.3 soil with a microbial biomass of 3.8 % of its organic carbon content was used for the study. The history of the soil batch demonstrated that no fertiliser and plant protection products were used over at least the last 4 years.

The effect of the test item RH-163353 on soil micro-organisms has been studied by investigation of the $NO_3$-N formation in a field fresh arable LUFA 2.3 soil amended with a nitrogen source, powdered Lucerne. Results for a test item treated soil were compared to the results of a non-treated soil.

Soil equivalent to 400 g dry weight was weighed out. The test substance, incorporated in RO water (2.92 mg/ stock solution), was mixed by means of an electric hand mixer along with further RO water to adjust the soil moisture to 45% maximum WHC. 2.0 g of powdered Lucerne (alfalfa) was also added (concentration in soil 0.5%). Application rates were 0.073 and 0.365 mg/kg dry soil. The test vessels were sealed and the lid perforated to allow air exchange. Triplicate samples of 100 g each per test rate and control were incubated for 28 days under environmentally controlled conditions.

After test item application on day 0, and after moisture adjustment on day 7, 14 and 28 of incubation, soil samples were taken on day 0 and at 7, 14 and 28 days after application from each test vessel. The samples were extracted with potassium chloride (0.1 M KCl, approximately 35 mL) and extract aliquots of 1.0 mL mixed with each 0.20 mL of Hach LCK 339 solution to analyse their $NO_3$-N content using a Hach Lange DR 3900 spectrophotometer. Two aliquots per sample were measured. The Limit of Quantification (LOQ) for this technique was 0.23 mg/L $NO_3$-N.

The data was subjected to statistical analysis (Dunnett Multiple Comparison Test) to determine the 28-day nitrate production NOEC and LOEC, using CETIS version 1.8.6.8. As there was no effect up to and including the highest test item concentration, $EC_{10}$, $EC_{25}$ and $EC_{50}$ values could not be calculated.

**Results and discussion**

Soil temperature remained within the required range of 20 ± 2 °C during incubation.

Over a 28-day period, the test substance had no significant effects on the amount of the nitrate produced in the soil type tested.

**Table A 112: Mean amount of nitrate measured over 28 days**

| Treatment (mg/kg dry soil) | Mean Nitrate-Nitrogen $NO_3^-N$ (mg N/kg dry soil) | | | | Mean Nitrate $NO_3^-$ (mg N/kg dry soil) | | | | Difference compared to the control ($NO_3^-$) (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day 0 | Day 7 | Day 14 | Day 28 | Day 0 | Day 7 | Day 14 | Day 28 | Day 7 | Day 14 | Day 28 |
| Control | 10.95 | 7.69 | 21.28 | 29.22 | 48.18 | 33.85 | 93.65 | 128.57 | N/A | | |
| 0.073 | 13.20 | 10.03 | 20.63 | 30.17 | 58.09 | 44.14 | 90.77 | 132.73 | 30.04 | -3.1 | 3.2 |
| 0.365 | 13.36 | 7.99 | 22.09 | 30.61 | 58.77 | 35.14 | 97.20 | 134.70 | 3.8 | 3.8 | 4.8 |

NA = not applicable
1 mg/kg nitrate nitrogen ($NO_3^-N$) = 4.4 mg/kg nitrate ($NO_3^-$)
Negative percentage difference compared to the control indicates lower nitrate production than the control.

**Table A 113: Mean amount of nitrate produced per day in the test soil**

| Treatment (mg/kg dry soil) | Mean Nitrate-Nitrogen $NO_3^-N$ (mg N/kg dry soil) | | | Mean Nitrate $NO_3^-$ (mg N/kg dry soil) | | | Difference compared to the Control (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Day 7 | Day 14 | Day 28 | Day 7 | Day 14 | Day 28 | Day 7 | Day 14 | Day 28 |
| Control | -0.47 | 0.74 | 0.65 | -2.05 | 3.25 | 2.87 | - | NA | - |
| 0.073 | -0.45 | 0.53 | 0.61 | -1.99 | 2.33 | 2.67 | -2.64 | -28.13 | -7.15 |
| 0.365 | -0.77 | 0.62 | 0.62 | -3.38 | 2.75 | 2.71 | 64.98 | -15.48 | -5.55 |

NA = not applicable
1 mg/kg nitrate nitrogen ($NO_3^-N$) = 4.4 mg/kg nitrate ($NO_3^-$)
Negative percentage difference compared to the control indicates lower nitrate production than the control.

The validity criterion was met:

The variation in $NO_3$-N concentrations between replicate control vessel samples was <15% on day 0, 7, 14 and 28. The coefficient of variation was calculated to be 4.28, 12.24, 4.86 and 3.10% for the Day 0, 7, 14 and 28 extractions, respectively.

**Conclusions**

The effect of the test substance RH-163353 on soil micro-organisms has been studied by investigation of the $NO_3$-N formation in a field fresh arable LUFA 2.3 soil amended with a nitrogen source, powdered Lucerne. Test results of the test item treated soil were compared to the results of a non-treated soil.

On day 28 after test start both treatment groups showed < 25% change in nitrate production compared to the control.

The NOEC for nitrate concentrations on day 28 was statistically determined to be at 0.365 mg/kg dry soil, the LOEC for nitrate concentrations on day 28 was statistically determined to be >0.365 mg/kg dry soil.

It was not possible to calculate $EC_{10}$, $EC_{25}$ and $EC_{50}$ values since there was no significant effect at the highest concentration in the test.

The study is valid.

(Jarrom R. 2020)

### A 2.5.1.1.4 Study 4 - Effects of CYMOXANIL 33% + ZOXAMIDE 33% WG on the activity of soil microflora (Nitrogen transformation test)

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 216 and according to the principles of GLP. The study is considered to be reliable and suitable for the risk assessment |

| | |
|---|---|
| **Reference:** | **KCP 10.5/04** |
| Report | Schulz, L., 2017: Effects of Cymoxanil 33% + Zoxamide 33% WG on the activity of soil microflora (Nitrogen transformation test)<br>Gowan Crop Protection Ltd., UK<br>BioChem Agrar, Germany, Report No. 17 48 SMO 0004, GLP, Not published |
| Guideline(s): | OECD 216 (2000) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | No |

## Materials and methods

| | |
|---|---|
| **Test material (Lot/Batch No.)** | Reboot (GWN-9823) (30AL5020) |
| **Active substance content (analysed)** | 33.0% cymoxanil<br>32.8% zoxamide |
| **Species:** | Micro-organisms naturally occurring in biologically active soil |
| **Test soil:** | loamy sand (DIN 4220) / sandy loam (USDA) |
| Source | Wassergut Canitz, Germany |
| Soil batch no.: | |
| Date of sampling: | 16.03.2017 |
| Fertilisers: | no since 2003 |
| Pesticides: | no since 2003 |
| Nitrogen content (% N): | 0.16 |
| Organic carbon content (% C): | 1.48 |
| Microbial biomass (% of organic C): | 47.47 = 3.21% of $C_{org}$ |
| Cation exchange capacity (meq/100g): | 9.9 |
| pH ($H_2O$) | 6.6 |

| | |
|---|---|
| WHC (%) | 38.00 |
| **Soil type (USDA)** | |
| Clay (%) (< 0.002mm) | 11.1 |
| Silt (%) (0.002-0.050mm) | 37.7 |
| Sand (%) (0.050-2.0mm) | 51.1 |
| **Test vessels** | 500 mL wide-mouth glass flasks |
| Filling: | 200 g soil dry weight per vessel, 3 replicates per treatment group |
| Pre-incubation: | Not specified |
| **Environmental conditions** | |
| Soil moisture: | approx. 45 % of its maximum water holding capacity |
| Temperature: | 19.7-21.3°C in a climatic room |
| Light conditions: | Darkness |
| **Application rate(s)** | Test item:<br>0.8 mg test item/kg soil dry weight<br>4.0 mg test item/kg soil dry weight<br>Control (deionised water only) |
| **Post exposure observation period** | 28 days |
| **Remarks** | None |

The incubation of the soil samples was performed as a series of individual and equal sub-samples of each treatment group. 200 g soil dry weight (= one sub-sample) per test vessel was weighed. The soil was mixed with 0.5% (i.e. 1.0 g/200 g soil d.w.) lucerne meal by means of a hand-stirrer (the C/N ratio of the lucerne meal was 13.2/1). One additional soil sample (without lucerne meal) was used for determination of the initial $NH_4$-N-content and $NO_3$-N-content. The $NH_4$-N-content and the $NO_3$-N-content was < LOQ and 2.69 mg/100 g soil d.w., respectively. The test item was mixed with deionised water and the test solution was subsequently mixed with the soil by means of a hand stirrer. Water was added to the soil to achieve a water content of approximately 45% of WHC.

The incubation of the prepared soil was carried out in wide-mouth glass flasks (500 mL) under the conditions mentioned above. The screw caps of the flasks used permitted air exchange. The water content of the soil in each test vessel was determined at test start (after application) and adjusted once a week to the required range of 40 - 50% of WHC.

Soil samples (10 g soil d.w. per replicate) were taken at intervals of 3 hours, 7, 14 and 28 days after application and the $NH_4$-N-, $NO_3$-N- and $NO_2$-N-contents were determined.

Soil was extracted by adding 50 mL 1 M KCl solution to the equivalent of 10 g soil d.w. and mixed on a rotator at 150 rpm for 60 minutes. The mixtures were centrifuged and stored deep-frozen prior to analysis at -20 ± 5 °C. The analysis was performed within one week following termination of the study at day 28.

For the quantitative determination of the mineralized part of nitrogen the Autoanalyzer produced by BRAN+LUEBBE, Hamburg, Germany was used.

The autoanalyzer was calibrated before each measurement series by establishing a calibration curve. After every 30 samples a standard was measured for recalibration and adjusting the calibration curve. The calibration curve was calculated with linear regression. The Limits of Quantification (LOQ) for NO3-N,

$NH_4$-N and $NO_2$-N were 0.84 mg/100 g soil d.w., 0.09 mg/100 g soil d.w. and 0.14 mg/100 g soil d.w., respectively.

## Results and discussion

Environmental parameters (pH, temperature and relative humidity) remained within acceptable limits throughout the study. The pH was ranged from 6.4-6.5, the temperature ranged from 19.7-21.3°C and relative humidity was in the range from 41.96 - 44.20% during of the study.

No adverse effects of the test item on nitrogen transformation in soil could be observed at both test concentrations (0.8 mg/kg soil dry weight and 4.0 mg/kg soil dry weight) after 28 days (time interval 14-28). The results are summarised in the table below. As no significant effects were seen at 28 days, the test was terminated at this time.

**Table A 114: Effects on nitrogen transformation in soil after treatment with the test item**

| Time Interval (days) | Control | 0.8 mg test item/kg soil dry weight | | 4.0 mg test item/kg soil dry weight | |
|---|---|---|---|---|---|
| | $NO_3$-N in mg/kg soil dry weight [1)] | $NO_3$-N in mg/kg soil dry weight [1)] | % difference to control | $NO_3$-N in mg/kg soil dry weight [1)] | % difference to control |
| 0-7 | 6.58 | 6.67 | +1.4 | 6.27 | -4.8 |
| 7-14 | 0.69 | 0.91 | +31.7 | 1.25 | +80.7 |
| 14-28 | 1.05 | 0.90 | -13.8 | 1.02 | -2.9 |

The calculations were performed with unrounded values

1) Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation

All validity criteria were met:

- The coefficients of variation in the control group of the nitrogen test were maximum 6.2% and thus fulfilled the demanded range ≤ 15%.
- In the most recent test dated 17.01. - 14.02.2017, the toxic standard Dinoterb caused effects of +35.4%, +28.2% and +126.8% (required ≥ 25%) on the nitrogen transformation in a field soil at the tested concentrations of 6.80 mg, 13.60 mg and 27.20 mg Dinoterb per kg soil dry weight, respectively, 28 days after application and thus demonstrates the sensitivity of the test system.

## Conclusions

Effects on soil nitrogen transformation (measured as $NO_3$-N-production) in the test item treatments of 0.8 mg and 4.0 mg test item/kg soil dry weight were below 25% at the end of the 28-day incubation period (time interval 14-28). Thus, no adverse effects on soil nitrogen transformation are expected at concentrations up to 4.0 mg test item/kg soil dry weight.

(Schulz L. 2017)

# A 2.6 KCP 10.6 Effects on terrestrial non-target higher plants

## A 2.6.1 KCP 10.6.1 Summary of screening data

## A 2.6.2 KCP 10.6.2 Testing on non-target plants

## A 2.6.2.1.1 Study 1 – Vegetative vigour study

| | |
|---|---|
| Comments of zRMS: | This study was previously evaluated and considered to be acceptable. Please refer to product fRR dated January 2012. |

| | |
|---|---|
| **Reference:** | **KCP 10.6.2/01** |
| Report | Colli, M., 2007: Vegetative vigour limit test for non-target plants following single rate application of 'CYMOXANIL 33% + ZOXAMIDE 33% WG'<br>Oxon Italia S.p.A., Italy<br>Biotecnologie BT S.r.l., Italy, Report No. BT034/06, GLP, Not published |
| Guideline(s): | OECD 227 (2006) |
| Deviations: | No |
| GLP: | No |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | No |

**Executive Summary**

Phytotoxicity of 'Cymoxanil 33% + Zoxamide 33% WG' on non-target plants was assessed through treatments of plants of 6 different species. A single concentration (1.35 kg/ha) of test item was tested on 30 plants for each species; the control group was treated with deionised water.
Effects on vigour and growth were evaluated 7, 14, 21 and 28 days after the application.
No significant effect of phytotoxicity was observed for all species tested; statistically significant inhibition of plant dry biomass was recorded for only one species (*Pisum sativum*) 28 days after the treatment.
According to EU guidance on terrestrial ecotoxicology and EPPO decision scheme, the formulation is classified as low risk to non target plants since all species showed biomass inhibition rate lower than 50%

### I. MATERIALS AND METHODS

**A. MATERIALS**

**1.Test Material:**

| | |
|---|---|
| **Description:** | Granules |
| **Lot/Batch #:** | Batch code BPL 212 |
| **Purity:** | Nominal: Cymoxanil 330 g/kg, Zoxamide 330 g/kg<br>Measured: Cymoxanil 322.9 g/kg, Zoxamide 333.2 g/kg |
| **Stability of test compound** | June 2008 (expiry date) |
| **2. Vehicle and/or positive control** | Deionised water as control |
| **3. Test Species** | *Cucumis sativa, Glicine max, Helianthus annus, Pisum sativum, Oryza sativa, Avena sativa.* |

**4. Environmental conditions**

| | |
|---|---|
| **Temperature:** | 9 - 27°C |
| **Humidity:** | 49 - 77% |
| **Air changes:** | Not relevant |
| **Photoperiod:** | 417 – 2501 lux |

**B. STUDY DESIGN AND METHODS**

| | |
|---|---|
| **1. In life dates** | 16 October – 6 December 2006 |

**2. Experimental treatment** Two mono- and four dicotyledon species at BBCH 12-14 were sprayed at nominal rate of 1.35 kg product/ha in 200 L water /ha in a fully controlled greenhouse. Controls were treated with de-ionised water. Each test group consisted of 6 (monocots) or 15 (dicots) replicates (pots), with each replicate containing 2 (dicots) or 5 (monocots) plants.

**3. Observations** Phytotoxicity was determined 7, 14 and 21 days after the application. The effects on plant fresh and dry weights were determined at the end of the test.

**4. Statistics** Student's T – Test, Anova and Manova Test were performed using the program Statistic – Stat Soft 1998

## II. RESULTS AND DISCUSSION

### A. FINDINGS

No significant symptoms of phytotoxicity were observed in all tested species.
The inhibition effect on biomass was evaluated in comparison with the control groups: no significant differences were measured for the fresh biomass, while for the dry one a low significant effect was recorded for the specie *Pisum sativum*.

**Table IIIA 10.8.1.2-1: Fresh and dry inhibition 28 day after the treatment.**

| Species | Fresh biomass | Dry biomass |
|---|---|---|
| | ***Inhibition (%)*** | ***Inhibition (%)*** |
| *Avena sativa* | - 6.89* | 1.36* |
| *Oryza sativa* | 15.24* | 0.45* |
| *Cucumis sativa* | - 6.95* | 0.84* |
| *Glycine max* | 7.48* | 8.25 |
| *Helianthus annus* | - 6.29* | - 10.73* |
| *Pisum sativum* | 10.95* | 16.26** |

* no significant differences in comparison with the control; p-value > 0.05
** significant differences in comparison with the control; p-value < 0.05

## III. CONCLUSIONS

Since for all tested species the phytotoxic effects were less than 50%, Cymoxanil 33% + Zoxamide 33% WG is classified as at low risk product.

(Colli M., 2007)

**A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants**

**A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)**

**A 2.8 KCP 10.8 Monitoring data**